

Sphingolipids: metabolism and cell signaling

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1. Introduction

Sphingolipids were first described by Johann L.W. Thudichum in *A Treatise on the Chemical Constitution of Brain* (1884) [1]. Among the described compounds were sphingomyelin, cerebroside, and cerebrosulfatide (Fig. 1), which encompass the three categories of sphingolipids known today (phosphosphingolipids, neutral and acidic glycosphingolipids). Thudichum noted that hydrolysis of these lipids produced a compound that “. . . is of an alkaloidal nature, and to which, in commemoration of the many enigmas which it has presented to the inquirer, I have given the name of *Sphingosin*.” Thus, this class of lipids became known as *sphingolipids*.

Thudichum, a practicing physician throughout most of his life, was searching for a better understanding of disease, but appreciated that “. . . to reach this goal of complete knowledge. . . the medicinal chemist must. . . not. . . carry on research by a kind of fishing for supposed disease-poisons, of which, according to my view of the subject, the attempt of the boy to catch a whale in his mother’s washing-tub is an appropriate parable.” Later studies fulfilled Thudichum’s faith in the value of basic research when

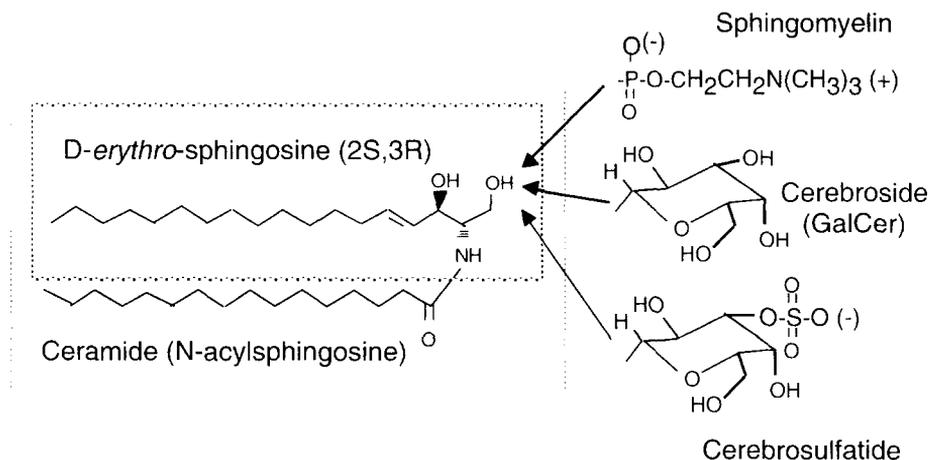


Fig. 1. Structures of sphingosine, ceramide, sphingomyelin, a cerebroside (galactosylceramide) and cerebro-sulfatide from human brain.

several genetic diseases were found to have elevated amounts of sphingolipids (such as sphingomyelin in Niemann–Pick’s disease and cerebrosides in Gaucher’s disease) arising from defects in enzymes responsible for sphingolipid turnover, activator proteins for such enzymes, or lipid trafficking (for reviews, see Hakomori [2] and Schuette et al. [3]). This knowledge allowed development of methods for diagnosis of such sphingolipid storage diseases (or ‘sphingolipidoses’), screening of families at risk, and, for at least Gaucher’s disease, some degree of correction of the disorder by enzyme replacement. Progress is also being made using inhibitors of sphingolipid synthesis, and gene replacement offers promise for the future.

For many years, the only diseases associated conclusively with sphingolipids involved defective sphingolipid turnover. Disruption of sphingolipid biosynthesis is now known to be the major mechanism of action of mycotoxins (fumonisins and alternaria toxins) that cause a wide spectrum of diseases of plants and animals (A.H. Merrill, 2001). And in 2001, genetic defects in sphingolipid biosynthesis were shown to cause the most common hereditary disorder of peripheral sensory neurons (hereditary sensory neuropathy type I) (J.L. Dawkins, 2001; K. Bejaoui, 2001). It is certain that additional genetic diseases due to abnormal sphingolipid biosynthesis will be found, and knockout mice defective in the biosynthesis of glucosylceramide and other glycolipids have severe defects, especially in the developing nervous system (T. Kolter, 2000). There are also indications that sphingolipids and sphingolipid analogs may be useful for prevention and treatment of disease, e.g., gangliosides (R. McKallip, 1999) and α -galactosylceramide (M. Taniguchi, 1997 and 1998) have potent effects as modulators of the immune system, ceramide-coated balloon catheters limit neointimal hyperplasia after stretch injury in carotid arteries (R. Charles, 2000), and dietary sphingolipids protect against colon tumorigenesis (E.M. Schmelz, 2001). These probably reflect just a few of the ways sphingolipids are relevant to pathology, nutrition and medicinal chemistry.

1.1. Biological significance of sphingolipids

Sphingolipids are found in essentially all animals, plants, and fungi, as well as some prokaryotic organisms and viruses. They are mostly in membranes, but are also major constituents of lipoproteins. The functions of sphingolipids are still being discovered, but there are at least three, i.e., structure, recognition and signal transduction, which have been summarized diagrammatically in Fig. 2.

1.1.1. Biological structures

Some glycosphingolipids and sphingomyelins tend to cluster rather than behave like typical ‘fluid’ membrane lipids. This behavior arises from the mostly saturated alkyl sidechains, which allow strong van der Waals interactions, and the ceramide hydroxyls, amide bond and polar headgroups that are capable of hydrogen bonding and dipolar interactions (it is common for sphingolipids to have phase transition temperatures $> 37^{\circ}\text{C}$). Sphingolipids contribute to the formation of regions of the plasma membrane termed ‘rafts’ and ‘caveolae’ ([5,6], Chapter 1), which are enriched in growth factor receptors, transporters and other proteins, especially proteins with a glycosylphosphatidylinositol-lipid anchor. Sphingolipids contribute to the stability of other types

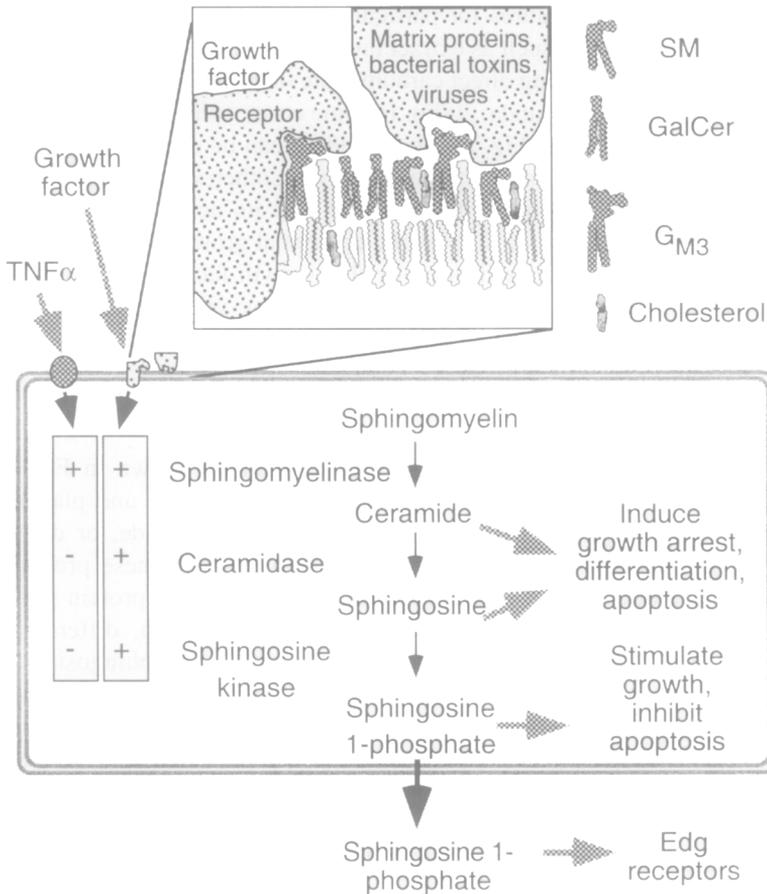


Fig. 2. Schematic representation of sphingolipid functions. Shown in the upper inset are sphingolipid- (and cholesterol-) enriched regions of the plasma membrane ('rafts'), and sphingolipids serving as ligands for extracellular proteins and receptors on the same cell. The lower diagram of a cell illustrates how agonists (such as tumor necrosis factor- α , TNF- α , and growth factors) can activate combinations of sphingolipid metabolizing enzymes to produce bioactive products that affect the shown cell behaviors. Sphingosine 1-phosphate is also secreted as an agonist for some members of the Edg family receptors (now named SIP).

of biological structures, such as the lamellar bodies that maintain the permeability barrier of skin (P. Wertz, 2000) and lipoproteins (S.L. Schissel, 1996). However, not all sphingolipids are so ordered, and some, such as sphingosine 1-phosphate and sphingosylphosphorylcholine (lysosphingomyelin), are sufficiently polar to exist in aqueous environments (Y. Yatomi, 1997).

1.1.2. Biological recognition

Membrane sphingolipids are located predominantly on the outer leaflet of the plasma membrane, the lumen of intracellular vesicles and organelles (endosomes, Golgi membranes, etc.), and in as yet undefined locations in mitochondria and nuclei. The complex

carbohydrate moieties are often signatures for particular cell types, and mediate interactions with complementary ligands, such as extracellular matrix proteins and receptors (S.I. Hakomori, 2000) (Fig. 2), including direct carbohydrate-carbohydrate binding with headgroups on neighboring cells (K. Handa, 2000). In some cases, sphingolipids interact with proteins on the same cell surface (Fig. 2). Such binding can be used to control the location of the protein (for example, in membrane rafts with other signaling proteins) as well as to modify the conformation of the receptor and its activity [7]. This is exemplified by the binding of ganglioside G_{M3} by the epidermal growth factor receptor, which makes the receptor refractory to activation by this growth factor (E.J. Meuillet, 2000; A.R. Zurita, 2001). Sphingolipids are also recognized by viruses, bacteria and bacterial toxins as a means of both attachment and entry into the cell via membrane trafficking (K.A. Karlsson, 1992; C.A. Lingwood, 1999).

1.1.3. Signal transduction

The sphingolipid backbones are members of a signaling paradigm shown in Fig. 2, wherein receptor activation by agonists such as tumor necrosis factor- α and platelet-derived growth factor induce sphingomyelin turnover to elevate ceramide, or downstream metabolites (sphingosine or sphingosine 1-phosphate) [8–10]. These products activate or inhibit multiple downstream targets (protein kinases, phosphoprotein phosphatases and others) that control cell behaviors as complex as growth, differentiation and programmed cell death (apoptosis). Because ceramide and sphingosine 1-phosphate often have opposing signaling functions (e.g., induction versus inhibition of apoptosis; inhibition versus stimulation of growth), Sarah Spiegel has proposed that cells utilize a ceramide/sphingosine 1-phosphate ‘rheostat’ in deciding between growth arrest/apoptosis versus proliferation/survival (S. Spiegel, 1999). Sphingosine 1-phosphate can be released from cells and serve as an agonist for SIP receptors [10], hence, this compound serves as both a first and second messenger!

The field of sphingolipid signaling is still relatively young and has many new facets that reveal the biochemical ‘logic’ of using such complex molecules to control cell behavior. For example, sphingomyelin turnover not only produces ‘signaling’ metabolites, but also alters the structure of membrane domains that depend on the presence of this lipid (furthermore, when ceramide accumulates, its biophysical properties can profoundly affect membrane structure and the behavior of associated receptors and other proteins) [5,6]. A similar paradigm can be envisioned for glycosphingolipids. Thus, sphingolipid ‘signaling’ is an ensemble of changes in membrane structure and dynamics, the production (and removal) of bioactive metabolites, and the activation and/or inhibition of downstream targets.

1.2. Structures and nomenclature of sphingolipids

More than 300 different types of complex sphingolipids have been reported, and this does not include differences in the ceramide backbone. It has become necessary to develop a system of nomenclature for sphingolipids so that individual species can be referred to in a logical manner [11]. Nonetheless, there is still considerable variability in the names that are used for these compounds. For example, ‘sphingosine’ is still

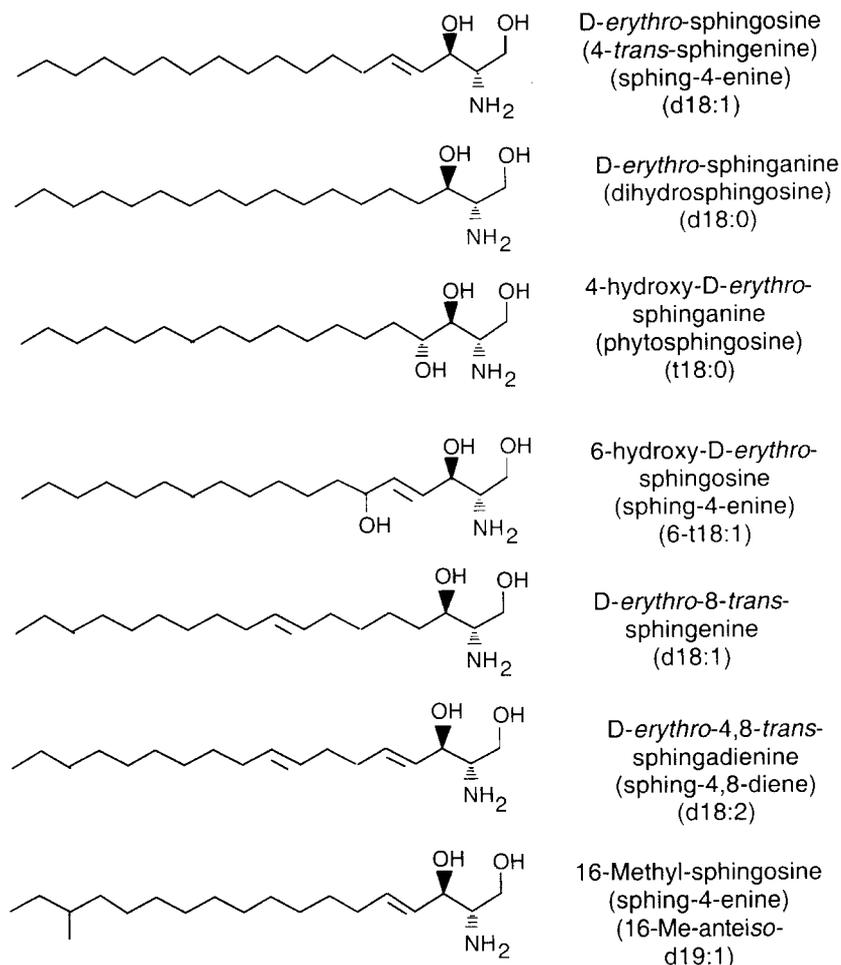


Fig. 3. Structures of some of the long-chain (sphingoid) bases that have been found in sphingolipids. Abbreviations for these compounds are shown in parentheses.

in common usage although the names recommended by the IUPAC are (*E*)-sphing-4-ene or (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol (by their recommendation, dihydro-‘sphingosine’ is sphinganine, and 4-hydroxysphinganine (also called phytosphingosine) is (2*S*,3*S*,4*R*)-2-aminooctadecane-1,3,4-triol). This chapter uses the most familiar names: sphingosine, sphinganine and 4-hydroxysphinganine.

Sphingosine is the prevalent backbone of most mammalian sphingolipids; however, over 60 different species of long-chain bases have been reported [12] and include compounds (Fig. 3) with (1) alkyl chain lengths from 14 to 22 carbon atoms, (2) different degrees of saturation at carbons 4 and 5, (3) a hydroxyl group at positions 4 or 6, (4) double bonds at other sites in the alkyl chain, and (5) branching (methyl groups) at the ω -1 (iso), ω -2 (anteiso), or other positions. Sphingoid bases are abbreviated by

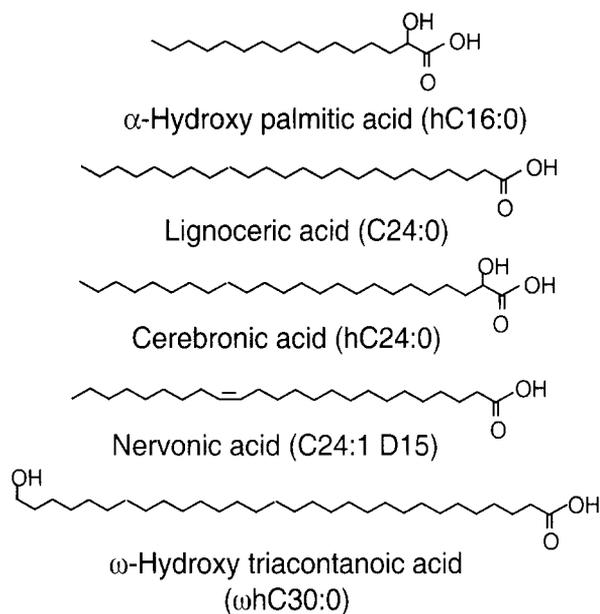


Fig. 4. Structures of representative fatty acids, including α - and ω -hydroxy fatty acids, that are found in mammalian sphingolipids. Abbreviations for these compounds are shown in parentheses.

citing (in order of appearance in the abbreviation) the number of hydroxyl groups (d and t for di- and tri-hydroxy, respectively), chain length and number of double bonds as shown in Fig. 3.

The majority of the sphingoid bases in cells are *N*-acylated with long-chain fatty acids to produce ceramides(s) (Fig. 1), although *O*-acylated (A. Abe, 1998), phosphorylated-(sphingosine 1-phosphate) and *N*-methylated-(*N,N*-dimethylsphingosine) derivatives also exist. The fatty acids of ceramide vary in chain length (14 to 30 carbon atoms), degree of unsaturation (but are mostly saturated), and presence or absence of a hydroxyl group on the α - or ω -carbon atom. Structures and abbreviations for some fatty acids are shown in Fig. 4.

Most sphingolipids have a polar headgroup at position 1 (Figs. 1 and 5). Sphingolipids are often grouped based on the headgroups into the phosphosphingolipids and glycosphingolipids; however, these categories are not mutually exclusive: the major sphingolipids of yeast are ceramide phosphorylinositols. Glycosphingolipids are classified into broad types on the basis of carbohydrate composition. Neutral glycosphingolipids contain uncharged sugars such as glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), and fucose (Fuc). Acidic glycosphingolipids contain ionized functional groups such as phosphate, sulfate (sulfatoglycosphingolipids), or charged sugar residues such as sialic acid (*N*-acetylneuraminic acid) in gangliosides or glucuronic acid in some plant glycosphingolipids. Further classification can be made on the basis of shared partial oligosaccharide sequences, sometimes referred to as 'root structures' as summarized in Table 1.

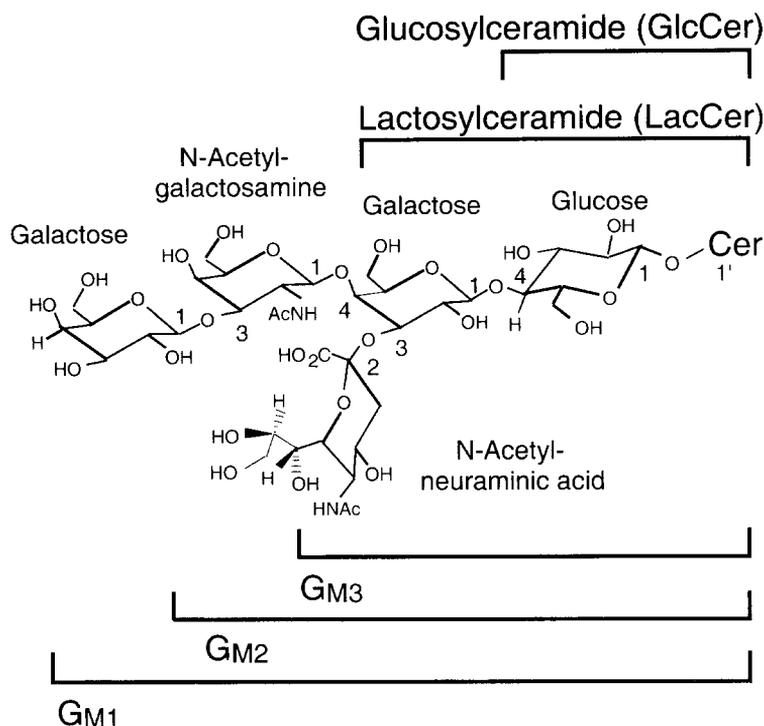


Fig. 5. Structures of some of the common neutral glycosphingolipids (GlcCer and LacCer) and gangliosides G_{M1} , G_{M2} and G_{M3} . 'Cer' refers to the ceramide backbone [for another useful overview structure, for ganglioside G_{D11} , see G. van Echten-Deckert (1999)].

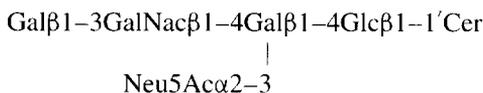
Table 1
Nomenclature for classification of glycosphingolipids

Root name	Abbreviation	Partial structure ^a			
		IV	III	II	I
Ganglio	Gg	Gal β 1-3GalNac β 1-4Gal β 1-4Glc β 1-1' Cer			
Lacto	Lc	Gal β 1-3GlcNac β 1-3Gal β 1-4Glc β 1-1' Cer			
Neolacto	nLc	Gal β 1-4GlcNac β 1-3Gal β 1-4Glc β 1-1' Cer			
Globo	Gb	GalNac β 1-3Gal α 1-4Gal β 1-4Glc β 1-1' Cer			
Isoglobo	iGb	GalNac β 1-3Gal α 1-3Gal β 1-4Glc β 1-1' Cer			
Mollu	Mu	GalNac β 1-2Man α 1-3Man β 1-4Glc β 1-1' Cer			
Arthro	At	GalNac β 1-4GlcNac β 1-3Man β 1-4Glc β 1-1' Cer			

^a Roman numerals define sugar positions in the 'root' structure.

Gangliosides are often denoted by the 'Svennerholm' nomenclature [11] that is based on the number of sialic acid residues (e.g., G_{M1} refers to a monosialo-ganglioside) and the relative position of the ganglioside upon thin-layer chromatography (thus, the order of migration of the series of monosialogangliosides in Fig. 5 is $G_{M3} > G_{M2} > G_{M1}$). By

commonly used nomenclatures, the same compound might be called ganglioside G_{M1} , $\text{II}^3\text{-}\alpha\text{-N-acetylneuraminosyl-gangliotetraosylCer}$, $\text{II}^3\text{-}\alpha\text{-Neu5NacGg}_4\text{Cer}$, or depicted as:



Note that the Roman numeral and Arabic superscript refer to the sugar in the root structure (cf. Table 1) that is substituted (counting from the ceramide toward the non-reducing end) and the position of that substitution, respectively.

A number of sphingolipids are referred to by their historic names as antigens and blood group structures, such as Forssman antigen ($\text{IV}^3\text{-}\alpha\text{-GalNac-Gb}_4\text{Cer}$), a globopentosylceramide that is found in many mammals (but it is unclear if humans express this antigen) and the Lewis blood group antigens, which correspond to a family of $\alpha 1-3$ -fucosylated glycan structures (Lewis \times , sialyl Lewis \times , etc.). For more information on these aspects of glycosphingolipidology see Varki et al. [13].

2. Chemistry and distribution

This section will summarize some of the properties of sphingolipids. More information is available in Merrill and Hannun [14].

2.1. Sphingoid bases

A distinctive feature of sphingoid bases is that they can bear a net positive charge at neutral pH, which is rare among naturally occurring lipids. Nonetheless, the $\text{p}K_a$ of the amino group is low for a simple amine (between 7 and 8) (A.H. Merrill, 1989), which means that a portion is uncharged at physiologic pH. This may help explain why sphingoid bases can readily move among membranes and across bilayers (in the uncharged state), unless transmembrane movement is impeded by acidic pH, such as in lysosomes.

Structural elucidation and quantitation of long-chain bases is possible using a variety of analytical techniques, including gas chromatography and high-performance liquid chromatography, mass spectroscopy, and nuclear magnetic resonance spectroscopy [13,14].

2.2. Ceramides

Ceramides per se are mostly found in small amounts in tissues, with the notable exception of the stratum corneum, where they are major determinates of the water permeability barrier of skin (P. Wertz, 2000). Many ceramides (even as part of complex sphingolipids) migrate on thin-layer chromatography as multiple bands due to the presence of at least several types of sphingoid bases and fatty acids. The molecular species can be analyzed by a number of techniques, such as gas chromatography, high-performance liquid chromatography (HPLC), or hydrolysis (or methanolysis) followed by analysis of the sphingoid bases and fatty acids [14]. However, the most information

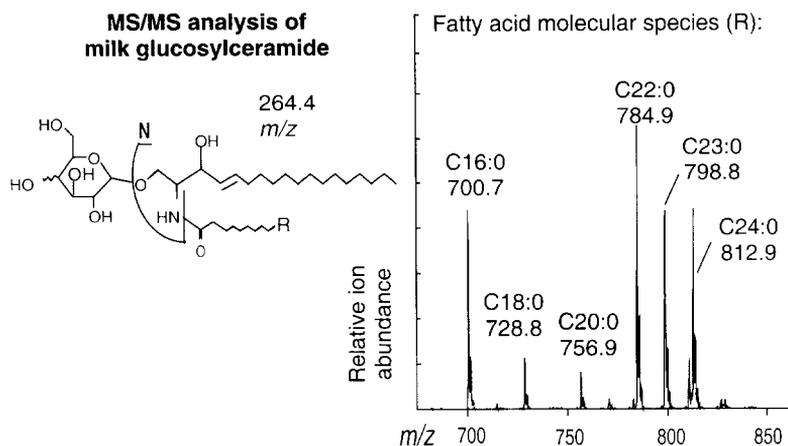


Fig. 6. Major fragmentation sites of a monohexosylceramide upon electrospray tandem mass spectrometry (left panel) and (right panel) a typical precursor ion spectrum (obtained with a bovine milk extract) monitoring m/z 264.4 (a signature fragment obtained with sphingolipids with a sphingosine backbone) over the range m/z 675–875. The labeled signals represent the various amide-linked fatty acids on the milk glucosylceramides. For more information see Sullards and Merrill [15].

is obtained by combining HPLC with electrospray tandem mass spectrometry (ESI MS/MS) [15]. In this method, the ceramides are separated as classes (free ceramides, sphingomyelins, glucosylceramides, etc.) by HPLC and the eluant is introduced directly into the ionizing chamber of the mass spectrometer, where the solvent is rapidly evaporated under high vacuum and the compounds are suspended in the gas phase as individual charged species. These ‘parent’ ions are separated by the first MS, then allowed to collide with a gas (such as N_2) to produce fragments that are separated by the second MS. Besides high sensitivity, the advantage of this instrumentation is the ability to focus on the compounds of interest in crude mixtures. For example, glucosylceramides containing sphingosine will fragment to m/z 264.4 (Fig. 6); therefore, the second MS can be set to detect m/z 264.4 and the first MS to identify the parent ions that produce this fragment. This is illustrated for the glucosylceramides in a milk lipid extract in Fig. 6. Quantitation is achieved by spiking the sample with an internal standard with a chemical composition sufficiently similar to the unknowns for them to fragment with similar efficiencies, as described in the legend to Fig. 7. More accurate and sensitive quantitation can be obtained using a specialized MS/MS technique known as multiple reaction monitoring (MRM), in which the mass spectrometer is programmed to maximize the time spent detecting specific precursor/product ion transitions, and the detection of each individual molecular species can be optimized with respect to ion formation and decomposition.

Use of such methods allows more accurate and facile quantitation of multiple sphingolipid species in cells and other biological materials. Fig. 7 gives a typical analysis of the sphingolipids of NIH 3T3 cells, which contain (nmol per 10^6 cells): SM (2.7), GlcCer (0.31), Cer (0.082), sphingosine (0.017), sphingosine-1-phosphate (0.011), sphinganine (0.160), and sphinganine-1-phosphate (<0.001). For compari-

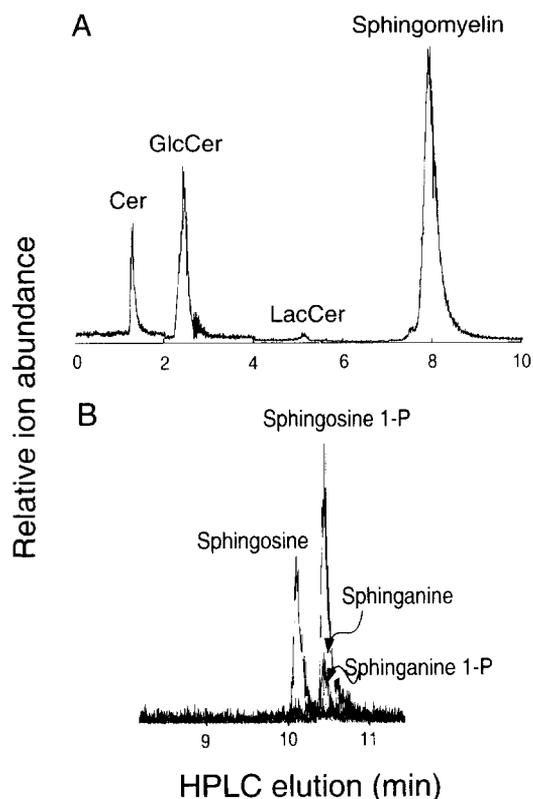


Fig. 7. A high-performance electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) total ion chromatogram of endogenous levels of the complex sphingoid bases ceramide (Cer), glucosylceramide (GlcCer), lactosylceramide (LacCer), and sphingomyelin (SM) from NIH 3T3 cells (upper panel A). The cells were treated with base to remove glycerolipids and the organic solvent-soluble compounds were separated on a normal phase column to produce the profile shown. The amounts of each species can be quantified by comparison with spiked internal standards (Cer, GlcCer and SM with C12 fatty acid [15]). The elution profile in panel B is the extracted ion chromatogram for the free sphingoid bases from these cells (separated by reversed-phase chromatography prior to ESI-MS/MS) from NIH 3T3 cells (dC20:0, dC20:1 and dC17:1-1-phosphate are used as internal standards) [15]. Panel C demonstrates that this methodology can also be used to analyze lysosphingolipids (lysosphingomyelin and psychosine) and *N*-methyl sphingosines (these were not detected in NIH 3T3 cells so the data are for mixtures of standards).

son, human monocytes contain (nmol per 10^6 cells): SM (1.2), GlcCer (0.032), Cer (0.027), sphingosine (0.024), sphingosine-1-phosphate (0.007), sphinganine (0.007), and sphinganine-1-phosphate (<0.001) (M.C. Sullards, unpublished). The amounts of the sphingolipid backbones (ceramides and sphingoid bases) are often small, but can be comparable to other sphingolipids in the cells (cf. GlcCer and Cer in monocytes).

Many studies add ceramides or ceramide analogs to cells in culture, enzyme assays, or other aqueous environments, but delivery is hampered by the hydrophobicity of long-chain ceramides, which have low critical micelle concentrations ($\leq 10^{-10}$ M). This is often circumvented by using short-chain ceramides (e.g., *N*-acetyl- or *N*-hexanoyl-

sphingosine) or by delivering long-chain ceramides in detergents, liposomes, or organic solvent mixtures (C. Luberto, 2000). Fluorescent ceramide analogs, such as *N*-[6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexanoylceramide (NBD-ceramide) and boron dipyrromethane difluoride ceramide (BODIPY-ceramide) are readily taken up by cells and have proven very useful in studies of sphingolipid transport and metabolism (A. Dagan, 2000; R.E. Pagano, 2000).

2.3. Phosphosphingolipids

Sphingomyelin is the major phosphosphingolipid in mammalian tissues. The biophysical properties of sphingomyelins have been described in Section 1.1.1 with respect to their role in rafts and caveolae [5] (see also Brugger, 1999 and 2000).

Animals additionally produce ceramide phosphorylethanolamines (M.N. Nikolova-Karakashian, 2000) and ceramide phosphate (S. Bajjalieh, 2000). Fungi such as *Saccharomyces cerevisiae* and *Sporothrix schenckii* contain ceramides derivatized with inositol phosphate with or without further addition of mannose and other carbohydrates (R.C. Dickson, 1999; C.V. Loureiro y Penha, 2001).

2.4. Glycosphingolipids

Plants and fungi often contain glycosphingolipids with relatively simple carbohydrate structures [16,17] whereas animals have a wide variety of simple to complex sphingolipids [18–20], including higher-order globosides, gangliosides and sulfatides.

2.4.1. Neutral glycosphingolipids

Glucosylceramide (Glc β 1–1'Cer, or GlcCer), galactosylceramide (Gal β 1–1'Cer, or GalCer), and lactosylceramide (Gal β 1–4Glc β 1–1'Cer, or LacCer) are the most common neutral glycosphingolipids in higher organisms (Figs. 1 and 5). The glycosidic linkage to ceramide is of the β configuration in these lipids. The *gala* type, such as galabiosylceramide (Gal α 1–4Gal β 1–1'Cer), is found primarily in kidney and pancreas. Other organisms utilize additional sugars in neutral glycosphingolipids; for example, the freshwater bivalve *Hyriopsis schlegelii* has Man β 1–1'Cer and Man β 1–2Man β 1–1'Cer, and Man β 1–4Glc β 1–1'Cer occurs in plants. More complex neutral glycosphingolipids are generally derived from LacCer or Man β 1–4Glc β 1–1'Cer. Numerous compounds also contain the oligosaccharide sequence –Gal β 1–4GlcNAc β 1–3– as seen at the non-reducing end of the paragloboside Gal β 1–4GlcNAc β 1–3Gal β 1–4Glc β 1–1'Cer and a Lewis blood group-specific antigen from human adenocarcinoma, Gal β 1–4[Fuc α 1–3]GlcNAc β 1–3Gal β 1–4Glc β 1–1'Cer.

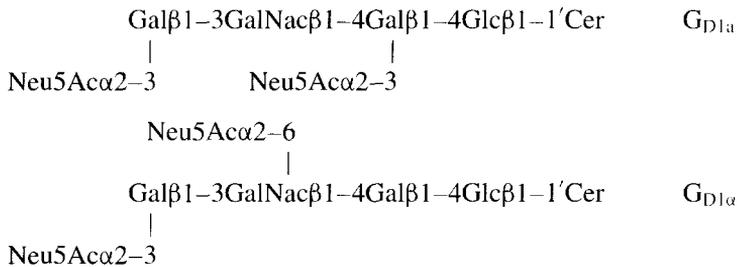
As discussed in Section 1.1.1, neutral glycolipids can form aggregates that may be important in establishing regions of the membrane with unique properties (see also T.E. Thompson, 1985).

2.4.2. Acidic glycosphingolipids

2.4.2.1. Gangliosides. Gangliosides are found in all cells of vertebrates, but in especially high amounts in the central nervous system. While diverse in structure, they

have in common one or more units of an acidic sugar called *N*-acetyl-neuraminic acid (more commonly called 'sialic acid') attached via α -glycosidic linkages to other sugars (Fig. 5). Sialic acids may have *N*-acetyl or *N*-glycolyl (i.e., hydroxyacetyl) groups at C5 (and less often C7, C8 or C9), and are distinguished by the names *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). The simplest gangliosides contain one sialic acid and galactose or glucose, such as Neu5Gc α 2-6Glc β 1-1'Cer (found in human brain). Most gangliosides are derivatives of LacCer (Fig. 5). The addition of one sialic acid gives G_{M3} ganglioside (Neu5Ac α 2-3Gal β 1-4Glc β 1-1'Cer), which is found in many biological sources. Gangliosides frequently contain a string of two or three sialic acid residues, attached to each other in α 2-8 glycosidic linkages, examples of which are G_{D3} (Neu5Ac α 2-8Neu5Ac α 2-3Gal β 1-4Glc β 1-1'Cer) and G_{T3} (Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac α 2-3Gal β 1-4Glc β 1-1'Cer).

Gangliosides were initially classified into a few related series, such as the *ganglio* and *neolacto* types (Table 1), with the sialic acids on one or both root galactose residues of the *ganglio* type as, for example, in ganglioside G_{D1a} (shown below) from human brain. However, additional kinds of naturally occurring structures (shown below) illustrate the structural diversity that can be obtained with glycosphingolipids of even the same carbohydrate composition.



The extensive carbohydrate chains and (poly)anionic charge make many gangliosides highly amphiphilic (with critical micelle concentrations of ca. 10^{-8} M); many will partition into the aqueous phase when extracted with organic solvents.

2.4.2.2. Phosphorus-containing glycosphingolipids. As described in Section 2.3, fungi, plants, and protozoa contain sphingolipids in which ceramide is attached to an oligosaccharide via a phosphodiester linkage to *myo*-inositol and other carbohydrates.

2.4.2.3. Sulfatoglycosphingolipids. Over a dozen sulfated glycosphingolipids have been isolated from vertebrates echinoderms and microorganisms [21]. Cerebrosulfatide (3'-sulfo-Gal β 1-1'Cer, or galactosylceramide-1³-sulfate) (Fig. 1) was the first such substance to be isolated, and is the major sulfoglycolipid of brain, kidney, the gastrointestinal tract and endometrium, and is a major glycolipid of mammalian male germ cells. Sulfatides are thought to be involved in neuronal cell differentiation, myelin formation and maintenance (F.B. Jungalwala, 1994; J.L. Dupree, 1999), and other processes (D. Mamelak, 2000). Two glucuronyl (GlcA) sulfatoglycolipids (3-*O*-SO₃-GlcA β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β 1-1'Cer and 3-*O*-SO₃-GlcA β 1-3(Gal β 1-4GlcNAc β 1-3)₂Gal β 1-4Glc β 1-1'Cer) occur in the peripheral nervous system.

2.5. *Lysosphingolipids*

Lysosphingolipids lack the amide-linked fatty acid of the ceramide backbone, which makes them highly water-soluble. A lysoglycosphingolipid with one hexose (e.g., glucosyl- or galactosyl-sphingosine) is colloquially referred to as a 'psychosine'. It has long been suspected that the appearance of psychosines, which are toxic to cells in culture, in some sphingolipid storage diseases implicates them in the pathology [22]. The cause of the toxicity is not known, but lysosphingolipids tend to form micelles and disrupt membranes, as well as inhibit protein kinase C (Y.A. Hannun, 1987) and bind to a recently discovered family of psychosine receptors (D.S. Im, 2001). Novel lysosphingolipids with alkyl groups on the sugar have been discovered and termed plasmalopsychosines (K.K. Sadozai, 1993; T. Hikita, 2001).

2.6. *Sphingolipids covalently linked to proteins*

At least two examples of covalent attachments between sphingolipids and proteins have been found. Structural proteins of the cornified cell envelope of the skin permeability barrier are covalently attached to ω -hydroxyceramides and ω -hydroxyglucosylceramides through the ω -hydroxyl groups (T. Doering, 1999; M.E. Stewart, 2001). And for some organisms, such as yeast, ceramides replace diacylglycerols in the covalently attached phosphatidylinositolglycan-linkage (Chapter 2) that is used to attach the proteins to membranes (A. Conzelmann, 1995).

2.7. *Sphingolipids in food*

Unlike other lipids, for which dietary consumption has long been known, the per capita consumption in the United States has only recently been estimated to be 115–140 g/year, or 0.3–0.4 g/day [23]. The amounts in any given food vary considerably, from a few $\mu\text{mol}/\text{kg}$ in fruits and some vegetables to ca. 2 mmol/kg (1–2 g/kg) in dairy products, egg and soybeans. Most foods of mammalian origin (beef, milk, poultry, etc.) have sphingomyelins, cerebroside, globosides, gangliosides, sulfatides, etc., that are comprised of ceramide backbones with sphingosine (d18:1 Δ^4) or 4-hydroxysphinganine (t18:0). The complex sphingolipids of plants are mainly cerebroside (mono- and oligohexosylceramides, with glucose the most common hexose), and ceramides that have relatively little d18:1 Δ^4 and mostly d18:1 Δ^8 , d18:2 $\Delta^{4,8}$, t18:0 and t18:1 Δ^8 sphingoid bases with α -hydroxy fatty acids.

3. *Biosynthesis of sphingolipids*

The general pathways for sphingolipid metabolism are reasonably well characterized [24] and their regulation is beginning to be understood through the development of specific inhibitors and identification of the genes for many of these enzymes.

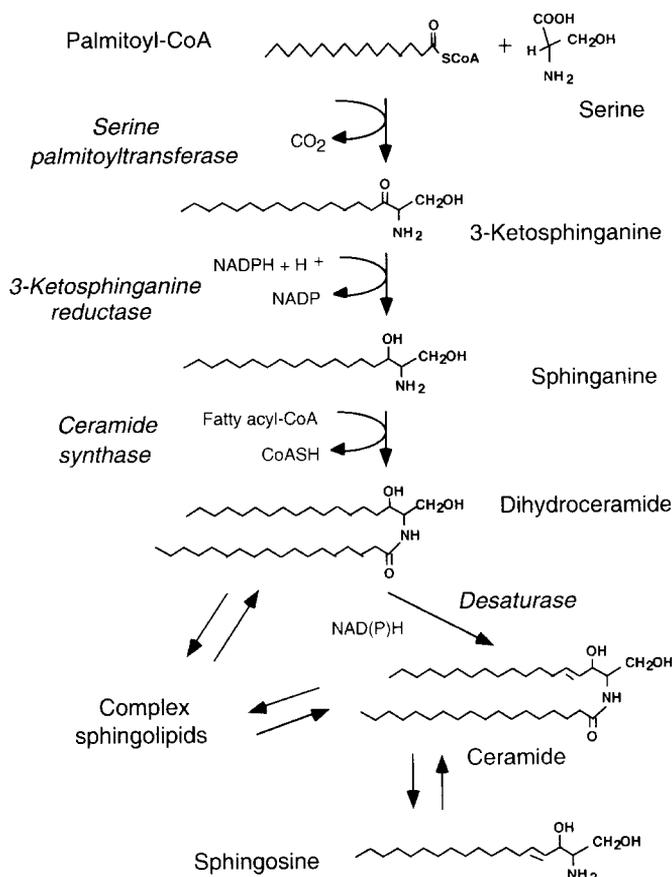


Fig. 8. Biosynthetic pathway for ceramide and sphingosine. Note that free sphingosine is formed after ceramide turnover, not as an intermediate of de novo biosynthesis.

3.1. Sphingoid bases and ceramide

3.1.1. Synthesis of the long-chain base backbone

Ceramide synthesis begins with the condensation of palmitoyl-CoA and L-serine (Fig. 8), catalyzed by the pyridoxal phosphate-dependent enzyme serine palmitoyltransferase. The reaction proceeds with overall retention of configuration of C2 of serine via the proposed mechanism shown in Fig. 9.

As would be predicted from this mechanism, serine palmitoyltransferase undergoes 'suicide' inhibition by β -halo-L-alanines and L-cycloserine. More potent and selective inhibitors have been isolated from microorganisms and are listed in Table 2. One is available commercially (myriocin or ISP-1) (Fig. 10), and is illustrated in Fig. 9 as the likely adduct that it forms with serine palmitoyltransferase.

For mammals and yeast, at least two gene products (termed SPTLC1 and SPTLC2, or sometimes SPT1 and SPT2) are necessary for activity (R.C. Dickson, 2000) and appear to be physically associated (K. Gable, 2000; K. Hanada, 2000). Human genetic

Table 2
Inhibitors of sphingolipid metabolism

Enzyme	Inhibitor	Reference
Serine palmitoyltransferase	Cycloserine	M. Lev, 1984
	β -Fluoroalanine	K.A. Medlock, 1988
	Sphingofungin C	M.M. Zweerink, 1992
	Lipoxamycins	S.M. Mandela, 1994
	ISP-1/myriocin	Y. Miyake, 1995
	Viridifungin A	S.M. Mandela, 1997
Ceramide synthase	Fumonisin B ₁ , B ₂ , etc.	E. Wang, 1991
	Alternaria toxin	A.H. Merrill, 1993
	Australifungins	S.M. Mandela, 1995
	<i>N</i> -acylaminopentols	H. Humpf, 1998
Glc : ceramide synthase	PDMP (D- <i>threo</i> -1-phenyl-2-decanoylamino-3-morpholino-1-propanol) and related analogs	N.S. Radin, 1990; A. Abe, 1995;
	<i>N</i> -butyldeoxynojirimycin	J.S. Shayman, 2000 F.M. Platt, 1997
Lactosylceramide synthase	Epoxy-glucosylceramide	C. Zacharias, 1994
Sphingomyelin synthase	PDMP (at very high concentrations)	A. Abe, 1995
	D609	C. Luberto, 1998
Inositolphosphorylceramide synthase	Khafrefungin	S.M. Mandala, 1997
	Aureobasidin A	M.M. Nagiec, 1997
	Rustimicin	S.M. Mandala, 1998
Sialidase	2-Deoxy-2,3-dehydro- <i>N</i> -acetylneuraminic acid	P. Meindl, 1969; S. Usuki, 1988
Glucocerebrosidase	Conduritol B-epoxide	G. Legler, 1977; S. Mahdiyoun, 1992
Acidic sphingomyelinase	Phosphatidylinositol 4',5'-bisphosphate, adenosine 3',5'-diphosphate, adenosine-9-beta-D-arabinofuranoside 5'-monophosphate	L.E. Quintern, 1987
	SR33557 ((2-isopropyl-1-(4-[3- <i>N</i> -methyl- <i>N</i> -(3,4-dimethoxy- β -phenethyl) amino]propyloxy)-benzene sulfonyl))indolizine	J.P. Jaffrezou, 1992
Neutral sphingomyelinase	3- <i>O</i> -methyl-sphingomyelin	M.D. Lister, 1995
	Glutathione	B. Liu, 1997
	Difluoromethylene sphingomyelin	T. Yokomatsu, 2001
	Scyphostatin and analogs	T. Izuhara, 2001; C. Arenz, 2001
Ceramidase	<i>N</i> -Oleoyl-ethanolamine	M. Sugita, 1975
	D-MAPP	A. Bielawska, 1996
Sphingosine kinase	D- and L- <i>threo</i> -sphingosine (and sphinganine)	B.M. Buehrer, 1993
	S-15183a and b	K. Kono, 2001
Sphingosine 1-phosphate lyase	4-Deoxypyridoxine-5' phosphate	W. Stoffel, 1969; P. Van Veldhoven, 1993

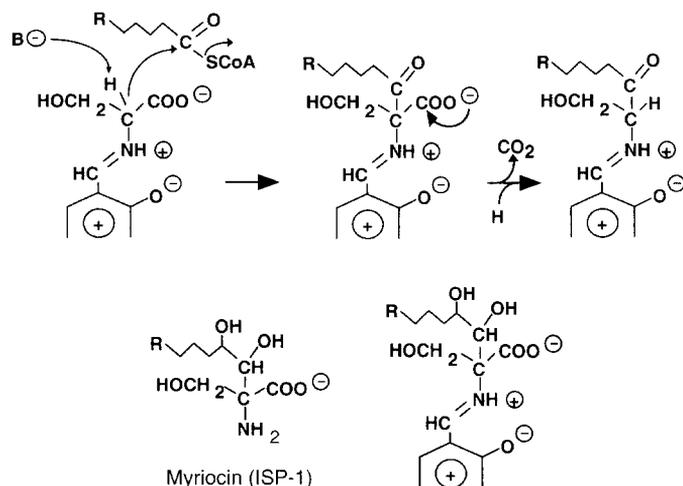
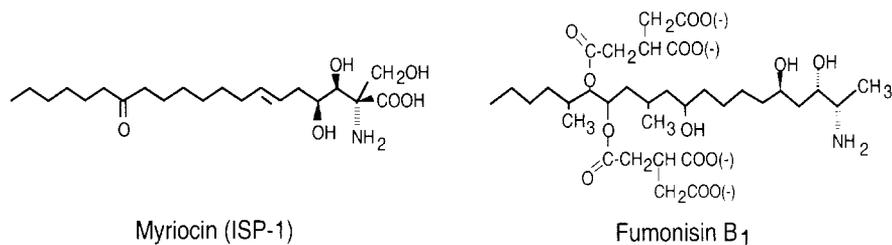


Fig. 9. A probable reaction mechanism for serine palmitoyltransferase (modified from K. Krisnangkura, 1976) and the adduct that presumably accounts for the high-affinity inhibition by myriocin (ISP1) (for the full structure of myriocin see Fig. 10).



Inhibitor of serine palmitoyltransferase

Inhibitor of ceramide synthase

Fig. 10. Representative inhibitors of sphingolipid biosynthesis that have been isolated from microorganisms. For more inhibitors and references for these compounds, see Table 2.

defects in SPTLC1 have been shown to cause hereditary sensory neuropathy type I. In yeast, an 80-amino acid polypeptide has been shown also to affect activity (K. Gable, 2000). Relatively little is known about the structure of serine palmitoyltransferase due to its membrane association; however, a soluble, homodimeric enzyme is produced by *Sphingomonas* (H. Ikushiro, 2001) and should be amenable to structural and mechanistic studies.

Sphingoid base synthesis can be suppressed by addition of lipoproteins or free sphingoid bases to cells in culture, and studies with a phosphorylated but poorly degraded analog (*cis*-4-methylsphingosine) indicates that sphingoid base 1-phosphate(s) down-regulate serine palmitoyltransferase activity (G. van Echten-Deckert, 1997). Regulation at a transcriptional level has been seen mostly with cells in culture (reviewed in Linn et al. [25]) but sometimes in vivo (R.A. Memon, 2001) and include endotoxin and

cytokines, UV irradiation, retinoic acid, corticosteroids, and phorbol esters. Transcriptional regulation appears to involve mainly changes in SPTLC2 mRNA. There is also post-translational activation of serine palmitoyltransferase in response to etoposide in mammalian cells (D.K. Perry, 2000) and heat shock in yeast (G.M. Jenkins, 2001). The heat shock response is interesting, not only with respect to the downstream pathways that are affected, such as amino acid transport (M.S. Skrzypek, 1998) and ubiquitin-dependent proteolysis (N. Chung, 2000), but also because the long-chain bases that are elevated are predominantly eicosasphingamines (i.e., 20-carbon atoms in length) (R.C. Dickson, 1997).

The next step of sphingoid base synthesis, the reduction of 3-keto-sphinganine (Fig. 8), is apparently rapid *in vivo* because the 3-keto-intermediate is rarely detected. The gene for this reductase has been identified in yeast (T. Beeler, 1998).

3.1.2. Synthesis of the *N*-acyl-derivatives of sphingoid bases

As shown in Fig. 8, free sphinganine is acylated to dihydroceramides by ceramide synthase(s), which utilize a wide variety of sphingoid bases and fatty acyl-CoAs, and may be a family of isoenzymes (E. Wang, 2000). Ceramide can also be made by reversal of ceramidase (S. El Bawab, 2001); however, this reaction appears to account for relatively little ceramide synthesis under normal physiological conditions. Recent studies (I. Guillas, 2001) have identified yeast (Lag1P and Lac1P) and mammalian (K. Venkataraman, 2002) genes that encode ceramide synthase or are obligatory for ceramide synthase activity.

Microorganisms produce a number of inhibitors of ceramide synthase (Table 2). Fumonisin (Fig. 10), which are produced by some species of *Fusaria*, were discovered as causes of human esophageal cancer, equine leukoencephalomalacia, and porcine pulmonary edema (W.F.O. Marasas, 2001), and are now known to induce multiple pathologies, including liver and kidney toxicity and carcinogenicity, immunosuppression (and in some cases immunostimulation), and birth defects (A.H. Merrill, 2001). Fumonisin not only block complex sphingolipid formation, but also cause sphinganine to accumulate, which is both key to fumonisin toxicity (E.M. Schmelz, 1998) and provides a useful biomarker for exposure of organisms to this mycotoxin. There can also be elevation of sphinganine 1-phosphate [15], which might explain how fumonisins can be toxic and mitogenic for different cell types.

The last step of ceramide synthesis is the insertion of the 4,5-*trans*-double bond into the sphingoid base backbone, which occurs at the level of dihydroceramide (Fig. 8) (J. Rother, 1992). Therefore, free sphingosine *per se* is not an intermediate of sphingolipid biosynthesis *de novo*. The genes for desaturases have been characterized in plants (P. Sperling, 2000) and mammals (P. Ternes, 2002). For 4-hydroxysphingamines (phytosphingosine), insertion of the 4-hydroxyl group occurs at the level of sphinganine in yeast (M.M. Grilley, 2000) and plants (P. Sperling, 2000).

3.2. Sphingomyelin and ceramide phosphorylethanolamine

Sphingomyelin is synthesized by transfer of phosphorylcholine from phosphatidylcholine to ceramide, liberating diacylglycerol. This reaction links glycerolipid and

sphingolipid signaling pathways, although it is not known if cells frequently capitalize on this relationship for signaling purposes. Most *de novo* sphingomyelin synthesis occurs in the Golgi apparatus (in liver); however, synthesis also occurs in the plasma membrane of many cell types and is probably a major site as well. Relatively little is known about the regulation of sphingomyelin biosynthesis, but it has interesting features such as stimulation by phorbol esters, 25-hydroxycholesterol, and Brefeldin A (G. Hatch, 1992; N. Ridgway, 1995), increases during development of the lung (C.A. Longo, 1997), decreases in aging (S.A. Lightle, 2001), and changes very early in the development of colon cancer (P.K. Dudeja, 1986).

Ceramide phosphorylethanolamine is synthesized from phosphatidylethanolamine and ceramide in a reaction analogous to sphingomyelin synthesis, and once formed can be methylated to sphingomyelin (M. Malgat, 1986; M.N. Nikolova-Karakashian, 2000). Inositolphosphoceramides are formed by transesterification (from phosphatidylinositol) (R.C. Dickson, 1999; A.S. Fischl, 2000) by transferases that share a conserved structural motif across yeast and pathogenic fungi, and which resembles somewhat a motif of lipid phosphatases (S.A. Heidler, 2000).

3.3. Neutral glycosphingolipids

Pathways for the biosynthesis of the different root glycosphingolipids (Figs. 11 and 12) appear complex, but are achieved by surprisingly few glycosyltransferases that commit precursors and intermediates to predictable products based on the specificities of the enzymes. The enzymes transfer a specific sugar from the appropriate sugar nucleotide (e.g., UDP-Glc, UDP-Gal, etc.) to ceramide or the non-reducing end of the growing carbohydrate chain attached to ceramide. The glycosyltransferases often recognize mainly the carbohydrate portion of the acceptor glycosphingolipid; however, ceramides with α -hydroxy-fatty acids are preferentially incorporated into GalCer (and sulfatides), whereas those with non-hydroxy-fatty acids are used to make GlcCer (I. van Genderen, 1995).

GalCer and GlcCer are synthesized with inversion of the configuration of the

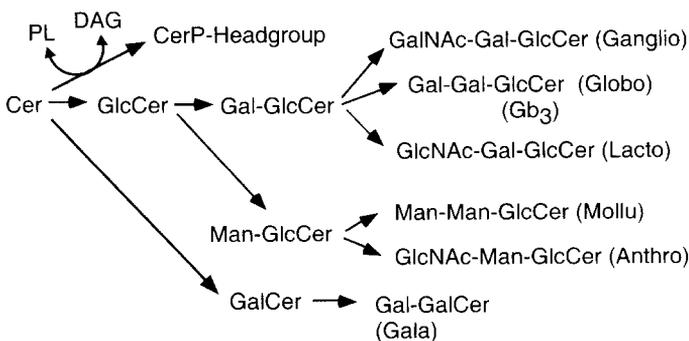


Fig. 11. Biosynthesis of phosphosphingolipids and 'root' glycosphingolipids. For phosphosphingolipids, the headgroup is transferred from a phosphoglycerolipid, such as phosphatidylcholine for sphingomyelin. For glycosphingolipids, the headgroup is transferred from a UDP-sugar.

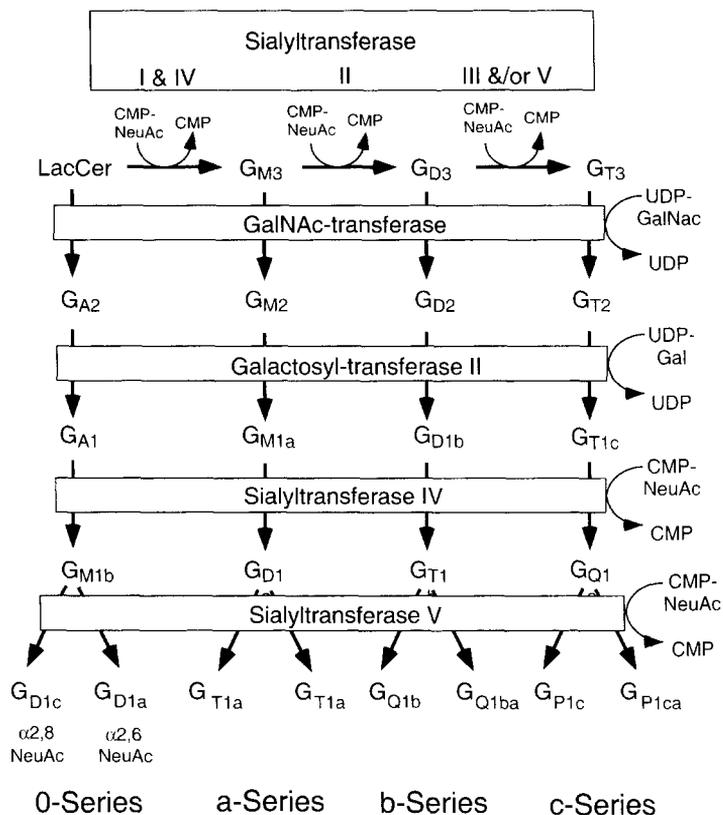


Fig. 12. Scheme for ganglioside biosynthesis from LacCer [24,26]. Due to cellular compartmentation sialyltransferases IV and V are probably not involved in GM_3 and GT_3 synthesis in vivo, respectively; however, they do so in vitro.

glycosidic bond (α to β) by UDP-Glc(or Gal) : ceramide glucosyltransferases, the genes for which have been identified in numerous organisms (S. Ichikawa, 1998; M. Leipelt, 2001; and reviewed in T. Tencomnao, 2001). Relatively little is yet known at a biochemical level about these enzymes; however, recent studies have identified amino acid residues of the glucosylceramide synthase active site that are essential for activity, and note that this enzyme and processive β -glucosyltransferases possess a conserved substrate-binding/catalytic domain (D.L. Marks, 2001).

A growing number of factors have been found to regulate expression of glucosylceramide synthase, including depletion of the amounts of GlcCer in the cell (I. Meivar-Levy, 1999), elevations in ceramide (H. Komori, 2000), endotoxin and acute phase response mediators (R.A. Memon, 2001), and basic fibroblast growth factor (although this appears to involve a post-translational mechanism) (S.A. Boldin, 2000). Glucosylceramide and protein-bound glucosylceramide play major roles in establishing the permeability barrier of skin, and GlcCer synthase is up-regulated during epidermal barrier development (K. Hanley, 1997; R. Watanabe, 1998; T. Doering, 1999).

The synthesis of GlcCer and GalCer can be inhibited by structural analogs of ceramide such as PDMP (Table 2), which decreases cellular levels of neutral glycosphingolipids and gangliosides and elevates ceramide, causing cell cycle arrest (C.S.S. Rani, 1995). GlcCer synthesis appears to be a major determinant of survival of tumor cells (by removal of ceramide and affecting multidrug resistance) (Y.Y. Liu, 2001; A. Senchenkov, 2001). But, the inability to make detectable glycolipids due to deficient GlcCer synthase is not lethal to some cell lines, although it slows growth and results in an elongated fibroblastic morphology (S. Ichikawa, 1994).

Additional glycosyltransferases are involved in the synthesis of the other neutral glycolipids as well as the addition of neutral sugars to gangliosides (Figs. 11 and 12), and many of the genes for these enzymes have been identified, including lactosylceramide synthase (T. Takizawa, 1999), globosylceramide (Gb3) synthases (Y. Kojima, 2000; J. Keusch, 2000), β -1,3-galactosyltransferases (M. Amado, 1999), β 1,3 *N*-acetylglucosaminyltransferase (Lc3 synthase) (T. Henion, 2001) as well as numerous fucosyltransferases for Lewis \times antigens. Labs are already beginning to engineer surface glycosphingolipids of desired composition by transfecting cells with combinations of these and other enzymes (E.G. Prati, 2000).

Synthesis of GlcCer occurs on the cytosolic aspect of the endoplasmic reticulum and/or early Golgi membranes (A.H. Futerman, 1991; D. Jeckel, 1992), whereas more complex neutral glycosphingolipids (beginning with LacCer) are made in the lumen of the Golgi apparatus (H. Lannert, 1994). Therefore, GlcCer and the sugar nucleotides must undergo transbilayer movement to the lumen of the Golgi for the synthesis of more complex sphingolipids. Sphingolipids are generally thought to reach their membrane locations via trafficking from Golgi; however, newly synthesized GlcCer can also be transported to the plasma membrane via a non-Golgi pathway (D.E. Warnock, 1994).

3.4. Gangliosides

The general pathway for the synthesis of gangliosides has evolved to the scheme depicted in Fig. 12 [24]. Gangliosides are synthesized by the stepwise transfer of neutral sugars and sialic acids by membrane-bound glycosyltransferases that are located in the regions of the Golgi apparatus that generally correspond to the order in which the sugars are added. For example, the sialyltransferase catalyzing the synthesis of G_{M3} ganglioside is in the *cis* Golgi, whereas the enzymes involved in terminal steps in ganglioside synthesis are localized in the more distal *trans* Golgi network. Ganglioside biosynthesis can also involve the introduction of *O*-acetyl groups on sialic acid and *N*-deacetylation to produce a free amino group on position 5 of sialic acid. Gangliosides are incorporated into the outer leaflet of the plasma membrane by vesicle-mediated transport.

There has been considerable progress in identification of the genes responsible for the key reactions in Fig. 12, allowing these relationships to be tested by transfecting cells with the cDNA for enzymes of this pathway and determining the types of glycosphingolipids that are made; for example (a) transfection of GalNac-transferase cDNA into Chinese hamster ovary cells, which normally make mainly G_{M3} , produced cells that now synthesize mainly G_{D2} , whereas transfection of cells that are defective in

sialylation yielded G_{A2} (M.S. Lutz, 1994), (b) transfection of several cell lines with a GalNac-transferase yielded G_{A2} , G_{M2} (the preferred product when both G_{M3} and LacCer were available), G_{D2} , GalNac sialylparagloboside and GalNac G_{D1a} (S. Yamashiro, 1995), and (c) transfection of the cDNA for G_{D3} synthase (sialyltransferase II in Fig. 12) into Neuro2a cells increased G_{D3} and G_{Q1b} (N. Kojima, 1994). The findings to date support the view that ganglioside synthesis can be viewed as 'combinatorial' reactions (Fig. 12) catalyzed by a conservative number of key enzymes such that the ultimate composition is determined by the relative activities of these enzymes and the availability of their substrates [27].

Regulation of ganglioside biosynthesis involves both transcriptional and post-transcriptional factors. Transcriptional control of key glycosyltransferases appears to account for many of the developmentally regulated, tissue-selective variations in ganglioside amounts and types in mammalian organs, including large changes with oncogenic transformation (R.W. Ledeen, 1998; S. Hakomori, 1998). The biosynthesis of gangliosides is also controlled through post-translational modification of glycosyltransferases (R.K. Yu, 2001); several sialyltransferases (X. Gu, 1995) are down-regulated by protein kinase C in cell-free and intact cell systems and *N*-acetylgalactosaminyl-transferase can be up-regulated by protein kinase A in cultured cells.

3.5. Sulfatoglycosphingolipids

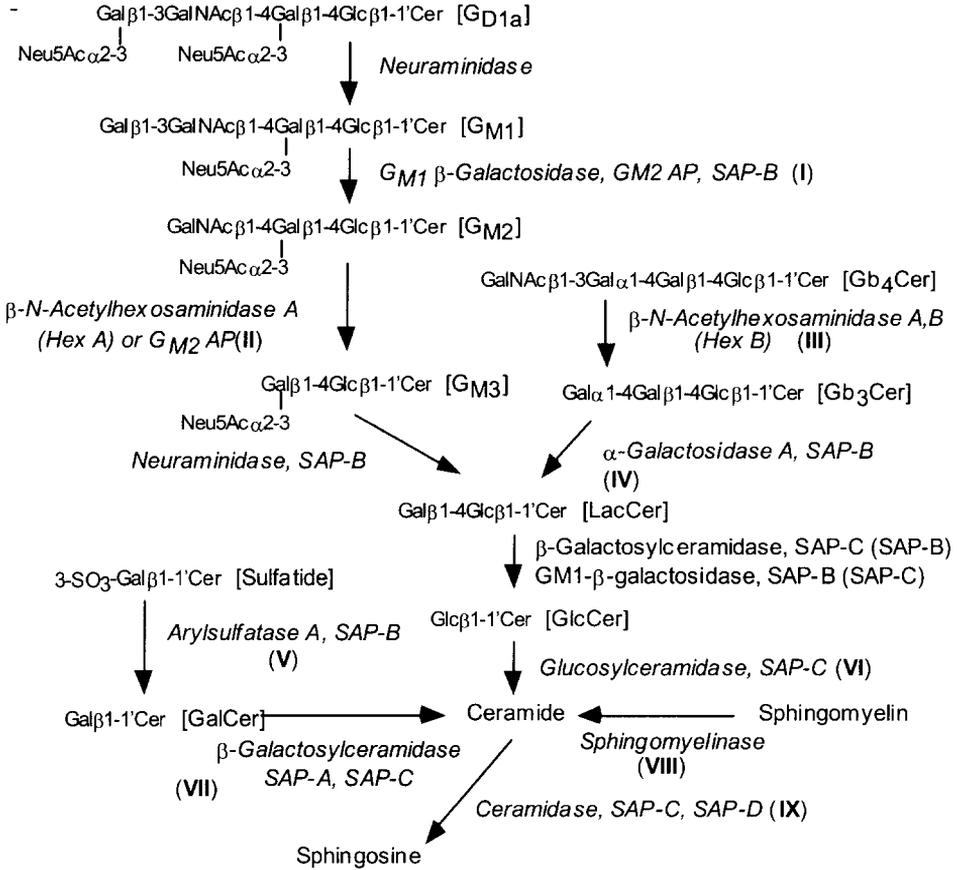
Sulfatide (3'-sulfo-galactosylceramide) synthesis is catalyzed by GalCer sulfatotransferase (3'-phosphoadenylylsulfate : galactosylceramide 3'-sulfotransferase), which utilizes the activated sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The cDNA encoding the sulfotransferase has been cloned (T. Honke, 1997). Regulation of sulfatide biosynthesis appears to reside in the activity of this sulfatotransferase.

4. Sphingolipid catabolism

Complex sphingolipids are lost from cells by (1) membrane internalization, recycling, and degradation, (2) hydrolysis to release bioactive products that participate in cell signaling, and (3) release from the cells by secretion or shedding.

In general, sphingolipids are internalized with endocytic vesicles, sorted in early endosomes, and recycled back to the plasma membrane (often with remodeling of the sphingolipid) or transported to lysosomes where they are degraded by specific acid hydrolases. Given that lysosomal membranes are rich in sphingolipids, it has been unclear why they, too, do not undergo hydrolysis if the endocytosed membranes and lysosomal membranes are simply fused. This dilemma was solved when it was shown (W. Furst, 1992; W. Mobius, 1999) that endocytosed sphingolipids (and presumably other components) become invaginated into intraendosomal vesicles that are delivered into the lumen of the lysosome. Thus, hydrolytic enzymes contact the sphingolipids to be digested in the lumen rather than as part of the lysosomal membrane, which is additionally protected by an elaborate glycocalyx that lines the inner leaflet.

The pathways for sphingolipid catabolism (Fig. 13) converge on ceramide; nonethe-



<u>Inherited disorders of sphingolipid catabolism:</u>	
I. Generalized gangliosidosis	VI. Gaucher disease
II. Tay-Sachs disease (Sandhoff AB variant)	VII. Globoid cell leukodystrophy;
III. Sandhoff disease	Krabbe disease
IV. Fabry disease	VIII. Niemann Pick disease
V. Metachromatic leukodystrophy	IX. Farber disease

Fig. 13. Catabolism of complex sphingolipids and associated diseases [3,24].

less, cells contain at least small amounts of other lysosphingolipids, such as galactosylsphingosine (psychosine) and sphingosylphosphorylcholine, implying that such compounds may arise from as-yet-to-be-characterized enzymatic activities analogous to phospholipase A₂, or by de novo biosynthesis.

4.1. Sphingomyelin

The lysosomal hydrolysis of sphingomyelin to ceramide and phosphocholine is catalyzed by acid sphingomyelinase, a water-soluble, lysosomal glycoprotein that interacts with a sphingolipid activator protein (SAP-C) and anionic lipids such as bis(monooacylglycero)phosphate (T. Linke, 2001). Individuals may display a number of different molecular defects that cause insufficient lysosomal sphingomyelinase activity and result in accumulation of sphingomyelin in reticuloendothelial cells scattered throughout the spleen, bone marrow, lymph nodes, liver, and lungs. These are called Niemann–Pick disease Types A and B, and sphingomyelin also accumulates in Type C Niemann–Pick disease, but not due to genetic defects in sphingomyelinase per se. Screening for human Niemann–Pick disease is used to identify carriers and for prenatal diagnosis of affected fetuses. Acid sphingomyelinase-deficient mice have been generated (K. Horinouchi, 1995) as models for Niemann–Pick disease.

Sphingolipid turnover in other cellular compartments involves both acid sphingomyelinase and sphingomyelinases with neutral to alkaline pH optima. Acidic sphingomyelinase is also secreted, and the secreted form requires supplemental Zn^{2+} for activity (I. Tabas, 1999). Neutral sphingomyelinases have been found in other cellular compartments, including the nuclear membrane, and at least one form resides in sphingolipid-enriched microdomains and is inhibited by the caveolin-scaffolding domain (R.J. Veldman, 2001). These are probably involved in cell signaling.

A sphingomyelinase D is found in the venom of brown recluse spiders, *Corynebacterium pseudotuberculosis* (which commonly infects sheep), *Vibrio damsela* (an aquatic bacterium that causes wound infections in humans) and the human pathogen *Arcanobacterium haemolyticum*. This enzyme, which yields ceramide 1-phosphate and choline, is able to produce much of the tissue damage caused by these organisms (A.P. Truett, 1993).

4.2. Glycosphingolipids

Glycosphingolipids are catabolized by the stepwise hydrolysis of the terminal monosaccharides through the concerted action of a series of specific exoglycosidases (Fig. 13). For the in vivo degradation of glycolipids with short oligosaccharide headgroups, i.e. of less than four carbohydrate residues, there is often a requirement for sphingolipid activator proteins (or saposins) SAP-A, -B, -C or -D. A number of inherited diseases are caused by mutations in the structural genes for these enzymes that result in reduced enzymatic activity, loss of the appropriate targeting signals for transport to lysosomes, or alteration of the domains that interact with other subunits of the enzyme and/or activator proteins (Fig. 13) [24].

That sphingolipidoses can result from several types of genetic defects is exemplified the G_{M2} gangliosidoses, which arise from mutations of β -*N*-acetylhexosaminidase (Hex A or Hex B) (Fig. 13) or G_{M2} activator proteins (R.A. Gravel, 1995) [24]. Hex A is an $\alpha\beta$ heterodimer and degrades negatively charged and uncharged substrates, whereas Hex B is the $\beta\beta$ homodimer and cleaves mainly *N*-acetylgalactosamine residues from uncharged substrates such as G_{A2} , globotetraosylceramide and oligosaccharides.

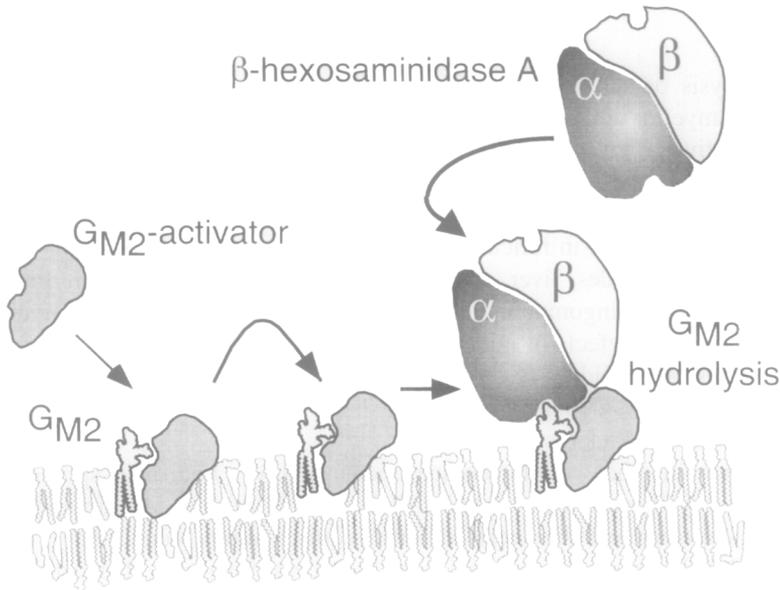


Fig. 14. A model for the role of G_{M2} activator protein (SAP) in the hydrolysis of ganglioside G_{M2} by β -hexosaminidase A (subunits α and β). For more information, see Schuette et al. [3] and Kolter and Sandhoff [24].

Therefore, mutations in the structural gene for the α subunit result in partial or complete loss of Hex A activity (Tay–Sachs disease), and mutations in the β subunit affect both Hex A and Hex B (Sandhoff disease). The phenotypes of these two gangliosidoses are similar, but they are easily distinguished by measuring Hex A and Hex B activities and by the accumulation of Gb_4Cer in Sandhoff disease but not in Tay–Sachs disease (G_{M2} accumulates in both disorders). The AB variant has normal levels of both Hex A and Hex B activity when measured *in vitro*, but there is a defective (or absent) G_{M2} activator protein. The G_{M2} activator protein is a membrane active protein that also binds ganglioside G_{M2} as well as structurally related gangliosides forming complexes (usually in a 1 : 1 molar ratio) that present them to hexosaminidase A as illustrated in Fig. 14.

4.3. Ceramide

The major pathway for catabolism of the ceramide backbone is shown in Fig. 15.

In lysosomes, ceramides are hydrolyzed to free sphingoid bases and long-chain fatty acids by a ceramidase that has an acidic pH optimum. The lysosomal ceramidase is a water-soluble glycoprotein that hydrolyzes membrane-bound ceramide in an interfacial reaction that needs the stimulation by SAP-D and anionic phospholipids such as the lysosomal bis(monoacylglycerol)phosphate (A. Klein, 1994; T. Linke, 2001). The human acidic ceramidase is a heterodimeric enzyme of 40 kDa and 13 kDa subunits synthesized as a single precursor polypeptide of approximately 53–55 kDa and targeted to the lysosome via the mannose 6-phosphate receptor (K. Ferlinz, 2001). Additional

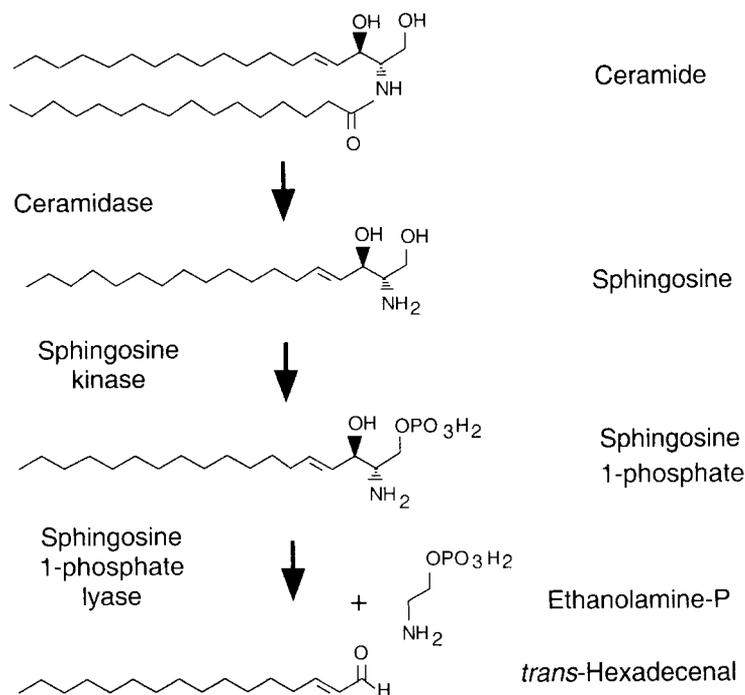


Fig. 15. Catabolism of ceramide and sphingosine [24].

ceramidases with neutral and alkaline pH optima have been found in various cell compartments (including mitochondria) and appear to be involved in signaling.

4.4. Sphingosine

Sphingosine undergoes reacylation by ceramide synthase (described in Section 3.2.2) or phosphorylation by sphingosine kinase. This family of ATP-dependent kinases is both cytosolic and membrane associated, and phosphorylates a wide range of sphingoid bases, although unnatural stereoisomers (e.g., *L-threo*-sphinganine) are inhibitors. The product can be dephosphorylated by specific sphingosine 1-phosphate phosphatases as well as more general lipid phosphatases, or cleaved to ethanolamine phosphate and *trans*-2-hexadecenal (Fig. 15) by a pyridoxal 5'-phosphate-dependent lyase (J. Zhou, 1998; P.P. Van Veldhoven, 2000). As shown first in the 1970s by W. Stoffel and co-workers, the phosphoethanolamine can be utilized for the synthesis of phosphatidylethanolamine, and *trans*-2-hexadecenal reduced to the alcohol and incorporated into alkyl ether lipids (Chapters 8 and 9). Under certain conditions, degradation of sphingoid bases can account for as much as one third of the phosphoethanolamine in phosphatidylethanolamine (E. Smith, 1995). In addition to being an intermediate in the degradation of sphingoid bases, sphingosine 1-phosphate is packaged in some cells (e.g., platelets) for secretion as discussed in Section 6.2.3.

5. Regulation of sphingolipid metabolism

Sphingolipid metabolism is regulated at a number of levels: (1) the control of ceramide biosynthesis *de novo* and by the recycling of existing sphingolipids; (2) the partitioning of ceramide toward the major classes of sphingolipids (e.g., sphingomyelin versus GlcCer and GalCer); (3) the partitioning of intermediates to determine the complex glycolipid profiles of different cell types; (4) the trafficking of sphingolipids to the appropriate cellular membranes as well as to specialized regions of a given membrane; (5) the secretion (and shedding) of some categories of sphingolipids; (6) the internalization of sphingolipids during endocytosis and other membrane functions; (7) the turnover of sphingolipids for cell signaling; and (8) degradation.

These events vary in different cell types, and in a given cell type at different stages of development and in response to varying environmental conditions [29]. These issues are obviously too complex to deal with fully in this chapter; therefore, we present only a few examples.

5.1. *De novo sphingolipid biosynthesis versus turnover in generating bioactive (signaling) metabolites*

The traditional 'signaling' paradigm for sphingolipids is that agonists trigger the turnover of sphingomyelin to ceramide, sphingosine and sphingosine 1-phosphate (Fig. 2); nonetheless, the *de novo* biosynthetic pathway also produces bioactive products such as sphinganine, ceramide, and sometimes sphinganine 1-phosphate. That these intermediates of *de novo* synthesis also affect cell behavior was first shown by the role of sphinganine in the toxicity of fumonisins (E. Wang, 1991) and the involvement of *de novo* synthesized ceramide in daunorubicin-induced apoptosis (R. Bose, 1995). There are now a large number of natural agonists, drugs, toxins and toxicants, and even intermediates of common metabolic pathways (such as palmitoyl-CoA) that can alter cell behavior at least in part by affecting sphingolipid biosynthesis, as illustrated in Fig. 16 (Y.A. Hannun, 2001; S.C. Linn, 2001).

5.2. *Complex sphingolipid formation in tissue development*

Glycosphingolipids undergo quantitative and qualitative changes with development [2,28], such as the stage-specific expression of the antigen SSEA-1, which appears at the 8-cell stage, is maximally expressed at the morulae stage, and disappears at the blastocyte stage. This antigenic determinant is actually present on several glycosphingolipids, all of which have a terminal Gal β 1-4[Fuc α 1-3]GlcNAc β 1-3Gal structure, as well as on cell surface glycoproteins. Globoside and Forssman antigen (IV³- α -GalNAc-Gb₄Cer) also appear at the morulae and blastocyte stages, respectively. It is likely that some of these changes contribute to cell-cell and cell-matrix interactions that affect the migration of cells to target locations within the developing embryo, and cell differentiation when the correct location(s) are found.

The requirement for glycosphingolipids in development has been established by targeted disruption of the gene encoding glucosylceramide synthase (T. Yamashita,

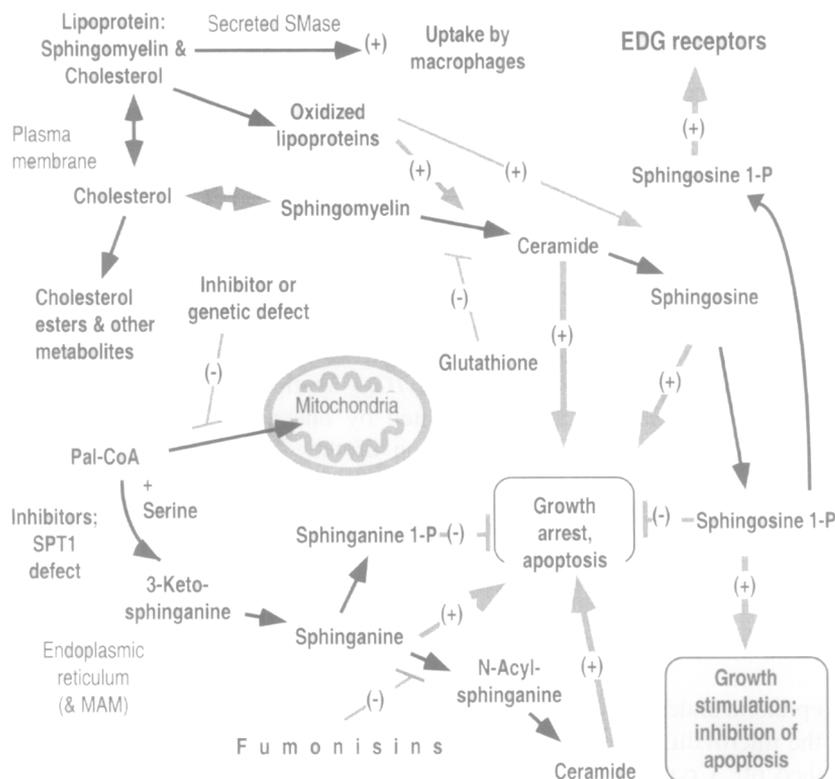


Fig. 16. Some of the factors that can modulate sphingolipid biosynthesis and turnover to impact cell behavior [23–25]. Agonist-induced changes in sphingolipid turnover were illustrated in Fig. 2; the additional pathways shown here are as follows (proceeding counter-clockwise from top left): (1) hydrolysis of lipoprotein sphingomyelin by secreted sphingomyelinase (SMase) to form particles that are taken up by macrophages; (2) induction of sphingomyelin turnover by oxidized lipoproteins to induce smooth muscle cell growth or death via sphingosine 1-phosphate versus ceramide, respectively; (3) associations between sphingomyelin and cholesterol that affect lipoprotein and membrane structure (as well as the efflux and metabolism of cholesterol, not shown); (4) perturbation of sphingolipid biosynthesis by factors that alter the amounts of the precursors (e.g., serine and palmitoyl-CoA) (Pal-CoA) including inhibitors and genetic defects of mitochondrial uptake of Pal-CoA; (5) inhibition of, or genetic defects in, serine palmitoyltransferase (SPT1); (6) inhibition of ceramide synthase (by fumonisins) to block complex sphingolipid formation and elevate sphinganine and sphinganine 1-phosphate; (7) induction of de novo sphingolipid biosynthesis at serine palmitoyltransferase and/or ceramide synthase by other stresses (irradiation, UV, cytokines, and heat shock, *inter alia*) (not shown); (8) the triggering of sphingomyelin hydrolysis by depletion of cytosolic glutathione, an inhibitor of neutral sphingomyelinase(s). More information about these factors is presented in the text.

1999). In the absence of glycosphingolipid synthesis, embryogenesis proceeded well into gastrulation with differentiation into primitive germ layers and patterning of the embryo, but was abruptly halted by a major apoptotic process. Embryonic stem cells deficient in glycosphingolipid synthesis were able to differentiate into endodermal, mesodermal, and ectodermal derivatives but were strikingly deficient in their ability to form well differentiated tissues.

5.3. Neural development and function

The ganglioside content of neuronal and glial cells changes quantitatively and qualitatively with development and aging (R.K. Yu, 1994) and it has been assumed that gangliosides help neuronal cells follow the appropriate path in development of neuronal networks, mediate cell–cell communication, and help regulate receptors and ion channels on neural cells, presumably by interactions with specific binding proteins (R.L. Schnaar, 1994).

These hypotheses have been confirmed by recent studies with mice lacking specific glycosyltransferases. Mice engineered to lack G_{M2}/G_{D2} synthase express only the simple gangliosides (G_{M3} and G_{D3}) and develop significant and progressive behavioral neuropathies (S. Chiavegatto, 2000). G_{M2}/G_{D2} synthase knockout mice lack a calcium regulatory mechanism that is modulated by one or more of the deleted gangliosides (G. Wu, 2001). Mice with disruption of the gene encoding G_{D3} synthase appear to undergo normal development and have a normal life span; however, when these mice are crossbred with mice carrying a disrupted gene encoding $\beta 1,4$ -*N*-acetylgalactosaminyltransferase, the double mutants display a sudden death phenotype and are extremely susceptible to induction of lethal seizures by sound stimulus.

5.4. Physiology (and pathophysiology) of the intestinal tract

The intestinal epithelia undergo constant renewal through cell proliferation in the crypts at the base of the microvilli, differentiation and migration up the villi, and death as the cells are sloughed off. Crypt cells have more ceramide and Gb_3 Cer, and less GlcCer and G_{M3} , than villus cells (J.-F. Bouhours, 1993), and differ also in their ceramide composition, with the crypt GlcCer having less hydroxy-fatty acids. All regions of the intestine contain relatively high levels of free ceramide (ca. 17% of the total sphingolipids). The glycosphingolipids of intestinal cells are part of the epithelial barrier, but are also used as attachment sites by microorganisms, viruses, and microbial toxins [23]; for example, *Candida albicans* binds to asialo- G_{M1} , HIV gp 120 to GalCer, cholera toxin to G_{M1} , and Shiga toxin and verotoxins to Gb_3 .

5.5. Oncogenic transformation, tumor antigens, and immunomodulation

Changes in the glycosphingolipid composition of cells have long been associated with oncogenic transformation [2]. There are multiple strategies that might utilize sphingolipids to prevent or treat neoplasia, such as (a) administration of sphingolipids or sphingolipid analogs via diet or other means (E.M. Schmelz, 2001), (b) modulation of sphingolipid metabolism to trigger apoptosis via endogenous ceramide (S. Selzner, 2001), (c) altering ceramide metabolism to overcome drug resistance (A. Senchenkov, 2001), (d) controlling the immunomodulatory effects of shed glycolipids (S. Ladisch, 1995), and (e) using abnormally expressed glycolipids as tumor markers and/or to target chemotherapeutic agents more effectively [2].

6. *Sphingolipids and signal transduction*

Findings over the last decade have provided evidence that sphingolipids are involved in essentially all aspects of cell regulation (many of which are summarized in Figs. 2 and 16). (1) Sphingolipids (particularly sphingomyelin in association with cholesterol) help define 'microdomains' of the membrane that influence receptor signaling as well as the functions of important transport proteins. (2) Complex sphingolipids serve as ligands for receptors on neighboring cells or the extracellular matrix, and mediate changes in cell behavior in response to a cell's environment. (3) Complex sphingolipids modulate the properties of receptors on the same cell, thereby controlling the responsiveness of the cell to external factors. (4) Sphingolipids are extensively involved in membrane trafficking and, therefore, influence receptor internalization, sorting and recycling, as well as the movement and fusion of secretory vesicles in response to stimuli. (5) Ceramide, sphingosine and sphingosine 1-phosphate (and possibly others) are produced from agonist- or stress-induced turnover of sphingomyelin (and de novo synthesis) to participate in cell signaling. (6) Sphingosine 1-phosphate is released from some cells to serve as an extracellular agonist for SIP receptors. Moreover, because diverse factors can affect sphingolipid metabolism (availability of precursor substrates, genetic mutations in related pathways, naturally occurring inhibitors, etc.), these pathways may play a central role in many aspects of cell physiology and pathophysiology (Fig. 16).

6.1. *Interactions between gangliosides and growth factor receptors*

Gangliosides are bimodal regulators of cell growth: both inhibitory (often as the complex species) and stimulatory (as catabolites). This is exemplified by ganglioside G_{M3} , which inhibits growth through extension of the G_1 phase of the cell cycle, and makes cells refractory to stimulation by epidermal growth factor [7]. Upon removal of the fatty acid, lyso- G_{M3} strongly inhibits cell proliferation whereas removal of the *N*-acetyl group from the sialic acid (producing de-*N*-acetyl- G_{M3}) enhances growth and epidermal growth factor receptor tyrosine kinase activity. The neutral glycolipid cores of G_{M3} , LacCer and GlcCer, also affect growth (K. Ogura, 1992).

6.2. *Hydrolysis to bioactive lipid backbones*

The finding that sphingosine is a potent inhibitor of protein kinase C (Y.A. Hannun, 1986) introduced the paradigm that the cellular functions of sphingolipids may reside not only in the complex species, but also in the lipid backbones. Soon thereafter, ceramide was found to be released from sphingomyelin in GH3 pituitary cells treated with diacylglycerol (R.N. Kolesnick, 1989) and HL-60 cells treated with $1\alpha,25$ -dihydroxyvitamin D_3 (which induces these cells to differentiate) (T. Okazaki, 1989). Furthermore, treatment of HL-60 cells with a short-chain ceramide, or exogenous sphingomyelinase, could mimic the effects of $1\alpha,25$ -dihydroxyvitamin D_3 , which linked the agonist-induced turnover of a sphingolipid to a metabolite that met many of the criteria for an intracellular mediator. A large number of studies have now explored how the lipid backbones of sphingolipids serve as second messengers, but caution must be exercised

in reading this literature because some of the effects of exogenous sphingolipids have not yet been linked to changes in endogenous mediators.

6.2.1. Ceramide

Sphingomyelin turnover to ceramide is now thought to mediate, at least in part, cellular responses to a wide spectrum of agents (cytokines, ionizing irradiation, corticosteroids, *inter alia*), many of which deal with some type of stress (R.N. Kolesnick, 1998; Y.A. Hannun, 2000; A. Huwiler, 2000). Both neutral and acidic (including a secreted acidic) sphingomyelinases are involved. The regulatory mechanisms for these enzymes are involved, and their mechanisms of activation and inhibition are still being uncovered. As an example, CD95-triggers translocation of acid sphingomyelinase to the plasma membrane outer surface, where it releases ceramide from sphingomyelin and enables the clustering of CD95 in sphingolipid-rich membrane rafts triggering apoptosis (H. Grassme, 2001).

At least five direct targets for ceramide have been identified: ceramide-activated protein phosphatases, which are both type 1 and 2A phosphoprotein phosphatases (C.E. Chalfant, 2000); ceramide-activated protein kinase, which has been identified as the kinase suppressor of ras (Y.H. Zhang, 1997; D.B. Polk, 2001); cathepsin D (M. Heinrich, 2000); protein kinase C zeta (N.A. Bourbon, 1998); and cytosolic phospholipase A₂ (A. Huwiler, 2001). In general, dihydroceramides (*N*-acylsphinganine) are ineffective in activating these targets, which makes them useful as controls. Elevations in cellular ceramide are usually associated with growth inhibition, differentiation and, in many cells, induction of apoptosis.

6.2.2. Sphingoid bases

A remarkable number of cellular systems are activated or inhibited by sphingoid bases, with the systems that are most likely to reflect direct activation being a 14-3-3 kinase (T. Megidish, 2000), and inhibition being protein kinase C (E.R. Smith, 2000), phosphatidic acid phosphohydrolase (D. Perry, 1992) and Akt kinase (H.C. Chang, 2001). There are also strong indications that sphingosine can affect ion transporters (C. Mathes, 1998; Y. Shin, 2000).

Relatively little is known about agonist-induced turnover of sphingolipids to sphingosine and the coupling of the sphingosine to intracellular responses. When added exogenously to cells, sphingoid bases are usually growth inhibitory and pro-apoptotic (V.L. Stevens, 1989; T. Shirahama, 1997). Agents affecting cellular levels of sphingosine include dexamethasone (R. Ricciolini, 1994), lipoproteins and phorbol esters (E. Wilson, 1988), platelet-derived growth factor (A. Olivera, 1993; E. Coroneos, 1995), Fas-induced apoptosis of type II Jurkat T cells (O. Cuvillier, 2000) and doxorubicin in MCF7 breast adenocarcinoma cells (O. Cuvillier, 2001). The *in vivo* correlates are few, but it has been recently reported that apoptosis in the skeletal muscle of rats with heart failure is associated with increased serum levels of tumor necrosis factor- α and sphingosine (L. Dalla Libera, 2001).

In addition, free sphingoid bases, sphingoid base 1-phosphates, and ceramides can be elevated by disruption of *de novo* sphingolipid biosynthesis by, for example, heat shock in yeast (R.C. Dickson, 1997; G.M. Jenkins, 1997) and fumonisins [4,15]. It should also

be borne in mind when studying cells in culture that many cell lines undergo a ‘burst’ of sphinganine and sphingosine formation from both increased de novo sphingolipid biosynthesis and turnover when ‘conditioned’ medium is removed (E.R. Smith, 1995). This may have a physiologic significance because cells produce a novel factor (named betrachamine) that suppresses the burst (L. Warden, 1999).

6.2.3. *Sphingosine 1-phosphate*

Platelet-derived growth factor induces rapid increases in cellular sphingosine and sphingosine 1-phosphate, and several lines of evidence indicate that sphingosine 1-phosphate is a mediator of growth stimulation by this growth factor (A. Olivera, 1993; Y. Su, 1994). The downstream responses are release of calcium from intracellular compartment(s) (S. Kim, 1995) as well as activation of AP1 transcription factor. Sphingosine 1-phosphate is also mitogenic when added to cells exogenously, and is thought to signal through a pertussis toxin-sensitive G protein (K.A. Goodemote, 1995) that activates the mitogen-activated protein kinase pathway (J. Wu, 1995). Progress in characterizing these receptors, members of the EDG family (now called S1P) receptors, has been rapid [10], and sphingosine 1-phosphate clearly qualifies as both an extracellular and an intracellular messenger.

Whereas ceramide and sphingosine are often growth inhibitory and pro-apoptotic, sphingosine 1-phosphate is mitogenic and anti-apoptotic, which has been likened to a rheostat that determines cell survival versus death pathways (S. Spiegel, 1999). Ceramides and sphingosine induce release of cytochrome *c* from mitochondria (an important event in apoptosis), and recent studies suggest that sphingosine 1-phosphate antagonizes apoptosis of human leukemia cells by inhibiting release of cytochrome *c* and Smac/DIABLO from mitochondria (O. Cuvillier, 2001). A major determinant of the type(s) of sphingolipids that are formed is whether the agonists activate only sphingomyelinase or a combination of sphingomyelinase and ceramidase with or without sphingosine kinase (as shown in Fig. 2) (A. Olivera, 1993; E. Coroneos, 1995; M.N. Nikolova-Karakashian, 1997; N. Augé, 1999).

6.2.3.1. Other bioactive lysosphingolipids. Considerable progress has been made in characterizing the occurrence and biological activities of other lysosphingolipids, but much work remains to elucidate their roles in cell regulation. For example, sphingosylphosphorylcholine (lysosphingomyelin) is a potent mitogen (T. Seufferlein, 1995), ceramide 1-phosphate is a potent calcium-mobilizing agent (S. Gijbsbers, 1999), and psychosine (lyso-GlcCer or -GalCer), which has long been known to be highly cytotoxic, is the agonist for an orphan G protein-coupled receptor, T cell death-associated gene 8 (D.S. Im, 2001).

7. *Bioactive sphingolipids appear to be at the heart of numerous aspects of cell regulation in normal and pathologic conditions*

Fig. 16 illustrates interrelationships between sphingolipid metabolism and some of the factors that can alter the amounts of key intermediates and/or products to impact cell

behavior. Beginning with the more familiar signaling paradigm:

- agonists, stress, oxidized lipoproteins and depletion of glutathione can induce sphingomyelinase activation to produce ceramide; and if ceramidase is also activated, sphingosine and/or sphingosine 1-phosphate (when sphingosine kinase is also active) (Section 6.2, and Fig. 2);
- a secreted acid sphingomyelinase hydrolyzes sphingomyelin associated with lipoproteins to form particles that are taken up by macrophages and promote foam cell formation (S. Marathe, 1998);
- hydrolysis of cellular sphingomyelin alters the structure of rafts and other membrane microdomains, as well as 'frees' cholesterol for efflux (Chapter 20), metabolism and down-regulation of de novo cholesterol synthesis (Chapter 15) [5,6];
- a number of factors can perturb sphingolipid biosynthesis by altering the amounts of the precursors (e.g., serine and palmitoyl-CoA) including inhibitors and genetic defects of mitochondrial uptake of palmitoyl-CoA (H. Vesper, 1999);
- genetic defects in serine palmitoyltransferase can, in an as yet to be elucidated way, result in sensory neuropathy (J.L. Dawkins, 2001; K. Bejaoui, 2001);
- a host of factors up-regulate de novo sphingolipid biosynthesis by transcriptional or post-translational activation of serine palmitoyltransferase and/or activation of ceramide synthase, often resulting in cell death due to elevations in pro-apoptotic intermediates (Section 5.1) [25];
- inhibition of ceramide synthase (by fumonisins) blocks complex sphingolipid formation and elevates sphinganine (to induce toxicity) and sometimes sphinganine 1-phosphate (which may induce a mitogenic response), whereas other factors (such as irradiation) can increase ceramide synthase and induce cell death (Section 3).

8. *Future directions*

Knowledge about basic 'sphingolipidology' — the physical properties of sphingolipids, sphingolipid metabolizing enzymes and targets regulated by sphingolipids — has at least doubled since the 1996 edition of this book. New ideas have also emerged about how sphingolipids are involved in disease etiology, and how naturally occurring and/or synthetic sphingolipids may be useful in disease prevention and treatment. Like other areas of biologic research, 'sphingolipidology' is rapidly evolving into 'sphingolipidomics', that is, a field where a rigorous evaluation of any one part requires a comprehensive analysis also of essentially all of the other components. For example, the 'sphingomyelin ceramide signaling pathway' was initially envisioned to involve agonist- (or stress-) induced turnover of sphingomyelin to ceramide to activate (or inhibit) intracellular target(s). As it is now understood, sphingomyelin turnover affects the organization (and intracellular trafficking) of important membrane microdomains (rafts and caveolae) and their associated receptors, transporters, and other bioactive lipids as well as the production of a cascade of products (ceramide, sphingosine and sphingosine 1-phosphate, etc.), each of which can interact with multiple intracellular (and sometimes extracellular) targets. Very often, a stimulus triggers not only sphingolipid turnover but also changes in de novo synthesis, including the formation of additional categories

of bioactive sphingolipids (such as GlcCer). Hence, to understand completely how sphingolipids regulate a given biological process, one must analyze essentially all of the sphingolipids in (and around!) the cells. It is likely that many of the seeming contradictions in the current research literature occur because each study has evaluated only one or a few of these factors, which may be analogous to the parable of the blind men trying to describe an elephant.

New technologies such as electrospray tandem mass spectrometry, DNA and protein microarrays, etc., are making ‘sphingolipidomic’ analyses feasible. Nonetheless, additional methods are still needed to evaluate temporal, localized changes in subcellular compartments, and to be able to study integrated systems such as tissues, organs, and intact organisms.

With better understanding comes greater utility, and sphingolipids are impacting almost every translational research field, from bioengineering to nutrition. It is intriguing that Thudichum’s interests included nutrition, and a century before ‘nutraceuticals’ and ‘functional foods’ were in vogue, he wrote in the preface to his last book, *Cookery. Its Art and Practice* (1895): “Physiologic deduction proves that . . . no cookery is rational which does not attain the utmost theoretically possible effect, namely, the production of the highest physiological force. . . . It is believed and hoped that the medical profession will find in this work many materials to assist them. . . .”. He surely had sphingolipids in mind.

Abbreviations

Cer	ceramide
ESI-MS/MS	electrospray tandem mass spectrometry
Fuc	fucose
Gal	galactose
G, with subscript for the subclass	ganglioside
Gb, with subscript for the number of carbohydrates	globoside
Glc	glucose
GlcA	glucuronic acid
GM2-AP	GM ₂ activator protein
Hex A or B	hexosaminidase A or B
Lac	lactose
Man	mannose
GalNAc	<i>N</i> -acetylgalactosamine
GlcNAc	<i>N</i> -acetylglucosamine
Neu5Ac	<i>N</i> -acetylneuraminic acid
Neu5Gc	<i>N</i> -glycolylneuraminic acid
Pal-CoA	palmitoyl-CoA
Ser	serine
SPT	serine palmitoyltransferase
SAP	sphingolipid activator protein

SM	sphingomyelin
SMase	sphingomyelinase
TNF α	tumor necrosis factor- α
UDP-sugar	uridine dinucleotide phosphate sugar
UV	ultraviolet

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