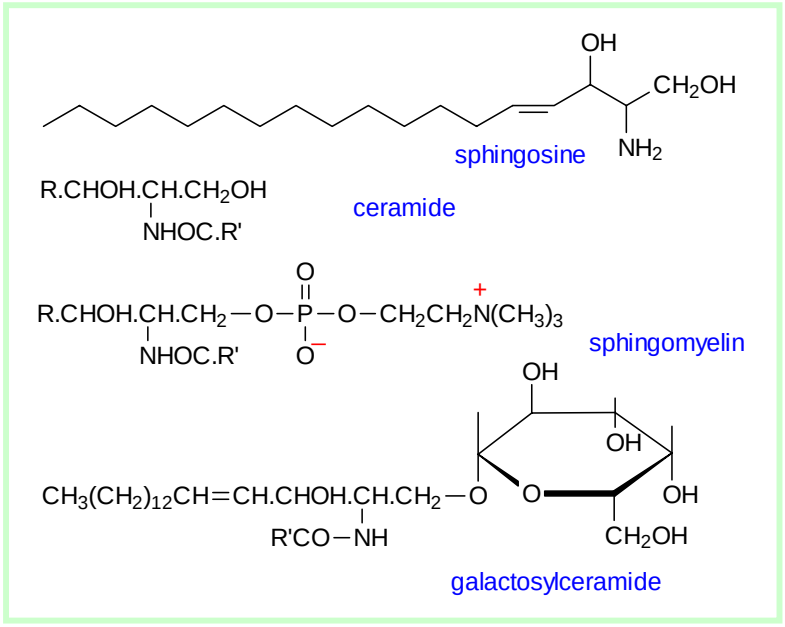
SPHINGOLIPIDS

INTRODUCTION TO SPHINGOLIPIDS AND RAFTS

1. Sphingolipids

The sphingolipids comprise a complex range of lipids in which fatty acids are linked via amide bonds to a long-chain base or sphingoid. The root term “sphingo-” was first coined by J.L.W. Thudichum in 1884 because the enigmatic nature of the molecules reminded him of the riddle of the sphinx. The term “sphingolipide” was introduced by Herbert Carter and colleagues in 1947. While they are perhaps less enigmatic than they once were, sphingolipids are extremely versatile molecules and surprises are certainly expected as new knowledge is gained of their functions in healthy and diseased animal and plant tissues. They are also found in one bacterial genus (*Sphingomonas*). The complex sphingolipids are located mainly in the plasma membrane of mammalian cells where they have a structural function, although they also serve as adhesion sites for proteins from the extracellular tissue. Similarly, they have analogous intracellular functions in all cellular compartments, including the nucleus. In addition, it has become evident that complex sphingolipids and their metabolites have important roles in signal transduction.



A **long-chain base**, such as sphingosine, is the simplest possible functional sphingolipid, but **ceramides**, which contain a fatty acid linked by an amide bond, are not only important molecules in their own right, but are the precursors of phospholipids and glycolipids with an immense range of functions in tissues. These are quite distinct from the properties of the complex glycerolipids. For example, **sphingomyelin** has structural similarities to phosphatidylcholine, but has very different physical and biological properties, while the complex oligoglycosylceramides and gangliosides have no true parallels among the glycerolipids.

In recent years, it has become apparent that sphingolipids are involved in many of the more common human diseases including diabetes, many different cancers, microbial infections, Alzheimer's disease and other neurological syndromes, and diseases of the cardiovascular and respiratory systems. Sphingolipids and their metabolism are therefore likely to prove of ever increasing interest to scientists.

2. Fatty acid Components of Sphingolipids

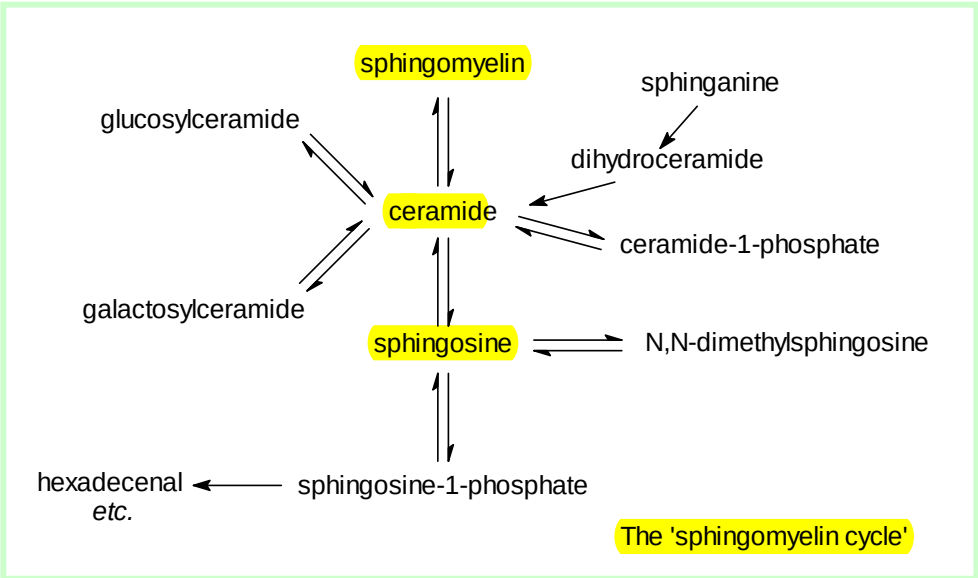
The **fatty acids** of sphingolipids are very different from those of glycerolipids, consisting of very-long-chain (up to C_{28}) odd- and even-numbered saturated or monoenoic and related 2-*D*-hydroxy components. In plants, 2-hydroxy acids predominate sometimes accompanied by small amounts of 2,3-dihydroxy acids. Polyunsaturated fatty acids are only rarely present, although sphingomyelins of testes and spermatozoa are exceptions in that contain polyunsaturated fatty acids, which are even longer in chain-length (up to 34 carbon atoms), including 28:4(n-6) and 30:5(n-6). Skin ceramides also contain unusual fatty acids, while yeast sphingolipids are distinctive in containing mainly C_{26} fatty acids.

Very-long-chain saturated and monoenoic fatty acids are produced by specific elongases, but there is only limited information on how this is coordinated with ceramide biosynthesis (see our webpage on **long-chain bases**). In plants, it seems probable that 2-hydroxyl groups are inserted into fatty acyl chains while they are linked to ceramide, as ceramide synthase does not accept hydroxy fatty acids *in vitro* at least. On the other hand, in the brain of mice, experimental evidence has been obtained that is consistent with 2-hydroxylation occurring at the fatty acid level prior to incorporation into ceramides.

Although the fatty acids are only occasionally considered in terms of the biological functions of sphingolipids, their influence is considerable, especially but not only in relation to their physical properties and function in membranes (see the comments on **rafts** below). For example, synthetic glycerolipids containing very-long-chain fatty acids (C_{26}) specifically allow growth in yeast mutants lacking sphingolipids, probably by stabilizing the proton-pumping enzyme H^+-ATPase. Similarly, ceramides containing different fatty acids can be used in highly specific ways. For example, in fungi, C_{16} or C_{18} hydroxy acids are used exclusively for synthesis of glucosylceramide, while those containing very-long-chain C_{24} and C_{26} hydroxy acids are used only for synthesis of **glycosyl inositol phosphorylceramide** anchors for proteins.

3. General Comments on Sphingolipid Metabolism

The biosynthesis and catabolism of sphingolipids involves a large number of intermediate metabolites, many of which have distinctive biological activities. In animals the relationships between these metabolites have been rationalized in terms of a 'sphingomyelin cycle'.

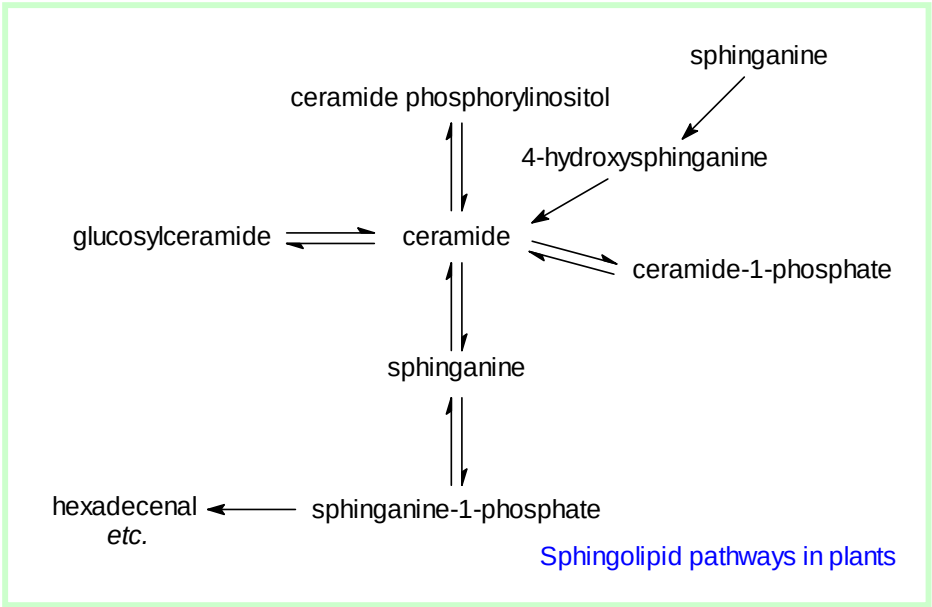


The 'sphingomyelin cycle'

Each of the various compounds in these pathways has characteristic metabolic properties, and these are discussed in more detail on the web pages on the individual compounds. Thus, free **sphingosine** and other long chain bases, which are the primary precursors of ceramides and thence of all the complex sphingolipids, function as mediators of many cellular events, for example by inhibiting the important enzyme protein kinase C. **Ceramides** are involved in cellular signalling, and especially in the regulation of apoptosis, and cell differentiation, transformation and proliferation, and most stress conditions. In contrast, **sphingosine-1-phosphate** and **ceramide-1-phosphate** promote cellular division (mitosis) as opposed to apoptosis, so that the balance between these lipids and ceramide and/or sphingosine levels in cells is critical.

Similarly, the 'structural' sphingolipids, such as **sphingomyelin**, **monoglycosylceramides**, **oligoglycosylceramides**, **gangliosides** and **sulfatides,** all have unique and characteristic biological functions, most of which are due to their physical properties and location within membranes (see below).

Metabolic pathways that are comparable to those of the sphingomyelin cycle are believed to occur in plants, although they have not been studied as extensively as those in animals (sphingomyelin itself does not occur in plants). However, sphingolipid metabolites such as sphingosine-1-phosphate have been linked to programmed cell death, signal transduction, membrane stability, host-pathogen interactions and stress responses, for example.



Sphingolipid pathways in plants

Plants also contain a unique range of complex lipids in their membranes, such as **ceramide phosphorylinositol and the phytoglycosphingolipids**, and these are now known to constitute a higher proportion of the total lipids than had hitherto been supposed. The functions of these have hardly been explored.

4. Rafts and Caveolae

It is impossible to understand the functions of sphingolipids without some understanding of their distinctive physical location within membranes. Sphingolipids are located only in the outer (exoplasmic) leaflet of the plasma membrane bilayer, while glycerophospholipids such as phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine occur only in the inner (cytoplasmic) leaflet. Cholesterol is believed to occur in roughly equal proportions in both leaflets. Further, sphingomyelin and other sphingolipids together with cholesterol are located in an intimate

association in specific sub-domains or '**rafts**' (or related structures termed '**caveolae**') of membranes. These are laterally segregated regions that form as a result of selective affinities between sphingolipids and membrane proteins, which act to compartmentalize the latter and thereby separate different biochemical functions.

The packing of cholesterol with the saturated acyl chains of sphingolipids is thermodynamically favoured over that with unsaturated acyl chains, and cholesterol is essential to the process of raft formation. If either the sphingolipid or cholesterol is depleted by any means, the other follows and *vice versa*. Indeed, there is evidence that in animal tissues sphingomyelin regulates the capacity of membranes to absorb cholesterol and thereby controls its flux between the plasma membrane and regulatory pathways in the endoplasmic reticulum.

A formal definition of rafts has been proposed, i.e.

> "Membrane rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions."

Earlier definitions have tended to depend on the methods used experimentally to isolate raft preparations and especially their resistance to non-ionic detergents, i.e. their insolubility in cold 1% Triton X-100, for example. They have then been described as detergent-resistant membranes or 'DRM'. This has resulted in much confusion and controversy in the literature. DRM certainly contain raft material, but it is considered that they should not be treated as rafts *per se*.

As much as 50% of the plasma membrane may consist of such rafts. As sphingolipids containing long, largely saturated acyl chains, they pack more tightly together, thus giving sphingolipids much higher melting temperatures than membrane glycerophospholipids. This tight acyl chain packing is essential for raft lipid organization, since the differential packing facility of sphingolipids and cholesterol in comparison with glycerophospholipids is believed to lead to phase separation in the membrane, giving rise to sphingolipid-rich regions ('liquid-ordered' phase) surrounded by glycerophospholipid-rich domains ('liquid-disordered' phase). This ordering is responsible for the resistance to attack by detergents. As these rafts are relatively small (approximately 50 nm diameter and containing roughly 3000 sphingomyelin molecules) and mobile, they are not easy to study by microscopic methods. They are believed to be thicker than normal membranes (46 versus 40 angstroms). Sphingolipids tend to have more free hydroxyl groups, both in the long-chain bases and fatty acid components than glycerolipids, and these enter into hydrogen bonding and contribute to the stability of rafts. The presence of very-long-chain fatty acid components (e.g. C_{26}) is believed to be essential. Cholesterol interacts particularly strongly with sphingomyelin, and much less with glycosphingolipids.

Membrane proteins are believed to play an important part in raft formation, and an important result of the process is that rafts contain many different proteins, including glycerophosphoinositol(GPI)-anchored proteins and tyrosine receptor kinases. These provide much of the important biological properties of rafts, and are also essential to maintain their stability. Thus, the interplay of lipid-based raft units together with protein-mediated assembly of specific protein complexes generates functional domains with high biological activity in cell membranes. From comparisons of membrane solubility in different detergents, it appears that there may exist subsets of membrane raft domains, which differ in their molecular compositions. In particular, they may contain distinct ganglioside species or glycosyl phosphatidylinositol (not a sphingolipid, of course) anchoring specific proteins.

Those subdomains in the plasma membrane related to rafts and termed '**caveolae**' lack glycosyl phosphatidylinositol-anchored proteins and are stabilized by particular membrane-spanning proteins, the caveolins, a family of palmitoylated hairpin-like proteins, which organize flask-shaped invaginations in membranes. In addition to the caveolins, caveolae are known to contain some

specific proteins not present in other raft microdomains. Other than glycosyl phosphatidylinositol, the lipid composition of caveolae is similar to that of rafts in general in that they contain appreciable amounts of sphingolipids and cholesterol. However, the gangliosides GM_1 and to some extent GM_3 appear to be concentrated in caveolae.

It is believed that there may be different classes of caveolae with different metabolic functions. Caveolae appear to have a role in controlling the level of free cholesterol in cells, and thence may affect signalling processes. They are especially abundant in adipocytes where they may regulate the flux of fatty acids across the plasma membrane. Also in adipocytes, insulin is the main hormone that affects metabolism, and the receptor at the plasma membrane is located in caveolae with possible implications for diabetes, obesity and other metabolic disorders. In addition, caveolae function as an important route by which nutrients, such as folate and glucose, are able to cross the plasma membrane, although the mechanism is still obscure.

It should be recognized that lipid rafts in general are dynamic structures, which can be formed or undergo compositional changes during signalling events and are short-lived (milliseconds or less). There may also be some form of cross-talk between different raft populations which can coalesce during activity. Thus in resting cells, sphingolipids may exist in small and highly dynamic domains, which on stimulation can stabilize and grow; in the process, they initiate biochemical reactions by promoting interactions between proteins.

Lipid rafts are believed to modulate signalling events in a number of different ways according to the composition of the specific subpopulations. Thus, the location of signalling molecules within one such micro-domain might in itself be a control mechanism, as a protein activated by phosphorylation within the raft and might be prevented from interacting with an inactivating phosphatase in another region of the membrane, for example. By concentrating all of the components of particular signalling pathways within one domain, lipid rafts could promote signalling in response to stimuli, while movement of signalling molecules in and out of the raft could control whether cells are able to respond to stimuli. Similarly, communication between different signalling pathways could be simplified if the relevant molecules were concentrated in the same lipid raft. In contrast, rafts might also regulate signals in a negative manner by sequestering signalling molecules in an inactive state. The physical properties of rafts may be key factors in these interactions. Thus, in response to receptor activation or other stimuli, sphingolipid compositions in rafts may be altered with effects on membrane architecture or morphology producing further downstream events.

Raft formation appears to be particularly important to the activity of T cells, i.e. lymphocytes derived from the thymus gland that are intimately involved in antibody production. Lipid rafts are a key element of membrane organization that appear to be crucial for the initiation of T cell signalling by enabling efficient interaction between antigens and receptors.

There is evidence that certain pathogens activate the acid sphingomyelinase that releases ceramide in membrane rafts transforming them into larger units. These can mediate the internalization of bacteria, viruses and parasites into host cells, to initiate programmed cell death (apoptosis) and release signalling molecules. They may also assist in the budding of viruses from infected cells. In effect, rafts and caveolae re-organize the receptor and intracellular signalling molecules in the cell membrane and enable the interaction of pathogens with cells. The lipids of viruses are derived from the host membranes, and for example, it has recently been demonstrated that the lipids of the HIV virus are enriched in sphingolipids that appear to be derived very specifically from rafts. Further, these lipids are essential for the infectivity of the virus. In contrast, rafts can assist cells to defeat infection, by activation of transcription factors and the release of cytokines.

Specific micro-domains or rafts that are enriched in the characteristic plant sterols, sterol glucosides and sphingolipids such as glucosylceramide have also been detected in the plasma

membrane and Golgi apparatus of plant cells. These contain distinct sets of proteins, including those anchored by glycosyl phosphatidylinositol or glycosyl inositol phosphorylceramide. As in animal cells, such rafts may assist in positioning proteins in specific regions of the cell where they can function in development and signalling. Similarly, micro-domains enriched in ergosterol and ceramides have been found in peroxisomal membranes of yeasts.

The problem of the actual size and lifetime of membrane rafts is not easily addressed. While a great deal of attention has been focused on rafts in the outer leaflet of the plasma membrane, little is known of the structural organization and properties of the corresponding inner leaflet or how the two layers interact.

A corollary of the existence of rafts, which are rich in cholesterol and with low levels of polyunsaturated fatty acids, is that microdomains must also exist that are depleted in cholesterol and enriched in polyunsaturated fatty acids. Indeed, the rigid structure of cholesterol and the highly flexible chains of docosahexaenoic acid, for example, are incompatible and promote the lateral segregation of membranes into rafts. Microdomains that are PUFA-rich/cholesterol-poor are technically less easy to study than rafts, but these may also contain distinctive proteins and have important biological functions.

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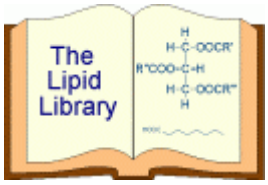

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