Glycolipids: Animal

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Glycolipids are carbohydrates linked to lipid (either ceramide or glyceride). They are found in animal cells and tissues.

Advanced article

Article Contents

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Introduction

Glycolipids are ubiquitous components of all animal cell membranes and are particularly abundant at the cell surface membrane. The majority of glycolipids belong to the class 'glycosphingolipids' (GSLs; also called sphingoglycolipids), which have a backbone lipid (termed 'ceramide') consisting of fatty acids and a long-chain aliphatic amino alcohol, discovered and named 'sphingosine' by JLW Thudichum in 1876. Sphingosine has the structure 1,3-dihydroxy-2-amino-octadecene, exhibiting the D-erythro stereoconfiguration with regard to the asymmetric carbon 1 (C1), C2 and C3 (**Figure 1a**). Fatty acids with various chain lengths are linked to the 2-amino group of sphingosine to form ceramide (**Figure 1b**). Various sugar residues are linked to the C1 primary hydroxyl group of the sphingosine moiety in ceramide to form galactosylceramide (GalCer) (**Figure 1c**), glucosylceramide (GlcCer) (**Figure 1d**), or a variety of more complex oligosaccharides, resulting in a wide variety of GSLs. One example of such a structure, 'GM3', which has sialic acid, galactose and glucose, is shown in **Figure 1e**. The sugar linkage to the C1 hydroxyl group of ceramide is always β, with only a single known exception – α-Gal ceramide, which is found in sea anemones.

GSLs are also found in plants, including yeast, although the ceramide and carbohydrate structures are distinctively different from those of animal GSLs. The ceramide of plant GSLs has a sphingosine analogue, termed 'phytosphingosine', which has an additional hydroxyl group at the C4 position. The carbohydrate moiety of plant GSLs has a novel glycan, termed 'phytoglycosphingolipid', consisting of phosphoinositol, glucosamine and mannose. GSLs are rarely found in bacteria, except for a novel group of 'sphingobacteria' that includes *Sphingomonas paucimobilis*.

A further class of glycolipids, termed 'glycoglycerolipids', has been found and characterized. They have 1,2-diacyl-*sn*-glycerol or 1–alkyl-2-acyl-*sn*-glycerol as a backbone lipid, to which a monosaccharide or relatively short oligosaccharide is linked through the primary hydroxyl group (**Figure 2**). Only two glycoglycerolipids have been well characterized as animal tissue components. Their distribution is limited to the nervous system (brain, spinal cord, peripheral nerves) and testis. In contrast to animal tissues, glycoglycerolipids are the major component in plants and bacteria.

Another class of glyceroglycolipids is the 'glycosylphosphatidylinositol anchor' (GPI anchor). A large number of functionally important cell-surface proteins are anchored through this class of glycoglycerolipids (see below). **See also**: Glycolipids: distribution and biological function

Structure

The most extensive studies on the structure and function of animal cell glycolipids have been focused on GSLs. GSLs consist of two distinct moieties: ceramide, which is hydrophobic, and carbohydrate, which is hydrophilic. A molecular model of GSL based on X-ray crystallography indicates that the axis of the ceramide is perpendicular to the axis of the carbohydrate chain. GSLs have a strong tendency to aggregate to form micelles in aqueous media, or to form microdomains in the cell membrane bilayer.

GSLs from animal tissues are classified according to two criteria: (1) the presence or absence of strongly acidic group (sialic acid or sulfate), or cationic amino group (very rarely present); and (2) differences in core carbohydrate structure. Four subclasses based on criterion (1) are neutral GSLs, gangliosides (GSLs containing sialic acid), sulfatide (sulfated GSL) and a few cationic GSLs having free amino group. Three subclasses based on criterion (2) are: ganglio-series, lacto-series and globo-series GSLs. In the current literature, approximately 50 ganglio-series, 80 lacto-series and 10 globo-series GSLs are known. For ganglio-series GSLs, 2 neutral, 7 sulfated and $\sim$40 sialylated species are known. For lacto-series, 14 neutral, 2 sulfated, $\sim$30 sialylated and $\sim$32 fucosylated species are known. In some cases, hybrid types between the lacto- and ganglio-series or between the globo- and lacto-series have been observed. In certain protozoa, parasites and marine invertebrates, novel GSL structures have been observed that cannot be assigned to any of the three subclasses described above.

Neutral glycosphingolipids

The most abundant GSL in animal tissues is galactosylceramide (GalCer; cerebroside) in brain, discovered by

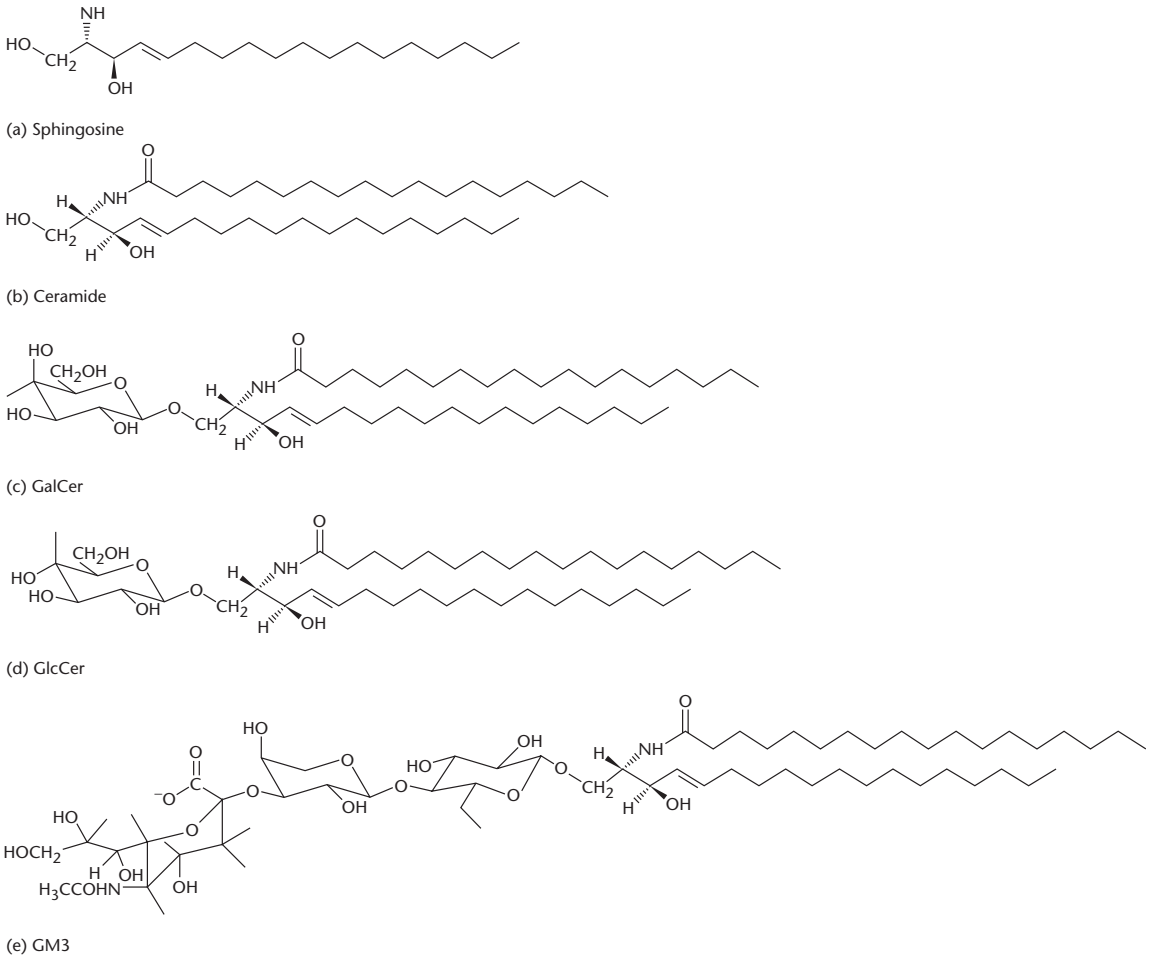

Figure 1 Structures of sphingosine (a), ceramide (b), galactosylceramide (c), glucosylceramide (d) and ganglioside GM3 (e).

Thudichum together with sphingosine in 1876. This was the only known glycolipid of animal cells until 1934, when Aghion found that glucosylceramide was accumulated in Gaucher disease. Lactosylceramide was discovered by Klenk and Rennkampf in bovine spleen in 1942, and globoside was discovered in human erythrocytes by Yamakawa and Suzuki, and Klenk and Lauenstein in 1951–1952. Since then, many variations derived from these basic structures have been found, and typical structures have been classified into globo-, lacto- and ganglioseries GSLs; representative examples of these are shown in **Figures 3**–**5**. These neutral GSLs constitute major membrane components of animal cells and tissues. Glucosylceramide and lactosylceramide are common to GSLs in animal cells and tissues, and all GSLs (except GalCer) are derived by the addition of various sugar residues to lactosylceramide.

Gangliosides

GSLs containing sialic acid (see below) are collectively termed 'gangliosides'. Sialic acid was found originally as a component of mucin, as well as of GSLs, by G. Blix and E. Klenk during 1935–1938. There was longstanding debate over its structure, but it was finally established by A. Gottschalk and S. Roseman as a condensation product of *N*-acetylmannosamine and pyruvic acid. Currently, the term sialic acid is applied to *N*- or *O*-acetyl or *N*-glycolyl derivatives of neuraminic acid (5-amino-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid), containing a novel nine-carbon sugar carboxylic acid as the backbone structure.

The first ganglioside was isolated and so named by Klenk from the brain of a child who died from Tay–Sachs amaurotic idiocy. It was composed of lactosylceramide,

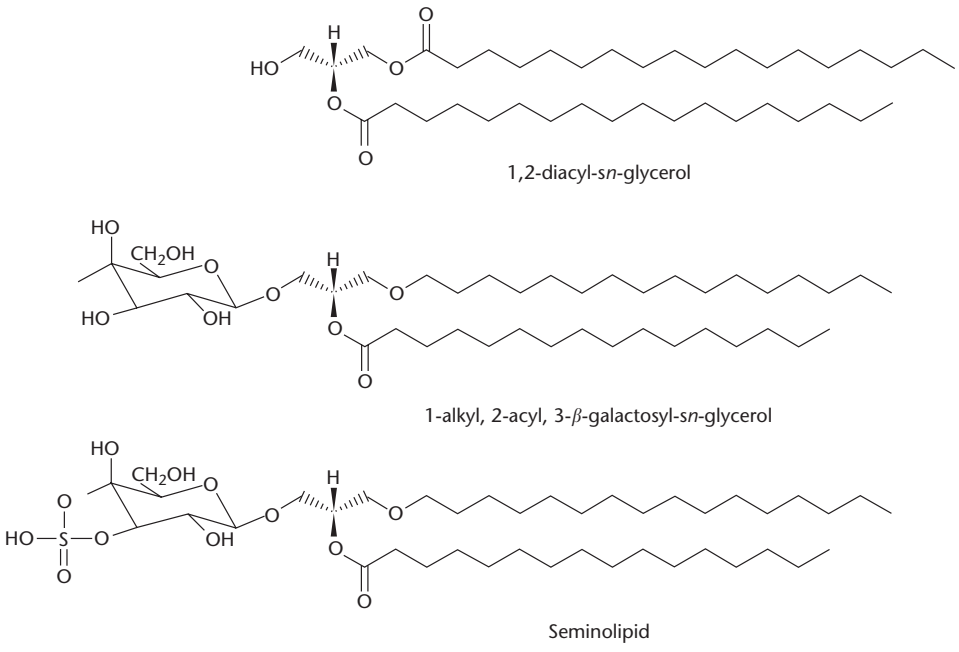

Figure 2 Structures of diacylglycerol, β-galactosyl diacylglycerol and seminolipid.

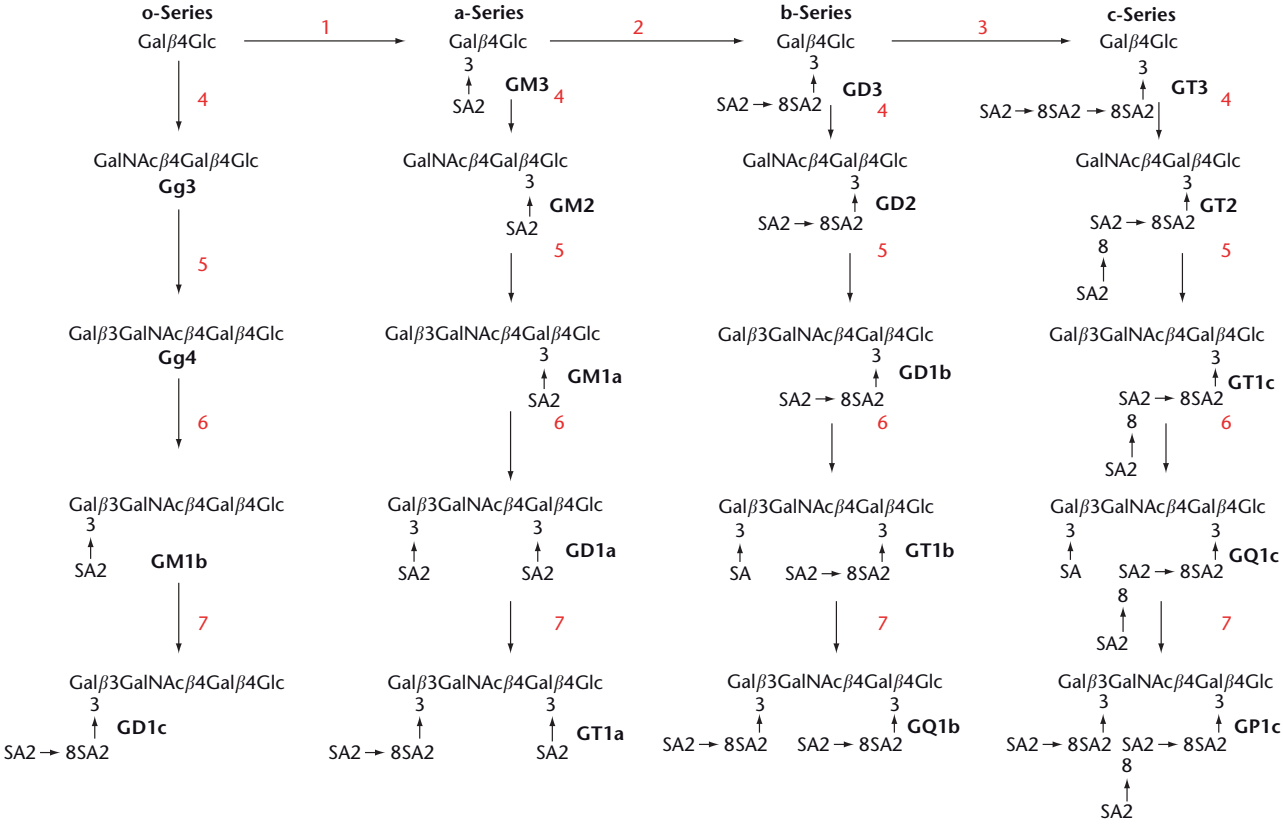

Figure 3 Ganglio-series GSLs, having o-, a-, b- and c- subseries as indicated. Enzymes and genes involved in each synthetic step (indicated by red numbers) are described under 'Synthesis and Degradation/(1) Ganglio-series pathway'

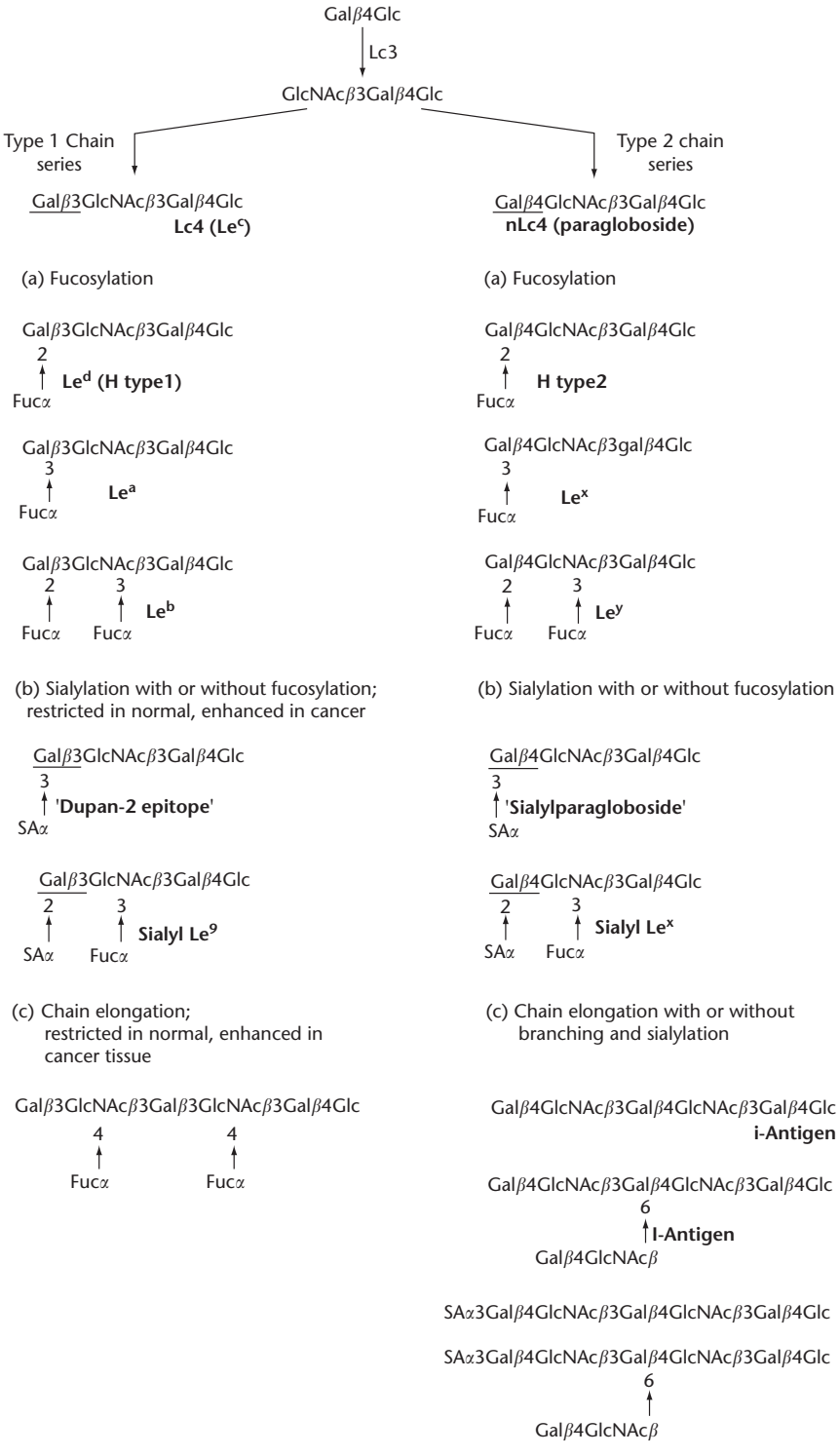

Figure 4 Lacto-series GSLs, having type 1 and type 2 chain subseries as indicated.

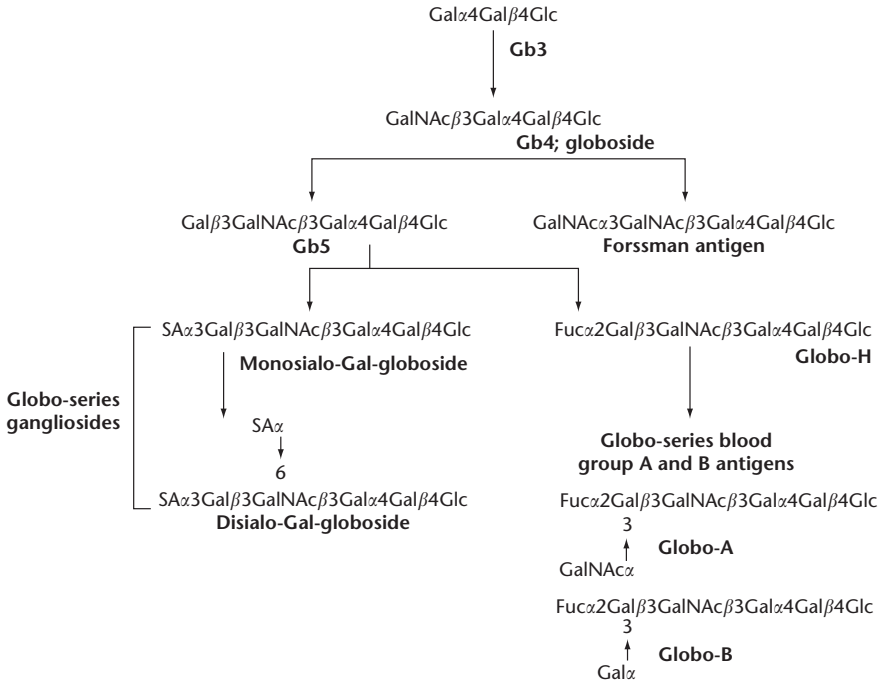

Figure 5 Globo-series GSLs.

N-acetylgalactosamine and sialic acid, often called 'GM2 ganglioside' (**Figure 3**). A systematic analysis of brain gangliosides was undertaken by R. Kuhn and H. Wiegandt, and the structures of GM1 ganglioside and its derivatives were clearly established in 1963. In contrast to ganglioside in the brain, a major ganglioside present in various other organs and blood cells had no galactosamine and consisted of lactosylceramide and sialic acid. This ganglioside, originally isolated from horse erythrocytes by Yamakawa and Suzuki in 1951 and termed 'haematoside', is often termed 'GM3'. Gangliosides GM3, GM2 and GM1 thus represent the basic backbone structure of a number of gangliosides derived therefrom by addition of different numbers of sialic acid at different positions. They are termed 'ganglio-series gangliosides'. Depending on the number of sialic acid substitutions, o-, a-, b- and c- series of ganglio-series gangliosides are distinguished. These gangliosides are usually named according to the system of Svennerholm (**Figure 3**).

There are two other types of gangliosides with different core structure. One type has the *N*-acetylglucosamine structure, and is known as the 'lacto-series gangliosides' (**Figure 4**). The other type has the same core structure as globoside (**Figure 5**), and is known as the 'globo-series ganglioside'. The carbohydrate structures of these two types are very different from those of ganglio-series gangliosides. A large number of different structures of lacto-series gangliosides have been found, whereas there are only a few globo-series structures (**Figure 5**).

Sulfated glycolipids

In comparison to neutral GSLs and gangliosides, there are a relatively small number of sulfated GSLs. The most abundant and widely distributed is 'sulfatide', i.e. sulfated GalCer, discovered originally by G. Blix in 1933. Its structure was identified as 3-*O*-sulfated GalCer by Yamakawa and independently by Stoffyn in 1962. Sulfatide is abundant in brain white matter, together with GalCer, and is the major lipid component in myelin sheath membrane. Sulfatide is also found in high concentration in kidney and gastrointestinal epithelia.

The second most abundant sulfated GSL is sulfated lactosylceramide (LacCer), which is absent in brain but rich in human kidney and in eggs of certain fish species. New types of sulfated GSL have been isolated and characterized by Ishizuka and colleagues from rat kidney: monosulfated or di(bis)-sulfated gangliotriaosylceramide, sulfated GM1, etc. Structures of typical sulfated glycolipids are shown in **Figure 6**. Similar types of sulfated GSLs were found in liver and intestine of mammals as well as in some malignant cell lines derived from glandular epithelial cells.

Another sulfated glycolipid found in animal tissues is seminolipid (see below).

Cationic glycolipids

Recently, GSLs with free amino group, showing cationic properties, have been isolated and characterized from

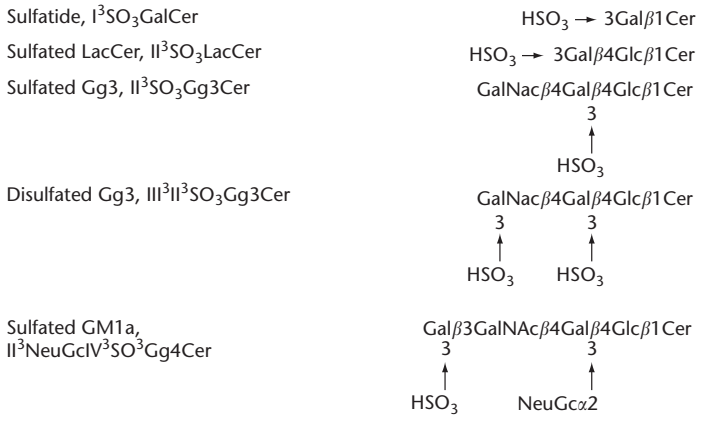

Figure 6 Sulfated glycolipids.

human and bovine brain. They are galactosylsphingosine (psychosine), plasmal (cyclic acetal) derivatives of psychosine (2,6- and 4,6-plasmalopsychosine) and psychosine conjugated with glycerol and another plasmal (glycero-plasmalopsychosine). In addition, some GSLs (e.g. Lc3) containing glucosamine with free amino group, without *N*-acetyl group, have been found. These cationic GSLs are minor components in brain, and we do not know whether they are present in other tissues. For further reading, see Hikita *et al*., 2002.

Glycoglycerolipids and glycosylphosphatidylinositol anchor

Glycoglycerolipids are the major glycolipid component in bacteria and plants. A 1985 review article by Ishizuka and Yamakawa (1985) lists 104 types of glycoglycerolipids. Since then, the total has increased, particularly for 'lipoteichoic acids'. Currently at least 150 kinds of glycoglycerolipids are known from various plants and microbes.

In striking contrast, animal cells and tissues have only three kinds of glycoglycerolipid: β-galactosyl-diglyceride, its sulfated derivative ('seminolipid'), and GPI as membrane anchor. The first two have the unique property of being present only in testis and nervous tissue, whereas GPI anchor is widely distributed in animal cells. Galβ1→3 1,2-diacyl-*sn*-glycerol or Galβ1→3 alkylacyl-*sn*-glycerol is found in bovine spinal cord and brain white matter, boar and human testis and rat brain. The same compound is widely distributed in various higher plants and bacteria. 3-*O*-Sulfated Galβ1→3 1,2-alkylacyl-*sn*-glycerol (termed 'seminolipid') was found abundantly in mammalian testis and spermatozoa by Ishizuka and Yamakawa. While testis seminolipid contains almost exclusively 1-alkyl-2-acyl-*sn*-glycerol as lipid component, nervous tissue seminolipid contains a mixture of 1,2-diacyl-*sn*-glycerol and 1-alkyl-2-acyl-*sn*-glycerol. The ratio of composition of these two glycoglycerolipids varies depending on species and developmental stage. In animal glycoglycerolipids, fatty aldehyde linked to glycerol as an alkenyl ether is essentially absent. Glycoglycerolipid having alk-1-enyl linkage has been found in bacteria.

Phosphatidylinositol is a well-known phospholipid widely distributed in animal and plant cells and tissues, but the majority is present as phosphatidylinositol 3-phosphate or 3,4-bisphosphate and is hydrolysed by phospholipase C to give 1,2-diacylglycerol, inositol 1,3-diphosphate and inositol 1,3,4-triphosphate. Among these hydrolysis products, 1,2-diacylglycerol and inositol 1,3,4-triphosphate are well known as second messengers affecting signal transduction.

Phosphatidylinositol without additional phosphorylation (i.e. absence of 3 and 4 phosphate) as above is linked to a novel tetrasaccharide core consisting of three mannoses (M1, M2, M3) and one glucosamine with a free amino group (not *N*-acetylated). The structure of this core is conserved from protozoa to mammalian cells, i.e. Manα1→2Manα1→6Manα1→4GlcNα1→6 inositol, in which terminal (nonreducing) Man is linked to the phosphate group of phosphoethanolamine (ETN). The *C*-terminus of the cell-surface protein is linked to the amino group of ETN, which plays a role as linker between GPI and the cell-surface protein. This basic, common structure for the 'GPI anchor' is shown in **Figure 7**. The fatty acid of phosphatidylinositol functioning as anchor is saturated. This type of glycolipid for anchoring cell-surface protein is clustered in the caveolae and/or GSL-enriched microdomain (GEM).

A large number of eukaryotic proteins are attached to the cell surface by this novel glycoglycerolipid. The first breakthrough regarding the presence of such an anchoring protein came from the identification of a bacterial phosphatidylinositol-specific phospholipase C (PI-PLC), which cleaves GPI-anchored protein from the cell surface. Another enzyme, GPI-phospholipase D (GPI-PLD), can also cleave GPI-anchored protein from the cell surface. A series of GPI-anchored proteins was originally found in

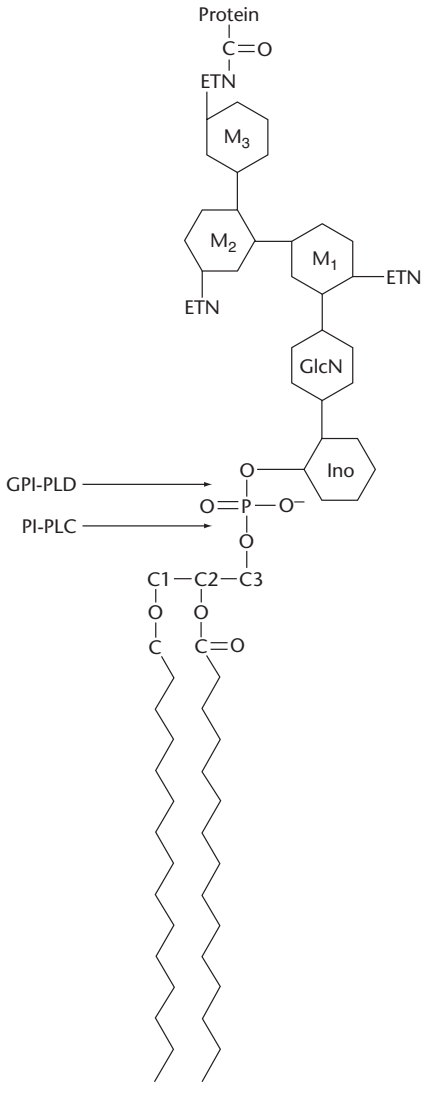

Figure 7 Core structure of the glycosylphosphatidylinositol (GPI) anchor. Arrows indicate cleavage sites for PI-phospholipase C and GPI-phospholipase D.

protozoa (e.g. *Trypanosoma*) and was subsequently found to be widely distributed in essentially all eukaryotic cell membranes. This is one of the major modes of association of cell-surface proteins, as opposed to other modes, i.e. transmembrane assembly. Currently, there is particular interest in the fact that many functional proteins in immunocytes (e.g. lymphocytes) are associated through GPI anchors. As many as 23 cell-surface proteins in lymphocytes (all named by CD series) are listed in current literature. Most remarkably, these GPI-anchored proteins are capable of delivering a core stimulatory signal to lymphocytes. For details on their structural variation and function, see Further Reading.

Synthesis and Degradation

The sphingosine backbone, a characteristic component of the lipid moiety of GSLs and sphingolipids, is synthesized from serine and activated palmitic acid, i.e. palmitoyl–coenzyme A. This reaction is catalysed by palmitoyl transferase, which is encoded by genes NM_009269 and NM_011479. Subsequently, the amino group of the sphingosine backbone is fatty acylated by various fatty acyl–coenzymes A to form dihydroceramide. A double bond is introduced by dihydroceramide desaturase acting only on ceramide in the sphingosine backbone, thus forming ceramide. These processes take place at the endoplasmic reticulum (ER) (**Figure 8**). Ceramide is also formed by hydrolysis of sphingomyelin by sphingomyelinase, or sphingomyelin is converted into ceramide by the reverse reaction. These reactions are catalysed by sphingomyelin phosphodiesterase 1 (SMPD1) and 2 (SMPD2), which are encoded respectively by genes NM_011421 and NM_009213. Newly synthesized or recycled ceramide is galactosylated or glucosylated by specific galactosyl- or glucosyltransferase, with uridine diphospho-Gal or uridine diphospho-Glc acting as the Gal or Glc donor, to form galactosylceramide (cerebroside) or glucosylceramide, catalysed by β-Gal transferase (GalCer synthase) (encoded by gene AK127970, AK057066 or AK128994) or β-glucosyltransferase (GlcCer synthase) (encoded by gene NM_011673), respectively. Galactosylceramide is rarely further glycosylated, but is often sulfated to form sulfatide, catalysed by sulfotransferase (encoded by gene AB040610).

Glucosylceramide is further glycosylated by a series of glycosyltransferases, with sugar nucleotides as sugar donors, to form a number of neutral GSLs and gangliosides (**Figures 3**–**5**). Each glycosyltransferase recognizes two substrates: the glycosyl residue of glycosylceramide, to which a sugar is transferred, and the sugar nucleotide, from which a sugar residue is donated. For synthesis of lactosylceramide (LacCer), β1–4Gal transferase-IV or β1–4Gal transferase-VI (encoded by gene NM_019835 or NM_019737, respectively) recognize GlcCer and UDP-Gal, and transfer Gal from UDP-Gal to GlcCer. LacCer, thus formed, is further converted via three pathways:

(1) *Ganglio-series pathway*. This is initiated by α2-3 sialylation catalysed by an enzyme capable of transferring sialic acid from cytidine monophospho (CMP)-sialic acid to LacCer. The enzyme (GM3 synthase) is encoded by gene NM_011375 (mouse), NM_003896 (human) or AY515255 (chicken). GM3 thus synthesized is converted into GM2 by β1-4GalNAc transferase (GM2 synthase) that recognizes UDP-GalNAc and GM3 ganglioside. The enzyme is encoded by gene NM_008080. Synthetic pathways of ganglio-series gangliosides, and genes encoding enzymes involved at each synthetic step, are summarized in **Figure 3**. GD3 synthase, which converts GM3 into GD3 (step 2), is encoded by gene NM_011375. GT3 synthase, which converts GD3 into GT3 (step 3), is encoded by gene

NM_013666. The same enzyme and gene involved in GM3-to-GM2 conversion (GM2 synthase as above) (step 4) are also involved in synthesis of Gg3, GD2 and GT2. β1-3Gal transferase for conversion of GM2 into GM1a (known as 'GM1 synthase') (step 5), is encoded by gene NM_019420. The same enzyme and gene are also involved in synthesis of Gg4, GD1b and GT1c. α2-3 sialyltransferase specific for transfer of sialic acid to terminal Gal of GM1a (SAT-IV) (step 6) is encoded by gene NM_009179. The same enzyme and gene are also involved in synthesis of GM1b, GT1b and GQ1c. Another α2-8 sialyltransferase (ST-8Sia–V), which transfers sialic acid to terminal sialic acid of GD1a for synthesis of GT1a (step 7), is encoded by gene NM_013666, the same gene that encodes GT3 synthase. The same enzyme and gene are also involved in synthesis of GD1c, GQ1b and GP1c.

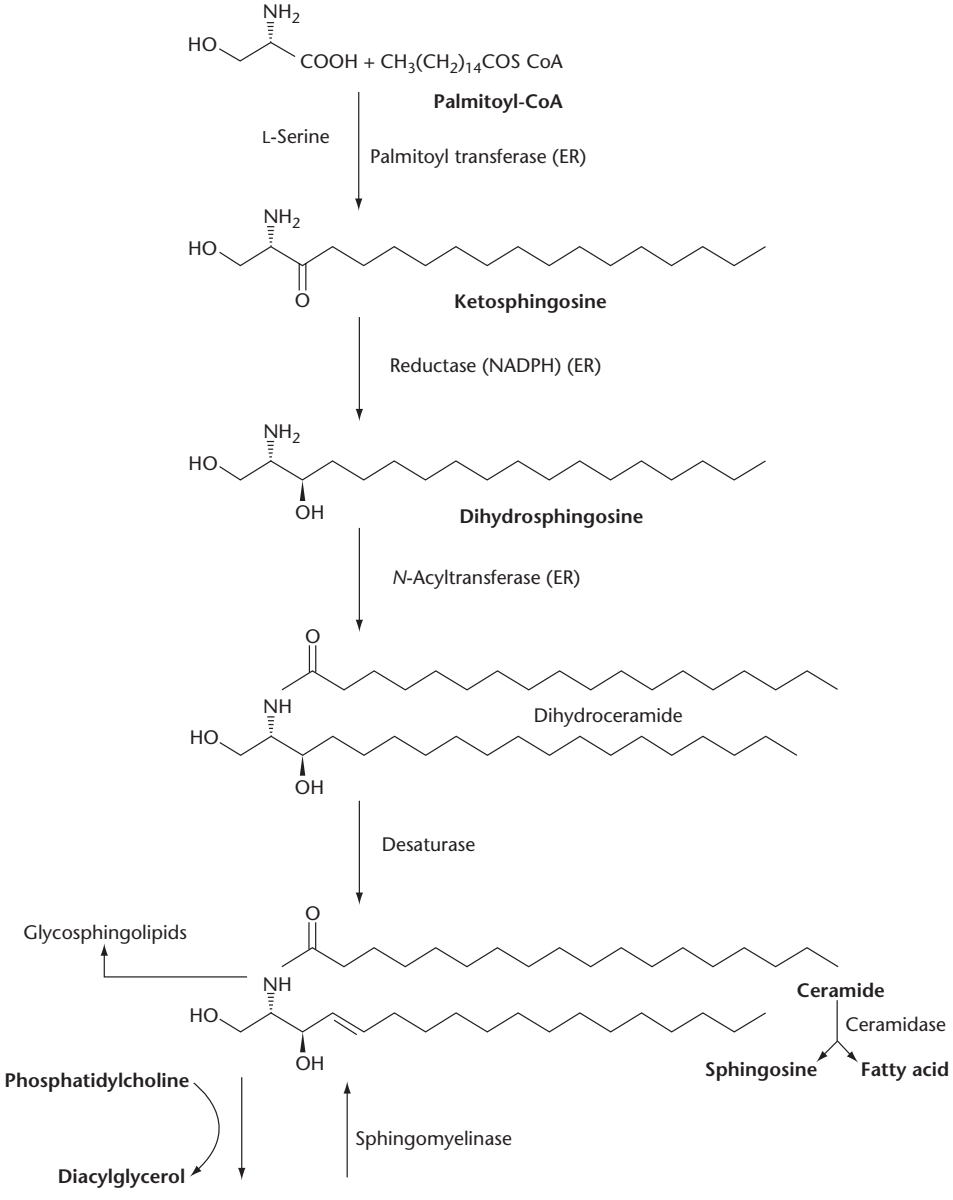

Figure 8 Basic synthetic pathway of ceramide and glycosphingolipids. The first four steps indicate the synthetic process from L-serine and palmitoyl–CoA to ceramide. Note that the double bond of sphingosine is introduced at ceramide. These processes take place at the endoplasmic reticulum (ER). Ceramide is converted into sphingomyelin by transfer of phosphorylcholine from phosphatidylcholine. Conversely, sphingomyelin is hydrolysed by sphingomyelinase and converted into ceramide. Subsequent ceramide glycosylation (glucose addition by β-glucosyltransferase, followed by addition of galactose by β-Gal transferase I to form LacCer) takes place on the Golgi membrane. Subsequent synthesis of ganglio-, globo- and lacto-series GSLs is initiated by β1,4 GalNAc-T, α1,4 Gal-T and β1,3 GlcNAc-T, respectively.

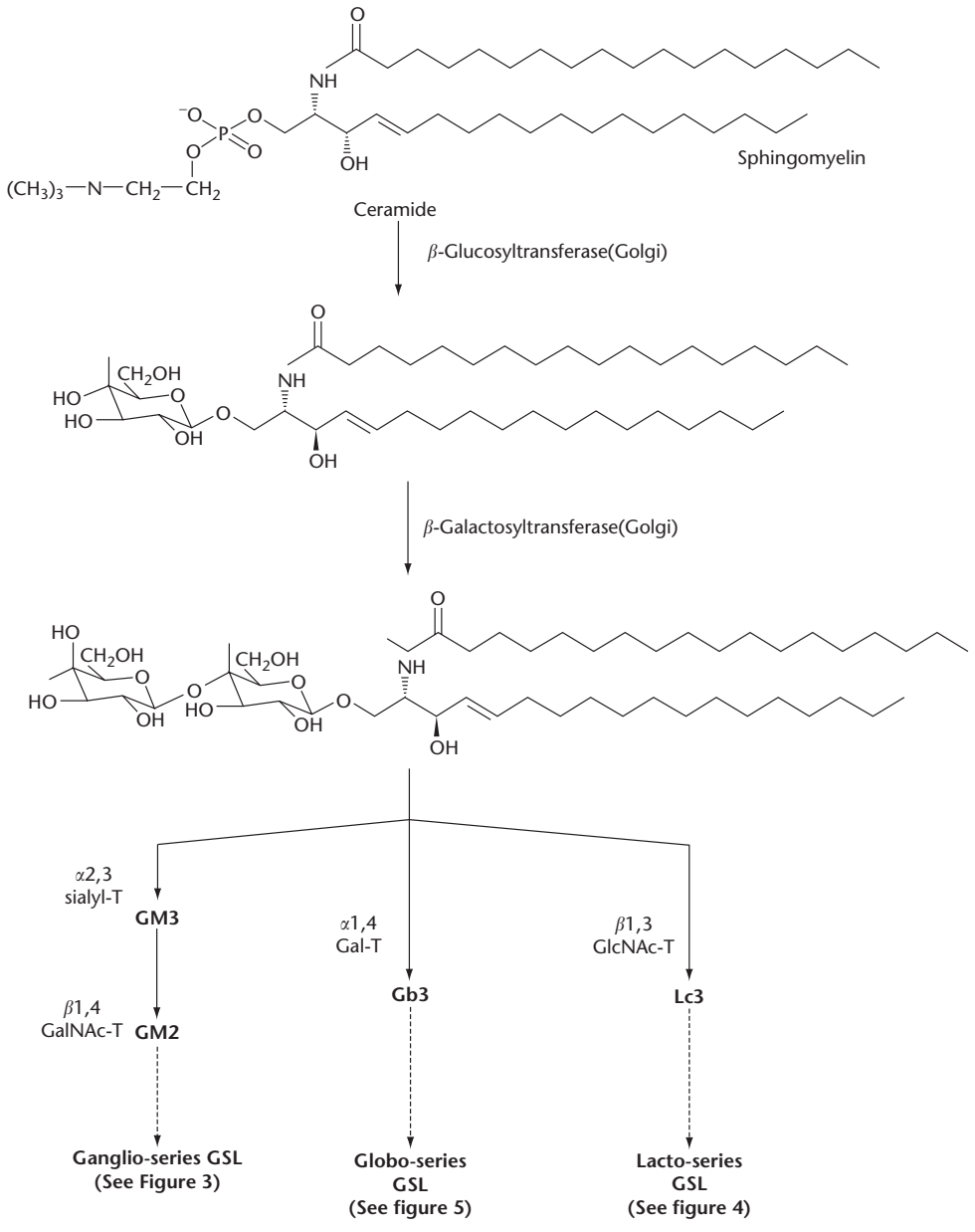

Figure 8 Continued

(2) *Globo-series pathway*. This is initiated by α1-4 galactosylation of terminal β–Gal residue of LacCer, catalysed by the enzyme (Gb3 synthase) recognizing LacCer and UDP-Gal, which is encoded by gene XM_139501 (recently replaced by NM_001004150/AY371179). Gb3 is converted into Gb4 (globoside) by β1-3GalNAc transferase (Gb4 synthase), encoded by genes NM_003781 ('variant 1'), NM_033169 ('variant 4'), etc. Gb4 is converted into Gb5 (SSEA–3) by β1-3Gal transferase (Gb5 synthase), encoded by a gene not yet entered in GeneBank, but presumably the same as β1-3Gal transferase for synthesis of GM1. Gb5 is converted by α2-3 sialylation catalysed by the enzyme ST3Gal-II (encoded by gene NM_173344), to sialyl-Gb5 (SSEA–4), defined by mAb RM1. Alternatively, the terminal Gal of Gb5 is α1-2 fucosylated to form globo-H. The α1-2 fucosyltransferase (FUT) involved in this reaction has not been clearly identified. It may be similar to lacto-series type 2 chain H synthase (see below).

(3) *Lacto-series pathway*. This is initiated by addition of β1-3GlcNAc to LacCer to form Lc3Cer, catalysed by β1-3GlcNAc transferase (Lc3Cer synthase) recognizing LacCer and UDP-GlcNAc. The enzyme is encoded by gene

NM_054052. Two types of GSL (lacto-series type 1 and type 2 chain) are synthesized from Lc3Cer:

a. Lacto-series type 1 chain (Galβ1-3GlcNAcβ1-3Galβ1-4GlcCer) is synthesized by β1-3Gal transferase recognizing Lc3 and UDP-Gal, encoded by genes NM_006057 ('variant 1'), NM_033172 ('variant 4'), etc. This structure is the precursor for type 1 chain H (Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4GlcCer), whose synthesis is catalysed by α1-2 fucosyltransferase–I (FUT-I), encoded by gene NM_000148. Type 1 chain H is an important precursor for synthesis of type 1 chain A and B, and Lewis b, in 'secretors'. Expression of these blood group antigens, their genes and allelic structures have been well elucidated (Hakomori, 1999; Watkins and Clarke, 2001). An important principle is that lacto-series type 1 chain structure is not extended or branched in normal cells and tissues, in contrast to lacto-series type 2 chain which is highly extended and branched (see b below). Lacto-series type 1 chain core structure remains mostly as tetrasaccharide as above. Interestingly, certain human cancer tissues have extended type 1 chain containing Le^a-on-Le^a (dimeric Le^a) or Le^b-on-Le^a (see **Table 3**).

b. Lacto-series type 2 chain (Galβ1-4GlcNAcβ1-3Galβ1-4GlcCer) is synthesized by β1-4Gal transferase-I, -II, and -III, which transfer Gal to Lc3, nLc5, nLc7, etc. to form repeating *N*-acetyllactosamine (LacNAc) units (Galβ1-4GlcNAc). These enzymes are encoded by genes β4GalT-I (NM_022305), β4GalT-II (NM_001005417, NM_003780) and β4GalT-III (NM_003779). These transferases are involved in synthesis of not only type 2 chain tetrasaccharide, but also extended and branched polylactosamine containing 10–20 LacNAc units, present in GSLs and in glycoproteins. Terminal Gal can be α1-2 fucosylated by FUT-II, to form type 2 chain H, the major blood group H antigen expressed in erythrocytes. Internal GlcNAc can be α1-3 fucosylated (by FUT-III, -IV, -V, -VI, -VII, -IX, etc. to form Le^x, dimeric Le^x or trimeric Le^x. In combination with terminal α2-3 sialylation and internal fucosylation, type 2 chain forms sialyl-Le^x, or sialyl internal dimeric Le^x (myeloglycan).

Essentially all enzymes and genes involved in these reactions have been identified, and are summarized in Taniguchi *et al*. (2002). GeneBank numbers mentioned in this section can be accessed through the U.S. National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/; search by 'Nucleotide').

Which type of GSL is synthesized depends on the type of cell – each type of cell has a different combination of activities of various glycosyltransferases, resulting in a cell-type-specific glycolipid pattern. The structure of the carbohydrate moiety of GSLs is therefore determined by the types of glycosyltransferases, and their encoding genes, expressed in the cell. Expression of each glycosyltransferase within the cell is gene defined, i.e. GSLs are secondary gene products. The primary gene products necessary for synthesis of GSL are the many enzymes responsible for the synthesis of ceramide, and the individual glycosyltransferases necessary for adding sugars step by step, as described in the preceding paragraph.

Arrays of glycosyltransferases organized and arranged in the Golgi membrane may be important in determining the types and quantities of GSLs produced. In addition, the degradation mechanism of GSLs through the lysosomal membrane, and connection with Golgi membrane, are also important (for recycling; see below). What mechanism determines termination of sugar chain elongation in GSLs within cells is not known.

Degradation of GSLs is catalysed by glycosidases specific for each type of glycosyl residue. The rate of degradation catalysed by a series of glycosidases is generally considered to be balanced with the synthesis of GSLs within cells, although metabolic turnover of GSLs is much slower than that of phospholipids or the carbohydrate moiety of glycoproteins in many types of cells. The turnover rate may also be different in cultured cells compared with that in cells *in vivo*. Little is known regarding types of glycosidases synthesized and stored in lysosomes, or the mechanism of their effect on GSLs.

It is generally assumed that plasma membrane components reach lysosomes by endocytotic membrane flow (creating 'coated pits') through the endosomal reticulum, which consists of early and late endosomes. GSLs thus reaching lysosomes are degraded to various components, and the final degradation products, sphingosine, dihydrosphingosine (sphinganine) and ceramide, are reutilized for synthesis of various GSLs and sphingomyelin in the Golgi membrane (*cis* and *trans* cisternae). Thus, the reutilization cycle of GSLs and sphingolipids (**Figure 9**, process 2) should be in balance with new synthesis (*de novo* synthesis) of sphingolipids at the ER initiated from serine and palmitoyl–coenzyme A (see above, and **Figure 9**, process 1). Another possibility is that some GSLs from the Golgi membrane are transported to early or late endosomes, and GSLs are transported from late endosomes to the Golgi membrane. Therefore, second recycling of GSLs is possible (**Figure 9**, process 3). The relative contribution of *de novo* synthesis versus recycling for maintenance of intracellular GSL level varies depending on the type of cell (Gillard *et al*., 1999).

Carbohydrate residues of GSLs are hydrolysed step by step by glycosidases organized at the lysosomal membrane, where coexisting 'sphingolipid-activating proteins' (SAP) assist in GSL hydrolysis. Thus, an important feature of GSL degradation is that it requires not only glycosidase, but also its activator protein. Activator proteins for some types of GSLs have been well studied because a disease causing accumulation of these GSLs is caused by a defect of a defined glycosidase in some cases, but caused by a defect of activator protein in other cases.

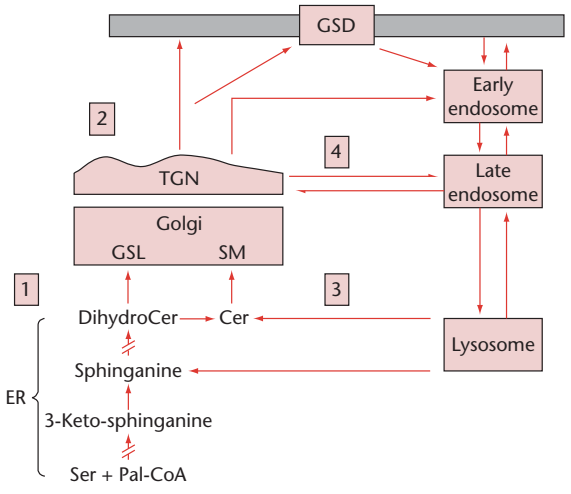

Figure 9 Balance of synthesis, degradation and recycling of sphingolipids and glycosphingolipids. New (*de novo*) synthesis of sphingosine and ceramide is catalysed by a series of enzymes associated with the ER ('1'), followed by glycosylation to form GSL or transfer of phosphorylcholine to form sphingomyelin (SM) at the Golgi membrane ('1'). GSLs are further processed by the *trans*-Golgi network (TGN), and finally incorporated in the plasma membrane and clustered in the glycosignalling domain (GSD) ('2'). GSLs are internalized to reach early endosome, then late endosome and are finally degraded at lysosome. Sphingosine and ceramide thus produced are reutilized for synthesis of GSLs and SM at the Golgi (recycling mechanism '3'). GSLs are 'shuttled' between late endosome and TGN by another recycling mechanism ('4'). GSLs of some animal cells are synthesized mainly by recycling mechanisms 2 and 3. GSLs in other cells depend on *de novo* synthesis (1). The idea of *de novo* synthesis versus recycling of GSLs comes from the data of Gillard *et al.* (1999).

Table 1 General functions of GSLs

1.	GSL antigens See **Table 2**
2.	GSL receptors • Choleratoxin (GM1) • Shigella or verotoxin (Gb3) • *Escherichia* coil infection (globo-series GSL) • *Staphylococcus* infection (Gg4; asialo-GM1)
3.	GSLs in cell adhesion/recognition (i) Mediated by GSL-binding protein • Neuronal cell adhesion to myelin sheath membrane mediated by gangliosides and myelin-associated glycoproteins • Myeloglycan GSLs to selectin • GM1 mediating adhesion through galectin-1 (ii) Mediated by GSL–GSL interaction • GM3–LacCer/Gg3 interaction mediating melanoma adhesion to endothelial cells (initiation of metastasis) • Gb4–GalGb4/ nLc$_4$interaction mediating human teratocarcinoma cell interaction (model of human embryo compaction) • Le^x–Le^x interaction mediating mouse embryogenesis
4.	GSLs controlling signal transduction See text and **Figure 10**

Function

An obvious and well-established role of GSLs is to serve as major cell surface antigens, such as allogeneic (blood group) antigens and developmentally regulated tumour-associated antigens (**Table 1**, item 1). Another role of GSLs is as receptors for bacterial or viral toxins, mediating the process of microbial infection (**Table 1**, item 2). A less well-defined function of GSLs is to mediate cell adhesion/recognition through carbohydrate-binding protein (lectin) or carbohydrate–carbohydrate interaction (**Table 1**, item 3). Recently, there has been increasing evidence that certain GSLs modulate or initiate a certain type of impulse from the cell surface to nucleus, termed 'signal transduction' (**Table 1**, item 4). Each of these functions will be explained below.

Antigens

Allogeneic antigens

Within a given species, different groups of individual have different antigens (called allogeneic antigens), typically found as blood groups, e.g. blood group ABH, blood group P_1 P_2 P^k p, and blood group Lewis a, b, c, d in humans. Each of these categories of antigens (cell-type-specific, species-specific, heterogenetic, allogeneic) has been identified as GSLs, and their carbohydrate structure represents the antigen specificity (see **Table 2**). **See also**: Blood group genetics

Heterogenetic antigens

Some antigens are found in common between a few groups of species, but are absent in other species. These are called heterogenetic antigens. A typical example is Forssman antigen, which is present in horse, sheep, goat, cow, dog and chicken erythrocytes, but absent in human, marmoset, rabbit and pig. Another carbohydrate antigen containing Galα1→3Galβ1→3/4GlcNAc (called di-Gal epitope or Galili antigen) is absent in human and Old World primates, but present in essentially all other mammals including New World monkeys (see **Table 2**).

Cell-type-specific antigens

Certain cells are capable of binding to a defined antibody (antigenicity) or of causing antibody response (immunogenicity), depending on the presence of a defined GSL antigen, termed cell-type-specific antigen. The real function of such antigens in a given cell type is not clear (see **Table 2**).

Table 2 GSL antigens

Antigen type	Antigens
Allogeneic antigens (blood group antigens)	ABH, Lewis (Le^a, Le^b, Le^c, Le^d; *cf*. Figure 4)
	I/i (*cf*. Figure 4)
	P, P^k (*cf*. Figure 5)
Heterogenetic antigens	Forssman antigen (*cf*. Figure 5)
	Positive: horse, sheep, goat, cow, dog, etc.
	Negative: human, marmoset, rabbit, pig, etc.
	Galα1→3Galβ1→3GlcNAc (di-Gal) antigen
	Positive: all mammals except as below
	Negative: human, Old World primates
Cell-type-specific antigens	Le^x for myeloid cells (CD15)
	2→6 sialyl poly-LacNAc for B cells (CD76; CDW75)
	LacCer for myeloid cells (CDW17)
	Sulfated glucuronyl paragloboside (HNK1 antigen) for peripheral nerves, human NK cells (CD57)
	Internally fucosylated sialyl Le^x (VIM2) for neutrophils, monocytes, myelocytes (CDW65)
Developmentally regulated antigens	Stage-specific embryonic antigen-1 (SSEA-1), Le^x, SSEA-3, SSEA-4, etc.
Tumour-associated antigens	See **Table 3**

Developmentally regulated and tumour-associated antigens

The presence of antigens in each type of cell, individual, group of individuals or species, is different when compared at different stages of development. Some antigens are present in large quantity at embryo or fetal stage and disappear in the infant or adult stages. Other antigens are absent at embryo stage, appear in the infant, and are present in large quantity in adult cells and tissues. These are called developmental or developmentally regulated antigens. Many antigens that are abundant in the fetal stage, that decline during development, and are absent (or minimally present) in adult cells, are also found in tumours derived from the same type of cell or tissue. These are called tumour-associated antigens, and are useful for detection of tumour cells (i.e. diagnosis) or treatment of cancer. Many tumour-associated antigens have been identified as GSLs (see **Table 3**). **See also**: Tumour antigens recognized by antibodies

Receptors

Many biologically active factors, such as hormones, toxins and growth factors, cannot enter cells directly because the cells are surrounded by a membrane (the plasma membrane). The factors first bind to specific molecules, termed 'receptors', on the plasma membrane. Many receptors are proteins, but some (particularly for bacterial toxins) have been known for years to be GSLs. Well-known examples are GM1 ganglioside (see **Figure 3**), which acts as the receptor for choleratoxin; GT1b and GQ1c gangliosides, which act as the receptors for botulism toxin; and globotriaosylceramide (Gb3), which acts as the receptor for shigella toxin or verotoxin. These toxins, which are released from bacteria and cause damage to the host cell, are termed 'exotoxins'. Because some GSLs constitute important receptors for exotoxins, specific GSLs can block binding of toxin to the cell surface and thus its entry into the cell. Carbohydrates having the same structure as the GSL receptor can inhibit binding and toxicity. If such carbohydrates are abundantly available, this could save the patient's life. **See also**: Toxin action: molecular mechanisms

A number of recent studies on the infection mechanism of bacteria and viruses indicate that there are two types of infectious process. In one, the pathogen has a protein (termed adhesin) that binds to GSLs on the surface of the host cell. For example, kidney and urinary tract epithelial cells have globoside or other globo-series structures, and the pathogen *Escherichia coli* binds to these structures, the initial step in pathogenic *E. coli* infection of urogenital epithelia. Another example is infection by pneumococci, which have a surface protein capable of binding to target cells having a certain GSL termed 'asialo-GM1'. Because of the crucial role played by globo-series or asialo-GM1 in binding, these specific GSLs or oligosaccharides can be used to inhibit the infectious process.

In the second type of infectious process, bacterial or viral surface carbohydrate is recognized by host cell protein. Chlamydia has a high level of glycan termed 'high-mannose-type glycan'. For chlamydial infection of various cells, surface mannose-binding protein plays an important role, and high-mannose oligosaccharide inhibits the infection. In this case, the high-mannose-type structure is not a GSL, but the principle is the same.

Table 3 Tumour-associated GSL antigens in human cancer

Name	Structure	Tumour expression
Globo-series antigens Gb3		
	Galα4Galβ4GlcCer	(a) Burkitt lymphoma (b) Ovarian cancer
Gal-Gb4	Galβ3GalNAcβ3Galβ4GlcCer	(a) Lung cancer (b) Seminoma
Globo-H	Fucα2Galβ3GalNAβ3Galα4Galβ4GlcCer	(a) Breast acncer (b) Ovarian cancer
Disialosyl Gal-Gb4	SAα3Galβ3GalNAcβ3Galα4Galβ4GlcCer 6 SAα	Renal cell carcinoma
Ganglio-series antigens		
GD3	SAα8SAα3Galβ4GlaCer	Melanoma
	9-O-Ac-SAα8SAα3Galβ4GlcCer	Melanoma
GD2	SAα8SAα3Galβ4GlcCer 4 GalNAcβ	Neuroblastoma
Fucosyl GM1	Fucα2Galβ3GalNAcβ4Galβ4GlcCer 3 SAα	Lung small cell carcinoma
Lacto-series antigens: Type 1 chain		
Sialosyl-Le^c	SAα3Galβ3GlcNAcβ3Galβ4GlcCer	(a) Lung small cell carcinoma (b) Expressed in mucin of pancreatic and ovarian cancer as DUPAN-2 epitope
Sialosyl-Le^a	Galβ3GlcNAcβ3Galβ4GlcCer 3 4 SAα Fucα	Expressed in mucin of pancreatic and other gastrointestinal, colorectal and lung cancers
Dimeric Le^a (Le^a–Le^a)	Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4GlcCer 4 4 Fucα Fucα	Gastric and colon cancers
(Le^b–Le^a)	Galβ3GlcNAcβ3Galβ3GlcNAcβ3Galβ4GlcCer 2 4 4 Fucα Fucα Fucα	Gastric and colon cancers
Le^a–Le^x (hybrid type)	Galβ3GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcCer 4 3 Fucα Fucα	Lung cancer
Lacto-series antigens: Type 2 chain		
Unbranched	Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcCer 2 ± SAα	Liver and colon cancers
Le^x	Galβ4GlcNAcβ3Galβ4GlcCer 3 Fucα	Gastrointestinal, colorectal, liver and lung cancers
Le^y	Galβ4GlcNAcβ3Galβ4GlcCer 2 3 Fucα Fucα	Gastrointestinal, colorectal, breast and lung cancers
Le^y–Le^x	Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcCer 2 3 3 Fucα Fucα Fucα	Colorectal and lung cancers

Continued

Table 3 Continued

Name	Structure	Tumour expression
SLe^x	Galβ4GlcNAcβ3Galβ4GlcCer 3 3 SAα Fucα	Gastrointestinal, colorectal, breast and lung cancers
SLe^x–Le^x	Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcCer 3 3 3 SAα Fucα Fucα	Gastrointestinal, colorectal, breast and lung cancers

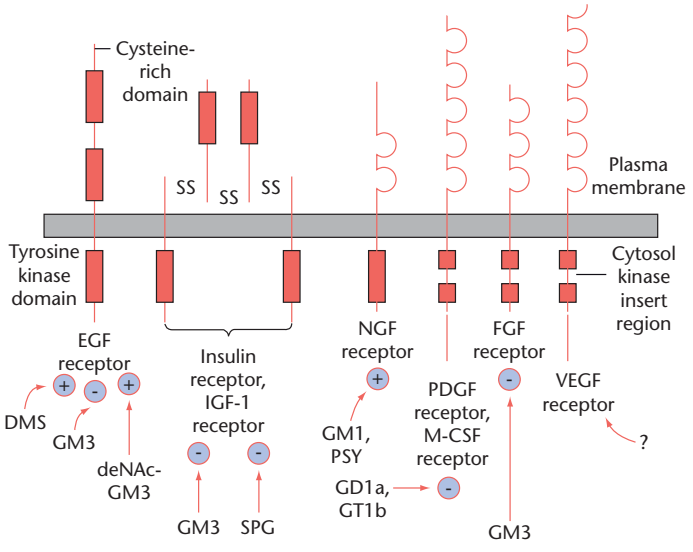

Figure 10 Possible modulation by GSLs of signal transduction through growth factor receptors. Signal transduction is initiated when growth factors or death-causing factors are bound to specific receptors. Receptors for growth factors are generally associated with cytoplasmic tyrosine kinases, which are activated upon binding of the factor to the receptor. Susceptibility of tyrosine kinase activity associated with each type of receptor to sphingolipids or GSLs is different. Known examples are shown schematically in this figure. Circled+and – signs represent, respectively, positive and negative effects of sphingolipid or GSL on the tyrosine kinase activity of the receptor. For example, tyrosine kinase associated with epidermal growth factor (EGF) receptor is promoted by *N*,*N*-dimethylsphingosine (DMS) and by de-*N*-acetyl-GM3, but inhibited by GM3 ganglioside. Tyrosine phosphorylation associated with the insulin receptor in human cells is inhibited by sialylparagloboside (SPG), and that in mice is inhibited by GM3. Other abbreviations: NGF, nerve growth factor; PSY, plasmalopsychosine; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor.

Cell adhesion sites

In higher animals and plants, cells are organized into tissues, and various tissues sometimes form organs. How individual cells are bound together and assembled to form tissues and organs is a basic biological question. Results from numerous recent studies show that certain proteins, termed 'integrins' or 'intercellular adhesion molecules' (ICAMs), have important functions in cell-to-cell or cell-to-matrix binding. Matrix consists of macromolecules that fill space between cells. While there is no question that integrin, ICAMs and matrix are essential components for cell adhesion for assembly into tissues, cell surface GSLs also contribute to cell-to-cell interaction in two different ways. In the first way, cell-surface proteins called 'lectins' bind to GSLs on the surface of the counterpart cell. Typical examples are seen in neuronal cell interaction, in which gangliosides are recognized by myelin-associated glycoprotein (MAG) or by another type of protein termed galectin. In the second way, GSLs present on the surface of one cell bind to other GSLs on the counterpart cell ('GSL-to-GSL binding'). In either case, certain types of GSLs involved in the adhesion can inhibit cell adhesion. Our knowledge of the role of GSLs in cell adhesion and tissue organization is fragmentary, and further systematic studies remain to be performed. **See also**: Integrins: signalling and disease; Lectins

Signal transduction

The life of a cell can be maintained by required nutrients, but cell proliferation does not occur unless the cell is stimulated by a growth factor or hormone. Cell proliferation requires cell-type-specific growth factor. In contrast, 'cell death' may be caused by a different type of factor, stimulated at the cell

surface, even when physiological conditions are optimal for cell life. The process of cell death is physiologically important during development, to form specific tissues and organs, and to maintain populations of functionally defined groups of cells.

On stimulation of cells by growth factor, through binding of the factor to the receptor, the receptors aggregate and activate the associated tyrosine kinase. This initiates transmission of an impulse to the nucleus, which is termed 'signal transduction', leading to cell proliferation. Similarly, a factor causing cell death binds to a specific receptor and leads to a different type of signal transduction instructing cell death. **See also**: Signal transduction: overview

GSLs or their derivatives that modulate signal transduction for cell proliferation are relatively well known. However, with the exception of sphingosine and *N*-methylsphingosine, few cases are known of GSLs involved in signal transduction for cell death.

Effect of GSLs on growth factor receptor function

Many types of growth factors and their receptors, specific for different types of cells, are known. Some types of cells require a growth factor abundantly present in platelets, termed 'platelet-derived growth factor' (PDGF). Others require 'epidermal growth factor' (EGF), found originally in salivary gland extract. Neuronal cell growth and differentiation to form neurites (long processes characteristic of neuronal cells that contribute to formation of networks) are maintained and stimulated by 'nerve growth factor' (NGF). These are only a few examples. In each case, specific receptors are present that are associated with specific tyrosine kinases. When a growth factor is bound to its receptor, it induces activation of tyrosine kinase to form tyrosine phosphate, which initiates signal transduction. This process is inhibited or promoted by GSLs, particularly gangliosides, although the mechanism for the effect of GSLs on tyrosine kinase is not fully understood. Examples and possible mechanisms are shown in **Figure 10**. **See also**: Protein kinases

GSL and sphingolipid components, ceramide or sphingosine, activate or inhibit specific protein kinases involved in signal transduction

When cells are stimulated, degradation of GSL or sphingolipid is enhanced, and the level of intracellular ceramide or sphingosine increases. Under certain conditions, the initial sphingosine catabolite, sphingosine 1-phosphate, also increases. Ceramide and sphingosine activate specific kinases called 'ceramide-activated kinase' or 'sphingosine-dependent kinase', leading to activation or inhibition of signal transduction. The mechanism for the entire process is still unclear. Sphingosine 1-phosphate activates proliferation of specific types of cells, but also inhibits motility of a large variety of cell types. The targets of sphingosine-dependent kinases are, in general, molecular chaperones or adapters of various molecules essential for maintenance of intracellular 'homeostasis'.

GSLs in specific microdomains at the cell surface initiate signalling

GSLs can also control signal transduction in a different way, through direct interaction of GSL with 'signal transducer molecules' present at the cytoplasmic cell surface. These molecules themselves are tyrosine kinases belonging to the 'Src kinase family', and are closely associated with specific organization ('microdomain') of GSLs in membrane, as described below.

Conformational Structure, Distribution and Organization of Glycosphingolipids in Membrane

Molecular models and distribution patterns

In molecular modelling studies, the most stable (minimum-energy) conformation of GSLs shows a structure in which the carbohydrate axis is perpendicular to the lipid axis (**Figure 11a**). The lipid moiety is inserted in the plasma membrane bilayer, and GSLs are present in the outer leaflet, similarly to sphingomyelin and phosphatidylcholine. In contrast, phosphatidylethanolamine and phosphatidylserine are present in the inner leaflet of the lipid bilayer. Typically, GSLs are enriched in plasma membrane as compared with intracellular membranes, although there are many exceptions. Neutrophils have a high quantity of lactosylceramide (70–80% of the total amount in the cell) in intracellular membranes, and only 20–30% in plasma membrane. The functions of GSLs are best known for those found in plasma membrane, whereas the significance of GSLs in intracellular membranes is almost unknown. **See also**: Cell membrane features

GSL clusters

GSLs and sphingolipids within the plane of the outer leaflet appear to be aggregated with themselves and segregated from phospholipids to form 'microdomains'. Such GSL microdomains can be present at the cell surface, separate from glycoprotein clusters, as evidenced by scanning electron microscopy (SEM) following labelling with anti-GSL antibodies (e.g. ferritin-labelled) or glycoprotein-binding lectins (e.g. gold sol label). The separation of microdomains enriched in GSLs from those enriched in glycoproteins is best demonstrated by a freeze-fracture technique followed by SEM. Clusters of GSLs are also revealed by labelled anti-GSL antibodies and transmission electron microscopy (TEM). GSL patches (microdomains)

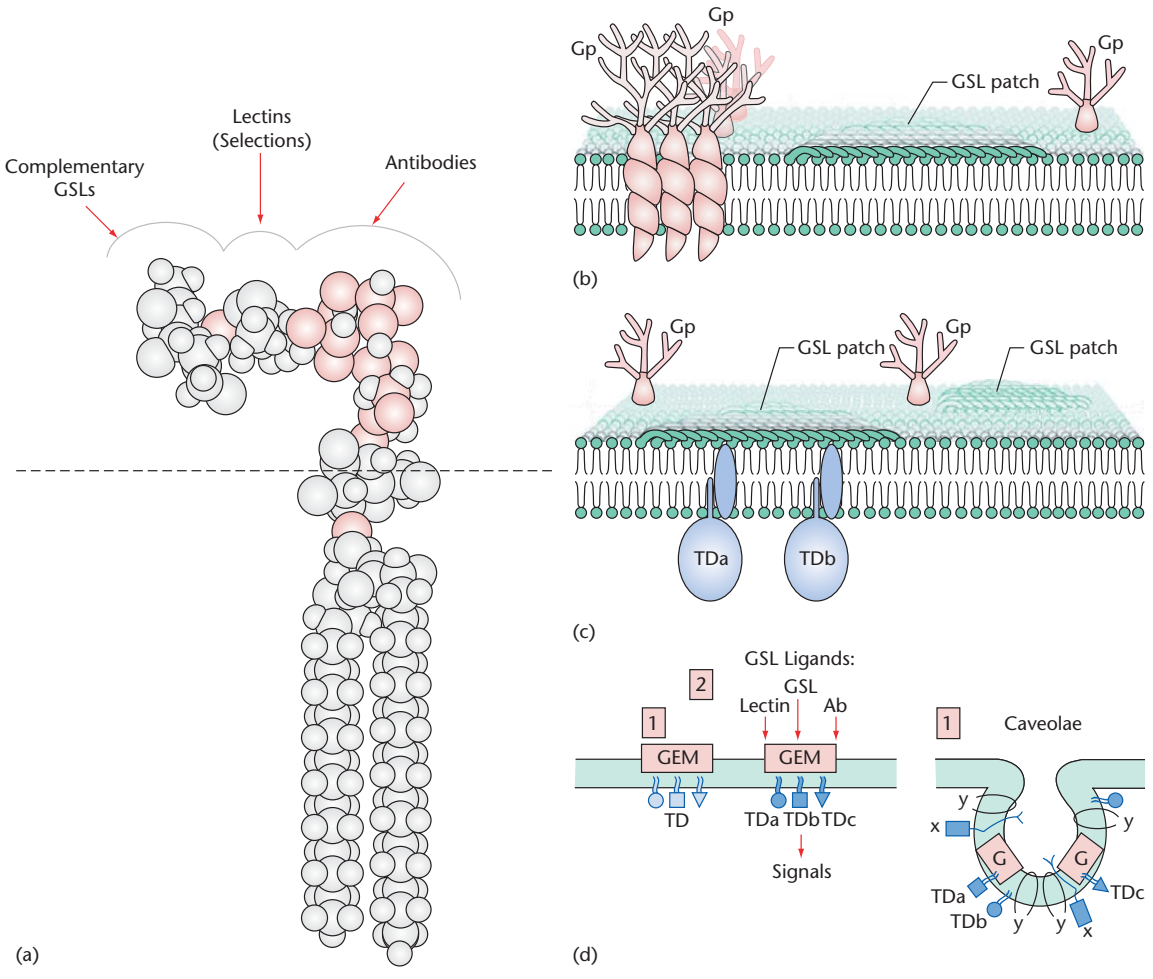

Figure 11 Schematic illustration of proposed function of GEM subfraction (glycosignalling domain) in cell adhesion coupled with signalling. (a) The minimum-energy conformational structure of a typical GSL (globoside) axis of the oligosaccharide is perpendicular to the axis of ceramide (*N*-fatty acyl sphingosine). Antibodies, lectins and complementary GSLs interact with the outer surface profile of oligosaccharide. (b) Proposed self-aggregation of GSLs in the lipid bilayer of the plasma membrane ('GSL patch'). The ceramide moiety holds GSL carbohydrates in defined orientation through insertion in the plasma membrane. GSL patches are separated from clusters of glycoprotein (Gp). (c) GSLs are self-assembled in microdomains, which constitute a major component of the GEM, and transducers are associated with these GSL microdomains. Glycoprotein clusters (Gp) are physically separate from GSL microdomains, as revealed by electron microscopy with a freeze-fracture technique. GSL microdomains are subject to binding by lectins, antibodies and complementary GSLs. These GSL microdomains, separated from caveolae, may contain transbilayer lipophilic proteins. Such proteins may mediate functional connections between GSLs and transducers (TDa, TDb). (d) GSLs in the GEM subfraction (glycosignalling domain) are associated with inactive TD (1). Stimulation of GSLs in GEM by GSL ligands (antibody, complementary GSL, lectin) induces conformational change of TDa, b, c, which triggers signal transduction (2). Caveolae (invaginations of plasma membrane) may contain GEM-like components (G), transducers (TDa, b, c), growth factor receptors (x), and caveolin (y). They are involved mainly in growth factor receptor-mediated signal transduction and not in cell adhesion (3).

separated from glycoprotein aggregates are shown schematically in **Figure 11b**. **See also**: Immuno-electron microscopy

Separation of the GSL-enriched microdomain and its properties

The GSL-enriched microdomain (GEM) of the plasma membrane can be separated by its resistance to detergent, as the low-density membrane fraction in sucrose, or by Ficoll density-gradient ultracentrifugation. This membrane fraction has been characterized as containing GSLs, sphingomyelin, cholesterol, signal transducer molecules such as c-Src and other Src family kinases, small G proteins (Ras, Rho, Rac), and focal adhesion kinase (FAK). These signal transducer molecules are associated with the GEM through long-chain alkyl groups (myristoyl or farnesyl) or through hydrophobic membrane proteins that connect the ceramide moiety of GSL patches to the transducer

molecules (TDa, TDb) (**Figure 11c**). **See also**: Plasma membranes: methods for preparation

Membrane components containing GSLs and c-Src, which maintain the function of GSL-dependent adhesion and the ability to initiate signal transduction to activate c-Src, can be separated from other membrane components that do not contain GSLs, c-Src or G-proteins, but which do contain caveolin, cholesterol and sphingomyelin. Membrane microdomains containing GSLs organized with certain types of signal transducer molecules are the functional and structural units of the plasma membrane responsible for the cell social functions of GSLs. When GSLs in the GEM are not stimulated, i.e. no ligands are bound, signal transducers (TD) are not activated and no signals are induced (**Figure 11d**, '1'). In contrast, when GSLs in the GEM are stimulated by binding to lectin, complementary GSL or antibody, transducers are activated and signals are induced (**Figure 11d**, '2').

The low-density membrane fraction also contains other functional and structural units representing 'caveolae', small invaginations of plasma membrane characterized by the structural protein caveolin and enriched in cholesterol. Caveolae isolated from certain cells do not contain GSLs, but do contain a large quantity of cholesterol, various growth factor receptors, GPI anchors and anchored proteins. They are involved in endocytosis and signal transduction (**Figure 11d**, '3'). GEMs and caveolae both show detergent resistance and coexist. However, under certain conditions GEM can be separated from the caveolin-containing fraction, i.e. caveolae, which does not contain GSL. The structure and function of caveolae depend on caveolin and cholesterol, in contrast to GEM.

GSLs in microdomains initiate signalling when the microdomain is stimulated by antibodies, complementary GSLs or lectins, through activation of signal transducer molecules, e.g. tyrosine phosphorylation or GTP binding. Therefore, some GSL microdomains complexed with signal transducers are regarded as signalling domains, and the term 'glycosignalling domain' (GSD) is applied (see Further Reading).

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