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Taming the Sphinx: Mechanisms of Cellular Sphingolipid Homeostasis

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Abstract

Sphingolipids are important structural membrane components of eukaryotic cells, and potent signaling molecules. As such, their levels must be maintained to optimize cellular functions in different cellular membranes. Here, we review the current knowledge of homeostatic sphingolipid regulation. We describe recent studies in *Saccharomyces cerevisiae* that have provided insights into how cells sense changes in sphingolipid levels in the plasma membrane and acutely regulate sphingolipid biosynthesis by altering signaling pathways. We also discuss how cellular trafficking has emerged as an important determinant of sphingolipid homeostasis. Finally, we highlight areas where work is still needed to elucidate the mechanisms of sphingolipid regulation and the physiological functions of such regulatory networks, especially in mammalian cells.

Introduction

Cellular membranes serve as essential barriers, either to separate the internal milieu from the extracellular environment or to compartmentalize the cytoplasm into organelles with different compositions and functions. In eukaryotes, membranes are composed primarily of lipids from three classes: glycerolipids, sterols, and sphingolipids. The lipid composition of membranes varies greatly in different organelles (1). Precise regulation of that composition is essential for proper growth, division, and responses to environmental stimuli to maintain cellular homeostasis. These regulatory mechanisms insure proper levels and distributions of the structurally and functionally diverse lipids that make up biological membranes.

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In this review, we focus on the regulation of sphingolipids. Sphingolipids were first identified in the 19th century as major constituents of brain tissue by Johann Ludwig Wilhelm Thudichum, who named them after the sphinx due to their enigmatic nature at the time (2). Subsequently, they have been found to be universal components of membranes in eukaryotes and also some bacteria (3).

Sphingolipids are synthesized in the endoplasmic reticulum (ER) and the Golgi apparatus membranes and are transported to the plasma membrane, where they are enriched in the outer leaflet and constitute 10–20% of the membrane lipids (1,4–6). In plasma membranes, sphingolipids have been proposed to form complexes with sterols, where they might form membrane nano-domains that may organize proteins for cell signaling (7–9). Sphingolipids are also important components of vesicles that traffic proteins to and from the plasma membrane (e.g., from the trans-Golgi network; (10,11). In addition, numerous intermediates and derivatives of sphingolipid metabolism, such as sphingosine-1-phosphate (S1P), ceramides, and some complex sphingolipids function as signaling molecules both within and between cells (12,13). Some sphingolipids, such as those produced by *Bacteroides* species in the human gut, mediate specific microbe-host interactions and may influence the host's immune system (14).

The diverse and distinct functions of sphingolipids require that cells tightly control their abundance in different compartments. We are only just beginning to understand the cellular pathways that regulate sphingolipid metabolism, but important paradigms are emerging. Here, we summarize pathways of sphingolipid biosynthesis and metabolism and review these new insights. Because much of the knowledge on sphingolipid homeostasis has been generated in yeast, we will focus on studies that have used this model system, but provide references to mammalian studies where data are available.

Sphingolipid Biosynthesis

De novo sphingolipid synthesis begins on the cytosolic face of the ER, where the two building blocks of sphingolipids, sphingosines, also known as long-chain bases (LCBs) and long chain fatty acids (LCFAs) and very long-chain fatty acids (VLCFAs), are synthesized (Fig. 1).

To generate sphingosines, serine palmitoyltransferase (SPT) catalyzes the rate-limiting condensation of the non-essential amino acid serine and fatty acyl Coenzyme A (CoA) (15–18). The SPT enzyme comprises a core membrane protein heterodimer in eukaryotes (Lcb1 and Lcb2 in yeast) and a number of smaller subunits required for maximal activity (Tsc3 in yeast; ssSPTa and ssSPTb in mammals; (19,20). Mutations in the human genes encoding core SPT subunits lead to hereditary sensory and autonomic neuropathy type 1 (HSAN1). HSAN1 is caused by an altered amino acid selectivity of the enzymes that leads to an accumulation of neurotoxic deoxysphingolipids (21–23). The product of the SPT reaction, 3-ketosphinganine, is reduced to form LCBs in the next step.

The other fatty acid moiety in sphingolipids is a LCFA or VLCFA. Also at the ER, a family of elongase enzymes extend long-chain fatty acids (C12-C16) to VLCFAs with 18 or more

carbons (24). *Saccharomyces cerevisiae* has three elongases (*ELO1-3),* but mammals have seven (*ELOVL1-7,* (24). The rate-limiting condensation reaction for fatty acid elongation is performed by isoenzymes producing fatty acyl CoAs of specific length (25). The length of the VLCFA product is determined by a molecular caliper mechanism and is based on the distance between the elongase active site and a lysine residue facing the cytosol (26). Sphingolipids in budding yeast contain primarily VLCFAs of 26 carbons, although LCFAs and VLCFAs of shorter length are also utilized. Sphingolipids in mammals have LCFAs and VLCFAs with more variation (24). VLCFAs are used primarily for sphingolipid synthesis, and it is thought that their length is essential for much of the sphingolipid function. Consistent with this notion, yeast cells lacking sphingolipids are viable when they incorporate VLCFAs into phosphatidylinositol lipids, which normally do not contain VLCFAs (27,28).

The two initial branches of sphingolipid synthesis, generating LCBs and VLCFAs, converge in an N-acylation reaction, catalyzed by ER-localized ceramide synthases, to produce ceramides. There are six ceramide synthases in mammals, and two in yeast (29,30). The products of this reaction are rich in complexity owing to variations in VLCFA length and hydroxylation and desaturation of the LCB and VLCFA components (31).

After ceramide synthesis, the remaining steps in sphingolipid synthesis occur in the Golgi apparatus. Ceramides are transported to the Golgi via vesicular (via COP-II vesicles) and non-vesicular transport (32,33). The latter mechanism has, so far, only been reported for mammals, in which ceramide transport occurs via a ceramide transport protein (CERT; (34)).

In the Golgi apparatus, a variety of head groups are added to ceramide to form complex sphingolipids. These reactions occur on the inner leaflet of the membrane, and the addition of head groups is thought to trap sphingolipids on that side of the membrane due to the slow trans-membrane flip-flop of lipids with hydrophilic head groups. The type of head groups in complex sphingolipids varies considerably between species. Yeast utilize phosphate groups and inositol and mannose sugars to produce three classes of complex sphingolipids: inositol phosphorylceramides (IPC), mannosylinositol phosphorylceramides, and mannosyldiinositol phosphorylceramides (18). Mammalian cells utilize a wider variety of molecules, including diverse sugars and choline, to produce a much more complex array of sphingolipid head groups. These include the phosphocholine headgroup to yield sphingomyelin, and numerous glucose- and galactose-based headgroups to generate complex glycosphingolipids, such as sulfatides, cerebrosides, and gangliosides (31,35).

After their assembly in the Golgi apparatus, complex sphingolipids are transported to the plasma membrane. In mammals, the intra-Golgi transport of glucosylceramide can be mediated by four-phosphate adapter protein 2 (FAPP2) for the production of globo-series sphingolipids (36,37). Evidence from mammalian cells and yeast suggests that trafficking of sphingolipids from the Golgi apparatus to the plasma membrane occurs in vesicles that are enriched in specific proteins and distinct from other vesicles (38,39). Such sorting of cargo proteins into sphingolipid-rich and other carrier vesicles may be important for apical versus

basolateral protein sorting in polarized cells, but the mechanisms of how different Golgi-derived vesicles reach different destinations is unclear.

Sphingolipid Degradation

For each step in the biosynthesis of sphingolipids, the reverse reaction can occur and is catalyzed by different enzymes. Whereas sphingolipid biosynthesis occurs in membranes of the secretory pathway, sphingolipid catabolism occurs primarily in membranes of the endosome-lysosomal compartments. The mechanisms of sphingolipid degradation in the yeast vacuole are unclear. Hydrolytic enzymes responsible for sphingolipid degradation have been localized to ER, Golgi, and mitochondria and include the mammalian homologue of neutral sphingomyelinase, the ceramidases Ydc1, and Ypc1, and the dihydrosphingosine phosphate lyase Dpl1. In mammals, complex sphingolipids are degraded in lysosomes by enzymes, such as acid sphingomyelinase, which recognize specific sphingolipid species (40). The ceramide products are then hydrolyzed by ceramidases to yield to LCBs, such as sphingosine and VLCFAs. LCBs, in turn, can be phosphorylated and degraded to yield an aldehyde, which is processed to a fatty acid (41–43). This pathway provides the only known process for complete degradation of sphingolipids. The breakdown of sphingolipids also occurs in subcellular locations other than the endo-lysosome, such as at the plasma membrane, where specific neutral sphinogmyelinases generate bioactive signaling molecules, such as ceramide (44).

Cells Control Sphingolipid Levels

Sphingolipids act both as structural components of membranes and as potent signaling molecules. So, it is not surprising that alterations in levels of different sphingolipids are linked to cellular dysfunction and human disease. For instance, changes in sphingolipid abundance are associated with common pathologies, including asthma, insulin resistance and diabetes, cancer, atherosclerosis, and neuro-degenerative diseases (45–48). Thus, cells appear to maintain sphingolipid homeostasis to ensure normal cellular function and physiology.

We are only beginning to understand how sphingolipid homeostasis is achieved in cells. In yeast, the total sphingolipid content is relatively constant throughout all growth phases (49). However, the relative amounts of specific sphingolipids do vary according to growth phase, temperature, and carbon sources (50). Moreover, specific growth conditions appear to require up- or down-regulation of total sphingolipid levels. For example, environmental changes, such as shifts in temperature and osmolarity, result in sphingolipid synthesis changes in yeast. Heat shock transiently increases the *de novo* synthesis of sphingolipid dramatically (51,52). In mammals, specific needs also demand changes in sphingolipid regulation. For example, specific stages of development, such as myelin sheath formation, need massive increases sphingomyelin synthesis by oligodendrocytes.

Given the importance of sphingolipid homeostasis, how do cells regulate sphingolipid levels? As a general principle, it appears that cells monitor the abundance of sphingolipids (e.g., in the plasma membrane) and transmit signals to the biosynthetic machinery in the ER

to initiate the appropriate response in sphingolipid biosynthesis. In this review, we focus on the molecular aspects of how sphingolipids levels are sensed at the plasma membrane, how cells regulate sphingolipid biosynthesis, and how membrane trafficking maintains proper sphingolipid homeostasis.

How are sphingolipids sensed at the plasma membrane?

Information on the mechanisms of cellular sphingolipid sensing in the plasma membrane is limited. Most directly, cells could sense plasma membrane sphingolipid levels by monitoring the presence of specific lipids (e.g., sphingolipids themselves or sphingolipid derivatives) that bind directly to sensing domains of proteins. A number of proteins bind sphingolipids, including enzymes of sphingolipid metabolism, sphingolipid transport proteins (e.g., CERT, FAPP2, ceramide-1-phosphate transport protein), and effector proteins that bind to bioactive sphingolipids, such as the S1P receptors (34, D'Angelo, 2007 #97,53,54). In addition, a sphingolipid binding motif (VXXTLXXIY) has been identified in the transmembrane domain of p24, a component of the COP-I machinery, which results in a highly specific interaction of p24 with sphingomyelin (55). Recent bioinformatic analysis of mammalian proteomes has uncovered multiple membrane proteins containing predicted sphingolipid binding motifs, including many G-protein-coupled receptors (56). These findings suggest that there are many more sphingolipid binding proteins yet to be discovered. However, to date, no sphingolipid sensors that affect homeostasis have been identified in this class of molecules.

Cells could also detect changes in sphingolipid levels in the plasma membrane indirectly by responding to changes of physical membrane properties, such as fluidity, thickness, or curvature due to changes of relative sphingolipid levels. Considerably more evidence exists for this type of regulation. For example, yeast cells appear to respond to membrane stretching or gradual depletion of plasma membrane sphingolipid by up-regulating sphingolipid biosynthesis (57,58). Lateral organization of the plasma membrane into domains of distinct protein and lipid composition is important for initiating signaling after changes of sphingolipid levels. For instance, when sphingolipid levels fall in yeast, Slm1/2 proteins re-localize within the plane of the membrane. At steady state, Slm1/2 proteins colocalize predominantly with static plasma membrane domains (known as membrane compartment containing Can1, or MCC; (59,60), organized by large protein complexes known as eisosomes (61–63). These domains are distinct from areas of the plasma membrane (known as membrane compartment containing TORC2, or MCT) where the kinase complex TORC2 localizes (64). When membrane sphingolipid levels fall, Slm1/2 proteins re-localize from the MCC/eisosome domain to associate with TORC2. This, in turn, triggers downstream signaling through Ypk-kinases to up-regulate sphingolipid synthesis (58,65–69). How the release of Slm1/2 from eisosomes is triggered is unknown. However, Slm1/2 proteins contain Bin1-Amphiphysin-Rvs161 (BAR) domains (63), which bind curved membranes, and MCC/eisosomes are curve furrows in the plasma membrane (63). An attractive model is that, upon sphingolipid depletion or membrane stretching, MCC/eisosome furrows flatten, thereby releasing Slm1/2 proteins. Because Slm1/2 also contain a pleckstrin homology domain for binding to phosphoinositol lipids, they may remain

associated with the plasma membrane and become available for TORC2 binding and activation.

Plasma membrane organization may be more broadly involved in sphingolipid regulation. When sphingolipid levels fall, the transmembrane protein Nce102 also relocalizes from MCC/eisosome domains to the surrounding membrane, and this change is associated with increased activity of Pkh1/2-kinases (homologues of mammalian Sgk1) that localize to MCC/eisosomes (70). However, deletion of Nce102 is not sufficient to alter sphingolipid levels (69). Downstream, Pkh1/2 kinases phosphorylate Ypk1/2 kinases at sites different from those targeted by TORC2. Both sites are required for full Ypk1/2 activation (68). Thus, a current model suggests that two signaling branches initiate new synthesis by re-localizing plasma membrane proteins Slm1/2 and Nce102, respectively, in response to changes of membrane sphingolipid levels to synergistically control sphingolipid biosynthesis.

In addition to the kinase network associated with eisosomes, signaling from the small GTPase Rho1 begins at the plasma membrane to regulate sphingolipid synthesis. In this case, signaling occurs through a kinase cascade known as the cell wall integrity pathway in yeast (71). Originally, a large-scale genetic interaction map identified the Rho1 guanidine nucleotide exchange factor Rom2 as a potential regulator of sphingolipid metabolism (72). It subsequently became clear that Rom2 acts by regulating phosphorylation of the Elo2 elongase. Elo2 phosphorylation is decreased when sphingolipid levels fall, which increases VLCFA synthesis (73). How Rho1 signaling is initiated is unknown, but it involves two potential transmembrane sensor proteins, Wsc1 and Wsc2 (73)

The principles of membrane organization and stretch might also be responsible for triggering signaling in mammalian cells. Although mammalian cells do not have eisosomes per se, MCC/eisosome domains share many conceptual similarities, such as static appearance and a lipid composition high in sterols at local invaginations, with caveolae of mammalian cells. Caveolae are important for plasma membrane organization, cholesterol transport and are primary responders to plasma membrane stretch (74–77), but it is not clear if mammalian cells use kinase systems originating at caveolae to control sphingolipid metabolism.

How do cells adjust sphingolipid synthesis?

Recent studies in yeast have shed light on the regulation of sphingolipid synthesis in the ER. Our knowledge of this regulation is incomplete and many details, including the relative contributions of different signaling pathways, remain to be elucidated.

An overview of the regulation of enzymes of sphingolipid biosynthesis is shown in Figure 2. A major discovery for the field was the identification of Orm1/2 as central regulators of sphingolipid metabolism (67). Ypk1/2 kinases phosphorylate the N-terminus of Orm1/2-proteins once activated by Pkh1/2 and TORC2 kinases (58,65–69,78). Orm1/2 proteins are in complex with and inhibit SPT. When phosphorylated, Orm1/2 proteins dissociate from SPT, leading to SPT activation and increased LCB synthesis (67,78). Because SPT catalyzes the rate-limiting step of sphingolipid synthesis, this action is predicted to increase overall flux through the sphingolipid pathway. A few mysteries of Orm1/2 regulation remain. For

example, the Orm1/2 complex also contains the phosphoinositide phosphatase Sac1 (67), suggesting an opportunity for cross-regulation between sphingolipid and phosphoinositide levels.

Ypk1/2 activity in this network is further adjusted by the Fpk1/2 kinases in a negative feedback loop, in which Ypk1/2 and Fpk1/2 phosphorylate each other (79). As both Ypk1/2 and Fpk1/2 have other targets, such as phospholipid flippases, cells might use this system to more broadly adjust plasma membrane composition according to need.

Among targets of the Ypk1/2 kinases, ceramide synthase stands out in the context of sphingolipid regulation (80). Sphingolipid depletion and shock stress increase Ypk1-dependent phosphorylation of the Lag1/Lac1 components of yeast ceramide synthase to increase ceramide production by an unclear mechanism.

This phosphorylation and possibly others are counteracted by the calcium-dependent phosphatase calcineurin. Specifically, calcineurin appears to counteract TORC2-Ypk-dependent regulation of sphingolipids (66). At least one mechanism for this effect is due to the downregulation of ceramide biosynthesis by dephosphorylation of yeast ceramide synthase Lag1/Lac1 by calcineurin (80). How this signaling is integrated during the response to changes in sphingolipid levels is not known. Further support for a link between Ca++ signaling and sphingolipid homeostasis is provided by calcium sensitivity of mutants in sphingolipid synthesis genes, such as Csg2 (81).

An increase in sphingolipid biosynthesis requires increased levels of VLCFAs. To provide these, Rom2-dependent signaling from the plasma membrane regulates the activity of Elo2 (73), the fatty acid elongase controlling VLCFA levels in yeast (82). In this case, depletion of sphingolipids leads to down-regulation of Rom2-signaling and de-phosphorylation of Elo2, which in turn increases VLCFA synthesis. How the signal is transduced from Rom2 to Elo2 is not well understood, but appears to involve only the initial kinases of the cell wall integrity pathway. It is not clear which kinases transduce the signal from this pathway to phosphorylate Elo2, but the GSK3 kinase Mck1 is required (73,83). Since mutants in downstream components of the pathway are nonetheless sensitive to sphingolipid synthesis inhibition, the pathway might have targets other than Elo2 that are important for sphingolipid homeostasis (73).

The available data suggest that at least two signaling branches, initiated by TORC2/Pkh1/2 and Rom2 signaling at the plasma membrane, act in parallel to regulate LCB and VLCFA synthesis in the ER. Consistent with this notion of two parallel, independent but convergent pathways, activation of Rho1, the target of Rom2, suppresses *tor2* mutations (84), and TORC2 inhibition leads to activation of the cell wall integrity pathway (57).

Signaling from organelles other than the plasma membrane may also contribute to sphingolipid homeostasis. For example, signaling by TORC1, which localizes to the yeast vacuole (64), may regulate sphingolipid biosynthesis. TORC1 inhibition of PP2A is suggested to regulate Npr1, a protein kinase implicated in phosphorylation of Orm1/2 (85). However, Npr1-dependent phosphorylation of Orm1/2 occurs at sites different than those responsive to active Ypk1/2 kinases. The Npr1 target sites on Orm1/2 do not appear to affect

SPT activity and LCB synthesis, but may affect downstream metabolism modulating ceramide or complex sphingolipid synthesis steps (85). However, how this may occur mechanistically is unclear.

Other mechanisms of regulating sphingolipid levels

In addition to signaling originating from the plasma membrane, sphingolipid metabolism intermediates might directly bind to and affect kinase and phosphatase activities that regulate sphingolipid synthesis. An example for such regulation is the response to heat stress in yeast. During an acute heat shock, both LCB and ceramide levels increase dramatically (51,52), as does Orm1/2 phosphorylation (86). This leads to a peak of LCB levels, after which Orm1/2-phosphorylation decreases (86), while ceramide levels reach their maximum approximately 10 minutes later during heat stress (51,52). Interestingly, no obvious changes in the levels of complex sphingolipids were observed within this time frame (52). This suggests that, during heat stress, changes of Orm1/2 phosphorylation might be due to changes in LCBs and/or ceramide levels. In support of this possibility, exogenously added LCBs induce Orm1/2 dephosphorylation without being converted to ceramide or to complex sphingolipids (86). Orm1/2 dephosphorylation may be mediated by regulation of the Cdc55-PP2A phosphatase, which was identified as a candidate for ceramide-activated protein phosphatase in yeast (87) and as a downstream target of C18:1-phytoceramide (88). These data suggest the hypothesis that the transient increase in LCB and ceramide levels during heat stress activates PP2A activity.

Another pathway that may directly respond to changes in sphingolipid metabolism intermediates is initiated by casein kinase 2 (CK2), a Ser/Thr protein kinase with roles in cell growth and proliferation. CK2 may directly phosphorylate ceramide synthase, since CK2 deletion mutants show increased levels of LCBs and decreased levels of ceramides (89). Moreover, a C-terminal phosphorylation of ceramide synthase subunits Lag1 and Lac1, essential for activity and ER-localization, depends on CK2 (90). CK2 localizes to many cellular compartments, including the ER. If CK2-dependent signaling is part of a homeostatic response to sphingolipid level changes, it will be important to determine where the signal originates.

Among sphingolipid metabolism intermediates, ceramide is a particularly potent signaling molecule. Sphingomyelin synthase-related enzyme (SMSr) might be a sensor for ceramides in the ER during sphingomyelin biosynthesis, and it might prevent cells from ceramide-induced mitochondrial apoptosis (91,92). In addition, ceramide also engages multiple downstream effectors of mammalian cells (93), including AKT, ceramide-activated protein kinase, ceramide-activated protein phosphatase, and protein kinase Cζ (93). It is unknown if any of these kinases or phosphatases regulate sphingolipid biosynthesis, analogous to the yeast AKT homologues Ypk1/2.

Membrane trafficking and regulation of sphingolipid levels

Although sphingolipids are enriched in the plasma membrane, they also have important functions in other organelles, such as the Golgi apparatus. Moreover, changes in the levels of

membrane sphingolipids could change membrane properties and potentially interfere with organelle function. Thus, sphingolipid levels must be maintained at set levels for different organelles to ensure normal cell physiology. This necessitates the controlled trafficking of sphingolipids between different cellular membranes (Fig. 3). In principle, sphingolipid trafficking can occur in vesicles or by non-vesicular means.

Non-vesicular trafficking is best characterized in mammalian cells, where CERT mediates trafficking of ceramide from the ER to the Golgi apparatus (34). Since the enzymes mediating the conversion of ceramide to complex sphingolipids are localized in the Golgi apparatus, this trafficking step is an important part of sphingolipid biosynthesis. In agreement with this, mice lacking functional CERT are embryonically lethal (e11.5) with ceramide accumulation, as well as degeneration of mitochondria and ER in different tissues (94). Interpretation of this phenotype is somewhat complicated by findings that another splice form of the gene encoding CERT appears to function as extracellular binding collagen, known in this context as Goodpasteur antigen binding protein (95,96).

Similarly to CERT, FAPP2 and ceramide-1-phosphate transport protein transport glucosylceramide and ceramide-1-phosphate within the Golgi apparatus and between the Golgi apparatus and the plasma membrane, respectively (36,37,53). Intriguingly, CERT is phosphorylated at multiple sites, indicating it might be regulated. Indeed, phosphorylation of CERT by PKD and casein kinase 1γ2 lower CERT activity and, as a consequence, reduce sphingomyelin synthesis (97,98).

Analogous non-vesicular transport routes for sphingolipid intermediates in the biosynthetic pathway have not been identified in yeast. Instead, their transport appears to rely more on vesicular trafficking (32). Such trafficking is mediated in part by the COPII and COPI pathways between ER and Golgi apparatus (32). Evidence suggests that these pathways are regulated by homologues of the oxysterol-binding homologous proteins (33), but the exact mechanism is unknown. At the Golgi apparatus, sphingolipids sort into vesicular carriers with distinct protein composition (11,99). How this sorting occurs is also unknown.

Membrane trafficking from the plasma membrane has a key role in sphingolipid homeostasis. Sphingolipids are internalized from the plasma membrane by endocytosis, which is coupled to their recycling and degradation. Sphingolipids are crucial for endocytosis, and in fact, initial screens for endocytosis turned up mutants in sphingolipid metabolism (100). Whether this requirement for sphingolipids in endocytosis primarily reflects their role in maintaining plasma membrane properties permissive for endocytic vesicle formation and/or is due to their signaling functions is unclear (57).

Once internalized, sphingolipids are either recycled from endosomes to the Golgi apparatus and the plasma membrane or routed to the lysosome/vacuole for degradation. Recycling in general is mediated by retromer and the Golgi-associated retrograde protein (GARP) trafficking complexes on endosomes and the Golgi membranes, respectively. Mutants in GARP appear to re-route sphingolipids to the vacuole, where they are degraded. Lack of recycling decreases sphingolipid levels in the plasma membrane, triggering up-regulation of the sphingolipid synthesis machinery (101). The current model posits that the massive mis-

sorting of sphingolipids from the plasma membrane to the vacuole/lysosome apparently overwhelms the degradation recycling machinery, leading to defects in cell growth in yeast. Consistent with the idea that vesicular trafficking is essential to maintain sphingolipids in the right compartments, retrograde endosome to Golgi recycling pathways become essential when complex sphingolipid synthesis is inhibited by Aur1 repression (102). How wild-type cells determine which fraction of sphingolipids should be recycled and which fraction should be degraded is unknown.

Intriguingly, in mice and humans, deficiencies in GARP are associated with severe neurological pathology. In humans, hypomorphic alleles of the Vps53 GARP complex subunit result in a rare, severe neurodegenerative disease, progressive cerebello-cerebral atrophy type 2 (PCCA2; (103)). In addition, mutations in retromer subunits cause Alzheimer's disease, and mutations in CHMP2b, a subunit of ESCRTIII required for lysosome trafficking, are associated with the development of ALS and frontotemporal dementia (104). Moreover, deficiencies in enzymes of sphingolipid degradation pathways lead to neurological disorders: mutation of B-hexosaminidase α-subunit *HEXA* leads to Tay-Sachs disease; deficiency in the B-hexosaminidase β subunit *HEXB* to Sandoff diseases; defects in β-galactosidase *GLB1* in GM1 gangliosidosis lead to storage defects associated with neurodegeneration (105). These findings suggest that lysosomal dysfunction associated with defects in sorting or turnover of sphingolipids is a common feature of a set of neurodegenerative diseases.

Outlook and Unsolved Mysteries

A clearer picture of sphingolipid regulation is emerging and prompting numerous important questions. Regarding the molecular mechanisms that regulate sphingolipid biosynthesis, a number of enzymes and key regulators, such as the Orm1/2 proteins, appear to be controlled by phosphorylation. We do not know how phosphorylation affects the activity, localization, or stability of any of these factors. Moreover, at least for Orm1/2, we know that their regulatory function is conserved in mammals (106), but their phosphorylation sites are not. Thus, phosphorylation may have evolved as an added complexity to modulate the primary, conserved inhibitory function of SPT.

Other major questions are whether and how sphingolipid degradation is regulated. TORC1-Sch9 signaling appears to repress transcription of the yeast ceramidases *YDC1* and *YPC1* (107). In addition, Sch9 affects the activity of the yeast neutral sphingomyelinase homologue Isc1. This might be due to regulating the relocalization of Isc1 from ER to mitochondria, which is necessary for the enzyme's function (107), yet detailed understanding of these processes is lacking. Since most of the available chemical inhibitors block steps in sphingolipid synthesis, it has been much easier to determine the cellular response to decreased sphingolipid synthesis than sphingolipid excess. It is unknown whether increased sphingolipid levels trigger homeostatic responses, but it seems likely.

We also know very little about the physiological role of sphingolipid homeostatic control. In part, this is due to our lack of knowledge of regulatory mechanisms in mammals. However, already interesting and important findings are emerging. Variants of the human homologue

of Orm1/2, ORMDL3, are a risk factor for developing asthma (108), and ORMDL3 expression appears to be up-regulated in asthma (109,110). In addition, data implicate sphingolipid intermediates, such as ceramides, as important mediators of physiology and pathology, including mediating lipotoxic effects in the development of type 2 diabetes (111) and heart disease (112). Intriguingly, the receptor for adiponectin, mediating many effects of this potent adipokines, might itself be a ceramidase (113). Ceramide is also an important component of the response to cancer therapeutics (44). Whether and how in these cases cells respond to changes in sphingolipid metabolites are not yet clear. Likewise, how mammalian cells maintain adequate ratios of complex sphingolipids with other lipids in the plasma membrane is an unexplored frontier.

As seen from this brief review, we have begun to tame the sphinx, with an increased understanding of the regulation of sphingolipid metabolism. However, the feral sphinx remains at large and enigmatic, challenging us with ongoing riddles of sphingolipid metabolism.

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References

1. Sankaram MB, Thompson TE. Modulation of phospholipid acyl chain order by cholesterol. A solid-state 2H nuclear magnetic resonance study. Biochemistry. 1990; 29:10676–10684. [PubMed: 2271675]
2. JLWT. Based Throughout upon original researches. London: London, Bailliere, Tindall, and Cox; 1884. A Treatise on the Chemical Constitution of the Brain.
3. Ingar Olsen EJ. Sphingolipids in bacteria and fungi. Anaerobe. 2001; 07:103–112.
4. Thewalt J, Kitson N, Araujo C, MacKay A, Bloom M. Models of stratum corneum intercellular membranes: the sphingolipid headgroup is a determinant of phase behavior in mixed lipid dispersions. Biochem Biophys Res Commun. 1992; 188:1247–1252. [PubMed: 1445357]
5. Vist MR, Davis JH. Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: 2H nuclear magnetic resonance and differential scanning calorimetry. Biochemistry. 1990; 29:451–464. [PubMed: 2302384]
6. Bird SS, Marur VR, Sniatynski MJ, Greenberg HK, Kristal BS. Lipidomics profiling by high-resolution LC-MS and high-energy collisional dissociation fragmentation: focus on characterization of mitochondrial cardiolipins and monolysocardiolipins. Anal Chem. 2011; 83:940–949. [PubMed: 21192696]
7. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010; 327:46–50. [PubMed: 20044567]
8. Schroeder R, London E, Brown D. Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. Proc Natl Acad Sci U S A. 1994; 91:12130–12134. [PubMed: 7991596]
9. Rietveld A, Simons K. The differential miscibility of lipids as the basis for the formation of functional membrane rafts. Biochim Biophys Acta. 1998; 1376:467–479. [PubMed: 9805010]
10. Gillon AD, Latham CF, Miller EA. Vesicle-mediated ER export of proteins and lipids. Biochim Biophys Acta. 2012; 1821:1040–1049. [PubMed: 22265716]

11. Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, Sampaio JL, de Robillard Q, Ferguson C, Proszynski TJ, Shevchenko A, Simons K. Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J Cell Biol. 2009; 185:601–612. [PubMed: 19433450]
12. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 2008; 9:139–150. [PubMed: 18216770]
13. Dickson RC. Thematic review series: sphingolipids. New insights into sphingolipid metabolism and function in budding yeast. J Lipid Res. 2008; 49:909–921. [PubMed: 18296751]
14. Wieland Brown LC, Penaranda C, Kashyap PC, Williams BB, Clardy J, Kronenberg M, Sonnenburg JL, Comstock LE, Bluestone JA, Fischbach MA. Production of alpha-galactosylceramide by a prominent member of the human gut microbiota. PLoS Biol. 2013; 11:e1001610. [PubMed: 23874157]
15. Hanada K, Nishijima M. Purification of mammalian serine palmitoyltransferase, a hetero-subunit enzyme for sphingolipid biosynthesis, by affinity-peptide chromatography. Methods Mol Biol. 2003; 228:163–174. [PubMed: 12824552]
16. Hanada K. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. Biochim Biophys Acta. 2003; 1632:16–30. [PubMed: 12782147]
17. Lowther J, Naismith JH, Dunn TM, Campopiano DJ. Structural, mechanistic and regulatory studies of serine palmitoyltransferase. Biochem Soc Trans. 2012; 40:547–554. [PubMed: 22616865]
18. Dickson RC, Sumanasekera C, Lester RL. Functions and metabolism of sphingolipids in Saccharomyces cerevisiae. Prog Lipid Res. 2006; 45:447–465. [PubMed: 16730802]
19. Gable K, Slife H, Bacikova D, Monaghan E, Dunn TM. Tsc3p is an 80-amino acid protein associated with serine palmitoyltransferase and required for optimal enzyme activity. J Biol Chem. 2000; 275:7597–7603. [PubMed: 10713067]
20. Han G, Gupta SD, Gable K, Niranjanakumari S, Moitra P, Eichler F, Brown RH Jr, Harmon JM, Dunn TM. Identification of small subunits of mammalian serine palmitoyltransferase that confer distinct acyl-CoA substrate specificities. Proc Natl Acad Sci U S A. 2009; 106:8186–8191. [PubMed: 19416851]
21. Penno A, Reilly MM, Houlden H, Laura M, Rentsch K, Niederkofler V, Stoeckli ET, Nicholson G, Eichler F, Brown RH Jr, von Eckardstein A, Hornemann T. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. J Biol Chem. 2010; 285:11178–11187. [PubMed: 20097765]
22. Gable K, Gupta SD, Han G, Niranjanakumari S, Harmon JM, Dunn TM. A disease-causing mutation in the active site of serine palmitoyltransferase causes catalytic promiscuity. J Biol Chem. 2010; 285:22846–22852. [PubMed: 20504773]
23. Bejaoui K, Wu C, Scheffler MD, Haan G, Ashby P, Wu L, de Jong P, Brown RH Jr. SPTLC1 is mutated in hereditary sensory neuropathy, type 1. Nat Genet. 2001; 27:261–262. [PubMed: 11242106]
24. Sassa T, Kihara A. Metabolism of very long-chain Fatty acids: genes and pathophysiology. Biomol Ther (Seoul). 2014; 22:83–92. [PubMed: 24753812]
25. Kihara A. Very long-chain fatty acids: elongation, physiology and related disorders. J Biochem. 2012; 152:387–395. [PubMed: 22984005]
26. Denic V, Weissman JS. A molecular caliper mechanism for determining very long-chain fatty acid length. Cell. 2007; 130:663–677. [PubMed: 17719544]
27. Nagiec MM, Wells GB, Lester RL, Dickson RC. A suppressor gene that enables Saccharomyces cerevisiae to grow without making sphingolipids encodes a protein that resembles an Escherichia coli fatty acyltransferase. J Biol Chem. 1993; 268:22156–22163. [PubMed: 8408076]
28. Lester RL, Wells GB, Oxford G, Dickson RC. Mutant strains of Saccharomyces cerevisiae lacking sphingolipids synthesize novel inositol glycerophospholipids that mimic sphingolipid structures. J Biol Chem. 1993; 268:845–856. [PubMed: 8419362]
29. Funato K, Lombardi R, Vallee B, Riezman H. Lcb4p is a key regulator of ceramide synthesis from exogenous long chain sphingoid base in Saccharomyces cerevisiae. J Biol Chem. 2003; 278:7325–7334. [PubMed: 12493772]

30. Levy M, Futerman AH. Mammalian ceramide synthases. IUBMB Life. 2010; 62:347–356. [PubMed: 20222015]
31. Degroote S, Wolthoorn J, van Meer G. The cell biology of glycosphingolipids. Semin Cell Dev Biol. 2004; 15:375–387. [PubMed: 15207828]
32. Funato K, Riezman H. Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. J Cell Biol. 2001; 155:949–959. [PubMed: 11733544]
33. Kajiwara K, Ikeda A, Aguilera-Romero A, Castillon GA, Kagiwada S, Hanada K, Riezman H, Muniz M, Funato K. Osh proteins regulate COPII-mediated vesicular transport of ceramide from the endoplasmic reticulum in budding yeast. J Cell Sci. 2014; 127:376–387. [PubMed: 24213531]
34. Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, Nishijima M. Molecular machinery for non-vesicular trafficking of ceramide. Nature. 2003; 426:803–809. [PubMed: 14685229]
35. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. Adv Exp Med Biol. 2010; 688:1–23. [PubMed: 20919643]
36. D'Angelo G, Polishchuk E, Di Tullio G, Santoro M, Di Campli A, Godi A, West G, Bielawski J, Chuang CC, van der Spoel AC, Platt FM, Hannun YA, Polishchuk R, Mattjus P, De Matteis MA. Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. Nature. 2007; 449:62–67. [PubMed: 17687330]
37. D'Angelo G, Uemura T, Chuang CC, Polishchuk E, Santoro M, Ohvo-Rekila H, Sato T, Di Tullio G, Varriale A, D'Auria S, Daniele T, Capuani F, Johannes L, Mattjus P, Monti M, Pucci P, Williams RL, Burke JE, Platt FM, Harada A, De Matteis MA. Vesicular and non-vesicular transport feed distinct glycosylation pathways in the Golgi. Nature. 2013; 501:116–120. [PubMed: 23913272]
38. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci U S A. 2009; 106:2136–2141. [PubMed: 19174513]
39. Schuck S, Simons K. Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. J Cell Sci. 2004; 117:5955–5964. [PubMed: 15564373]
40. Jenkins RW, Canals D, Hannun YA. Roles and regulation of secretory and lysosomal acid sphingomyelinase. Cell Signal. 2009; 21:836–846. [PubMed: 19385042]
41. Mao C, Obeid LM. Ceramidases: regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate. Biochim Biophys Acta. 2008; 1781:424–434. [PubMed: 18619555]
42. Fyrst H, Saba JD. An update on sphingosine-1-phosphate and other sphingolipid mediators. Nat Chem Biol. 2010; 6:489–497. [PubMed: 20559316]
43. Nakahara K, Ohkuni A, Kitamura T, Abe K, Naganuma T, Ohno Y, Zoeller RA, Kihara A. The Sjogren-Larsson syndrome gene encodes a hexadecenal dehydrogenase of the sphingosine 1-phosphate degradation pathway. Mol Cell. 2012; 46:461–471. [PubMed: 22633490]
44. Newton J, Lima S, Maceyka M, Spiegel S. Revisiting the sphingolipid rheostat: Evolving concepts in cancer therapy. Exp Cell Res. 2015; 333:195–200. [PubMed: 25770011]
45. Bikman BT, Summers SA. Sphingolipids and hepatic steatosis. Adv Exp Med Biol. 2011; 721:87–97. [PubMed: 21910084]
46. Ryland LK, Fox TE, Liu X, Loughran TP, Kester M. Dysregulation of sphingolipid metabolism in cancer. Cancer Biol Ther. 2011; 11:138–149. [PubMed: 21209555]
47. Horres CR, Hannun YA. The roles of neutral sphingomyelinases in neurological pathologies. Neurochem Res. 2012; 37:1137–1149. [PubMed: 22237969]
48. Yuyama K, Mitsutake S, Igarashi Y. Pathological roles of ceramide and its metabolites in metabolic syndrome and Alzheimer's disease. Biochim Biophys Acta. 2014; 1841:793–798. [PubMed: 23948264]
49. Casanovas A, Sprenger RR, Tarasov K, Ruckerbauer DE, Hannibal-Bach HK, Zanghellini J, Jensen ON, Ejsing CS. Quantitative analysis of proteome and lipidome dynamics reveals functional regulation of global lipid metabolism. Chem Biol. 2015; 22:412–425. [PubMed: 25794437]

50. Klose C, Surma MA, Gerl MJ, Meyenhofer F, Shevchenko A, Simons K. Flexibility of a eukaryotic lipidome--insights from yeast lipidomics. PLoS One. 2012; 7:e35063. [PubMed: 22529973]
51. Dickson RC, Nagiec EE, Skrzypek M, Tillman P, Wells GB, Lester RL. Sphingolipids are potential heat stress signals in Saccharomyces. J Biol Chem. 1997; 272:30196–30200. [PubMed: 9374502]
52. Jenkins GM, Richards A, Wahl T, Mao C, Obeid L, Hannun Y. Involvement of yeast sphingolipids in the heat stress response of Saccharomyces cerevisiae. J Biol Chem. 1997; 272:32566–32572. [PubMed: 9405471]
53. Simanshu DK, Kamlekar RK, Wijesinghe DS, Zou X, Zhai X, Mishra SK, Molotkovsky JG, Malinina L, Hinchcliffe EH, Chalfant CE, Brown RE, Patel DJ. Non-vesicular trafficking by a ceramide-1-phosphate transfer protein regulates eicosanoids. Nature. 2013; 500:463–467. [PubMed: 23863933]
54. Postma FR, Jalink K, Hengeveld T, Moolenaar WH. Sphingosine-1-phosphate rapidly induces Rho-dependent neurite retraction: action through a specific cell surface receptor. EMBO J. 1996; 15:2388–2392. [PubMed: 8665846]
55. Contreras FX, Ernst AM, Haberkant P, Bjorkholm P, Lindahl E, Gonen B, Tischer C, Elofsson A, von Heijne G, Thiele C, Pepperkok R, Wieland F, Brugger B. Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. Nature. 2012; 481:525–529. [PubMed: 22230960]
56. Bjorkholm P, Ernst AM, Hacke M, Wieland F, Brugger B, von Heijne G. Identification of novel sphingolipid-binding motifs in mammalian membrane proteins. Biochim Biophys Acta. 2014; 1838:2066–2070. [PubMed: 24796501]
57. Rispal D, Eltschinger S, Stahl M, Vaga S, Bodenmiller B, Abraham Y, Filipuzzi I, Movva NR, Aebersold R, Helliwell SB, Loewith R. Target of Rapamycin Complex 2 Regulates Actin Polarization and Endocytosis via Multiple Pathways. The Journal of biological chemistry. 2015; 290:14963–14978. [PubMed: 25882841]
58. Berchtold D, Piccolis M, Chiaruttini N, Riezman I, Riezman H, Roux A, Walther TC, Loewith R. Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. Nat Cell Biol. 2012; 14:542–547. [PubMed: 22504275]
59. Malinska K, Malinsky J, Opekarova M, Tanner W. Distribution of Can1p into stable domains reflects lateral protein segregation within the plasma membrane of living S. cerevisiae cells. J Cell Sci. 2004; 117:6031–6041. [PubMed: 15536122]
60. Malinska K, Malinsky J, Opekarova M, Tanner W. Visualization of protein compartmentation within the plasma membrane of living yeast cells. Mol Biol Cell. 2003; 14:4427–4436. [PubMed: 14551254]
61. Walther TC, Brickner JH, Aguilar PS, Bernales S, Pantoja C, Walter P. Eisosomes mark static sites of endocytosis. Nature. 2006; 439:998–1003. [PubMed: 16496001]
62. Stradalova V, Stahlschmidt W, Grossmann G, Blazikova M, Rachel R, Tanner W, Malinsky J. Furrow-like invaginations of the yeast plasma membrane correspond to membrane compartment of Can1. J Cell Sci. 2009; 122:2887–2894. [PubMed: 19638406]
63. Karotki L, Huiskonen JT, Stefan CJ, Ziolkowska NE, Roth R, Surma MA, Krogan NJ, Emr SD, Heuser J, Grunewald K, Walther TC. Eisosome proteins assemble into a membrane scaffold. J Cell Biol. 2011; 195:889–902. [PubMed: 22123866]
64. Berchtold D, Walther TC. TORC2 plasma membrane localization is essential for cell viability and restricted to a distinct domain. Mol Biol Cell. 2009; 20:1565–1575. [PubMed: 19144819]
65. Tabuchi M, Audhya A, Parsons AB, Boone C, Emr SD. The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. Mol Cell Biol. 2006; 26:5861–5875. [PubMed: 16847337]
66. Aronova S, Wedaman K, Aronov PA, Fontes K, Ramos K, Hammock BD, Powers T. Regulation of ceramide biosynthesis by TOR complex 2. Cell Metab. 2008; 7:148–158. [PubMed: 18249174]
67. Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, Ejsing CS, Weissman JS. Orm family proteins mediate sphingolipid homeostasis. Nature. 2010; 463:1048–1053. [PubMed: 20182505]

68. Niles BJ, Powers T. Plasma membrane proteins Slm1 and Slm2 mediate activation of the AGC kinase Ypk1 by TORC2 and sphingolipids in S. cerevisiae. Cell Cycle. 2012; 11:3745–3749. [PubMed: 22895050]
69. Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J. Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 2011; 108:19222–19227. [PubMed: 22080611]
70. Frohlich F, Moreira K, Aguilar PS, Hubner NC, Mann M, Walter P, Walther TC. A genome-wide screen for genes affecting eisosomes reveals Nce102 function in sphingolipid signaling. J Cell Biol. 2009; 185:1227–1242. [PubMed: 19564405]
71. Levin DE. Regulation of cell wall biogenesis in Saccharomyces cerevisiae: the cell wall integrity signaling pathway. Genetics. 2011; 189:1145–1175. [PubMed: 22174182]
72. Aguilar PS, Frohlich F, Rehman M, Shales M, Ulitsky I, Olivera-Couto A, Braberg H, Shamir R, Walter P, Mann M, Ejsing CS, Krogan NJ, Walther TC. A plasma-membrane E-MAP reveals links of the eisosome with sphingolipid metabolism and endosomal trafficking. Nat Struct Mol Biol. 2010; 17:901–908. [PubMed: 20526336]
73. Olson DK, Frohlich F, Christiano R, Hannibal-Bach HK, Ejsing CS, Walther TC. Rom2-dependent phosphorylation of Elo2 controls the abundance of very long chain fatty acids. J Biol Chem. 2014
74. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Vedie B, Johannes L, Morone N, Parton RG, Raposo G, Sens P, Lamaze C, Nassoy P. Cells respond to mechanical stress by rapid disassembly of caveolae. Cell. 2011; 144:402–413. [PubMed: 21295700]
75. Zhang B, Peng F, Wu D, Ingram AJ, Gao B, Krepinsky JC. Caveolin-1 phosphorylation is required for stretch-induced EGFR and Akt activation in mesangial cells. Cell Signal. 2007; 19:1690–1700. [PubMed: 17446044]
76. Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI, Drab M, Kurzchalia TV, Stan RV, Sessa WC. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. J Clin Invest. 2006; 116:1284–1291. [PubMed: 16670769]
77. Sedding DG, Hermsen J, Seay U, Eickelberg O, Kummer W, Schwencke C, Strasser RH, Tillmanns H, Braun-Dullaeus RC. Caveolin-1 facilitates mechanosensitive protein kinase B (Akt) signaling in vitro and in vivo. Circ Res. 2005; 96:635–642. [PubMed: 15731459]
78. Han S, Lone MA, Schneiter R, Chang A. Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. Proc Natl Acad Sci U S A. 2010; 107:5851–5856. [PubMed: 20212121]
79. Roelants FM, Baltz AG, Trott AE, Fereres S, Thorner J. A protein kinase network regulates the function of aminophospholipid flippases. Proc Natl Acad Sci U S A. 2010; 107:34–39. [PubMed: 19966303]
80. Muir A, Ramachandran S, Roelants FM, Timmons G, Thorner J. TORC2-dependent protein kinase Ypk1 phosphorylates ceramide synthase to stimulate synthesis of complex sphingolipids. Elife. 2014; 3
81. Beeler T, Bacikova D, Gable K, Hopkins L, Johnson C, Slife H, Dunn T. The Saccharomyces cerevisiae TSC10/YBR265w gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca2+-sensitive csg2Delta mutant. J Biol Chem. 1998; 273:30688–30694. [PubMed: 9804843]
82. Oh CS, Toke DA, Mandala S, Martin CE. ELO2 and ELO3, homologues of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. J Biol Chem. 1997; 272:17376–17384. [PubMed: 9211877]
83. Zimmermann C, Santos A, Gable K, Epstein S, Gururaj C, Chymkowitch P, Pultz D, Rodkaer SV, Clay L, Bjoras M, Barral Y, Chang A, Faergeman NJ, Dunn TM, Riezman H, Enserink JM. TORC1 inhibits GSK3-mediated Elo2 phosphorylation to regulate very long chain fatty acid synthesis and autophagy. Cell Rep. 2013; 5:1036–1046. [PubMed: 24239358]
84. Schmidt A, Bickle M, Beck T, Hall MN. The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. Cell. 1997; 88:531–542. [PubMed: 9038344]

85. Shimobayashi M, Oppliger W, Moes S, Jeno P, Hall MN. TORC1-regulated protein kinase Npr1 phosphorylates Orm to stimulate complex sphingolipid synthesis. Mol Biol Cell. 2013; 24:870–881. [PubMed: 23363605]

86. Sun Y, Miao Y, Yamane Y, Zhang C, Shokat KM, Takematsu H, Kozutsumi Y, Drubin DG. Orm protein phosphoregulation mediates transient sphingolipid biosynthesis response to heat stress via the Pkh-Ypk and Cdc55-PP2A pathways. Mol Biol Cell. 2012; 23:2388–2398. [PubMed: 22535525]

87. Nickels JT, Broach JR. A ceramide-activated protein phosphatase mediates ceramide-induced G1 arrest of Saccharomyces cerevisiae. Genes Dev. 1996; 10:382–394. [PubMed: 8600023]

88. Matmati N, Metelli A, Tripathi K, Yan S, Mohanty BK, Hannun YA. Identification of C18:1-phytoceramide as the candidate lipid mediator for hydroxyurea resistance in yeast. J Biol Chem. 2013; 288:17272–17284. [PubMed: 23620586]

89. Kobayashi SD, Nagiec MM. Ceramide/long-chain base phosphate rheostat in Saccharomyces cerevisiae: regulation of ceramide synthesis by Elo3p and Cka2p. Eukaryot Cell. 2003; 2:284–294. [PubMed: 12684378]

90. Fresques T, Niles B, Aronova S, Mogri H, Rakhshandehroo T, Powers T. Regulation of ceramide synthase by casein kinase 2-dependent phosphorylation in Saccharomyces cerevisiae. The Journal of biological chemistry. 2015; 290:1395–1403. [PubMed: 25429105]

91. Tafesse FG, Vacaru AM, Bosma EF, Hermansson M, Jain A, Hilderink A, Somerharju P, Holthuis JC. Sphingomyelin synthase-related protein SMSr is a suppressor of ceramide-induced mitochondrial apoptosis. J Cell Sci. 2014; 127:445–454. [PubMed: 24259670]

92. Vacaru AM, Tafesse FG, Ternes P, Kondylis V, Hermansson M, Brouwers JF, Somerharju P, Rabouille C, Holthuis JC. Sphingomyelin synthase-related protein SMSr controls ceramide homeostasis in the ER. The Journal of cell biology. 2009; 185:1013–1027. [PubMed: 19506037]

93. Morad SA, Cabot MC. Ceramide-orchestrated signalling in cancer cells. Nat Rev Cancer. 2013; 13:51–65. [PubMed: 23235911]

94. Wang X, Rao RP, Kosakowska-Cholody T, Masood MA, Southon E, Zhang H, Berthet C, Nagashim K, Veenstra TK, Tessarollo L, Acharya U, Acharya JK. Mitochondrial degeneration and not apoptosis is the primary cause of embryonic lethality in ceramide transfer protein mutant mice. J Cell Biol. 2009; 184:143–158. [PubMed: 19139267]

95. Raya A, Revert F, Navarro S, Saus J. Characterization of a novel type of serine/threonine kinase that specifically phosphorylates the human goodpasture antigen. J Biol Chem. 1999; 274:12642–12649. [PubMed: 10212244]

96. Mencarelli C, Losen M, Hammels C, De Vry J, Hesselink MK, Steinbusch HW, De Baets MH, Martinez-Martinez P. The ceramide transporter and the Goodpasture antigen binding protein: one protein--one function? J Neurochem. 2010; 113:1369–1386. [PubMed: 20236389]

97. Kumagai K, Kawano M, Shinkai-Ouchi F, Nishijima M, Hanada K. Interorganelle trafficking of ceramide is regulated by phosphorylation-dependent cooperativity between the PH and START domains of CERT. J Biol Chem. 2007; 282:17758–17766. [PubMed: 17442665]

98. Fugmann T, Hausser A, Schoffler P, Schmid S, Pfizenmaier K, Olayioye MA. Regulation of secretory transport by protein kinase D-mediated phosphorylation of the ceramide transfer protein. J Cell Biol. 2007; 178:15–22. [PubMed: 17591919]

99. Surma MA, Klose C, Klemm RW, Ejsing CS, Simons K. Generic sorting of raft lipids into secretory vesicles in yeast. Traffic. 2011; 12:1139–1147. [PubMed: 21575114]

100. Munn AL, Riezman H. Endocytosis is required for the growth of vacuolar H(+)-ATPase-defective yeast: identification of six new END genes. J Cell Biol. 1994; 127:373–386. [PubMed: 7929582]

101. Frohlich F, Petit C, Kory N, Christiano R, Hannibal-Bach HK, Graham M, Liu X, Ejsing CS, Farese RV, Walther TC. The GARP complex is required for cellular sphingolipid homeostasis. Elife. 2015; 4

102. Voynova NS, Roubaty C, Vazquez HM, Mallela SK, Ejsing CS, Conzelmann A. Saccharomyces cerevisiae Is Dependent on Vesicular Traffic between the Golgi Apparatus and the Vacuole When Inositolphosphorylceramide Synthase Aur1 Is Inactivated. Eukaryot Cell. 2015; 14:1203–1216. [PubMed: 26432633]

103. Feinstein M, Flusser H, Lerman-Sagie T, Ben-Zeev B, Lev D, Agamy O, Cohen I, Kadir R, Sivan S, Leshinsky-Silver E, Markus B, Birk OS. VPS53 mutations cause progressive cerebello-cerebral atrophy type 2 (PCCA2). Journal of medical genetics. 2014; 51:303–308. [PubMed: 24577744]
104. Hu YB, Dammer EB, Ren RJ, Wang G. The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. Transl Neurodegener. 2015; 4:18. [PubMed: 26448863]
105. Platt FM. Sphingolipid lysosomal storage disorders. Nature. 2014; 510:68–75. [PubMed: 24899306]
106. Siow DL, Wattenberg BW. Mammalian ORMDL proteins mediate the feedback response in ceramide biosynthesis. The Journal of biological chemistry. 2012; 287:40198–40204. [PubMed: 23066021]
107. Swinnen E, Wilms T, Idkowiak-Baldys J, Smets B, De Snijder P, Accardo S, Ghillebert R, Thevissen K, Cammue B, De Vos D, Bielawski J, Hannun YA, Winderickx J. The protein kinase Sch9 is a key regulator of sphingolipid metabolism in Saccharomyces cerevisiae. Mol Biol Cell. 2014; 25:196–211. [PubMed: 24196832]
108. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature. 2007; 448:470–473. [PubMed: 17611496]
109. Wu H, Romieu I, Sienra-Monge JJ, Li H, del Rio-Navarro BE, London SJ. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. Allergy. 2009; 64:629–635. [PubMed: 19133921]
110. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Lariviere M, Moussette S, Grundberg E, Kwan T, Ouimet M, Ge B, Hoberman R, Swiatek M, Dias J, Lam KC, Koka V, Harmsen E, Soto-Quiros M, Avila L, Celedon JC, Weiss ST, Dewar K, Sinnett D, Laprise C, Raby BA, Pastinen T, Naumova AK. Allele-specific chromatin remodeling in the ZPBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. American journal of human genetics. 2009; 85:377–393. [PubMed: 19732864]
111. Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. Trends Endocrinol Metab. 2015; 26:538–550. [PubMed: 26412155]
112. Park TS, Goldberg IJ. Sphingolipids, lipotoxic cardiomyopathy, and cardiac failure. Heart Fail Clin. 2012; 8:633–641. [PubMed: 22999245]
113. Villa NY, Kupchak BR, Garitaonandia I, Smith JL, Alonso E, Alford C, Cowart LA, Hannun YA, Lyons TJ. Sphingolipids function as downstream effectors of a fungal PAQR. Mol Pharmacol. 2009; 75:866–875. [PubMed: 19066337]

Highlights

- sphingolipids are structural membrane components and signaling molecules
- cells sense changes in sphingolipid levels in the plasma membrane
- cells acutely regulate sphingolipid biosynthesis
- membrane trafficking is an important for sphingolipid homeostasis

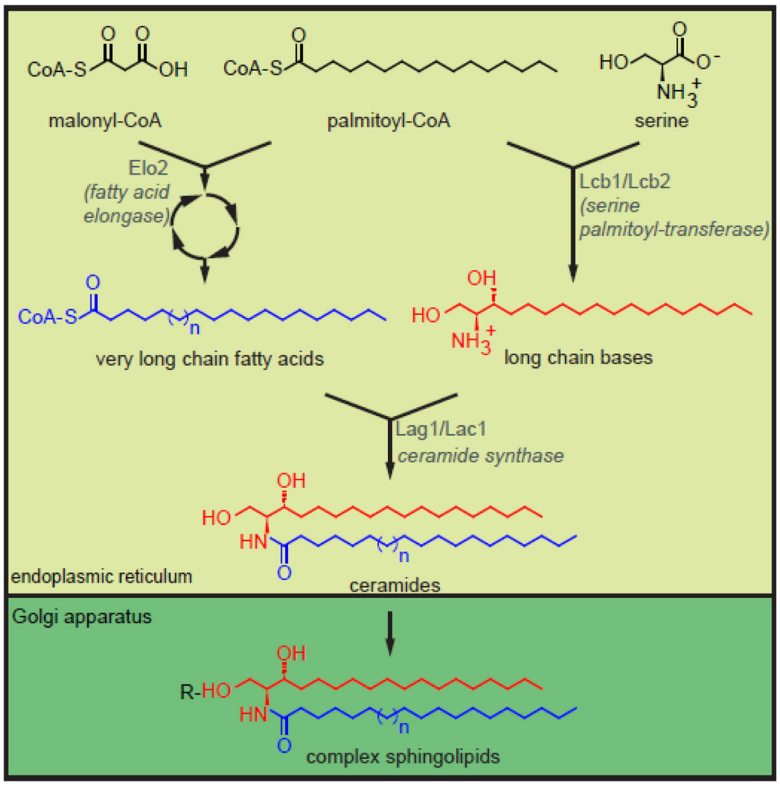

Figure 1.

Key steps in the synthesis of sphingolipids. See text for details. Precursors are shown in black, very long-chain fatty acids in blue, long-chain bases in red. Representative very long-chain fatty acids and long-chain bases are shown, and do not depict all possible variations that arise due to hydroxylation, desaturation, and varied chain length. Key enzymes that are known to be regulated in yeast are depicted.

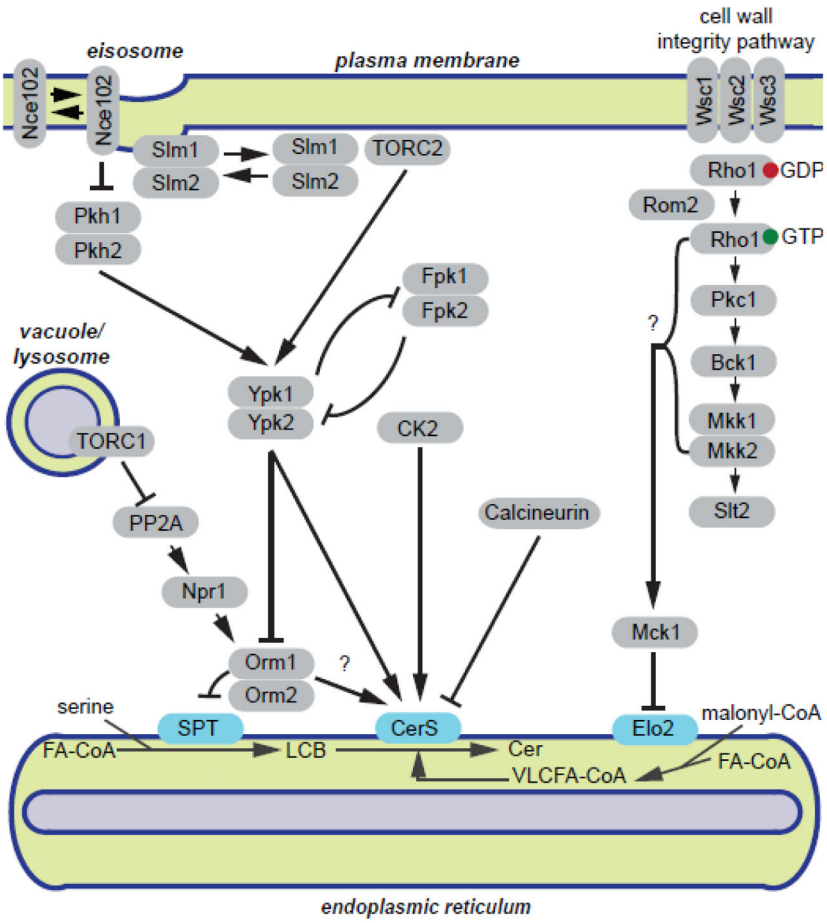

Figure 2.

Regulation of sphingolipid biosynthesis. See text for details. Kinase and phosphatase signaling from the plasma membrane and other intracellular locations regulate enzymes of the early stages of sphingolipid synthesis. Fatty acid elongase (Elo2), serine palmitoyltransferase (SPT), ceramide synthase (CerS).

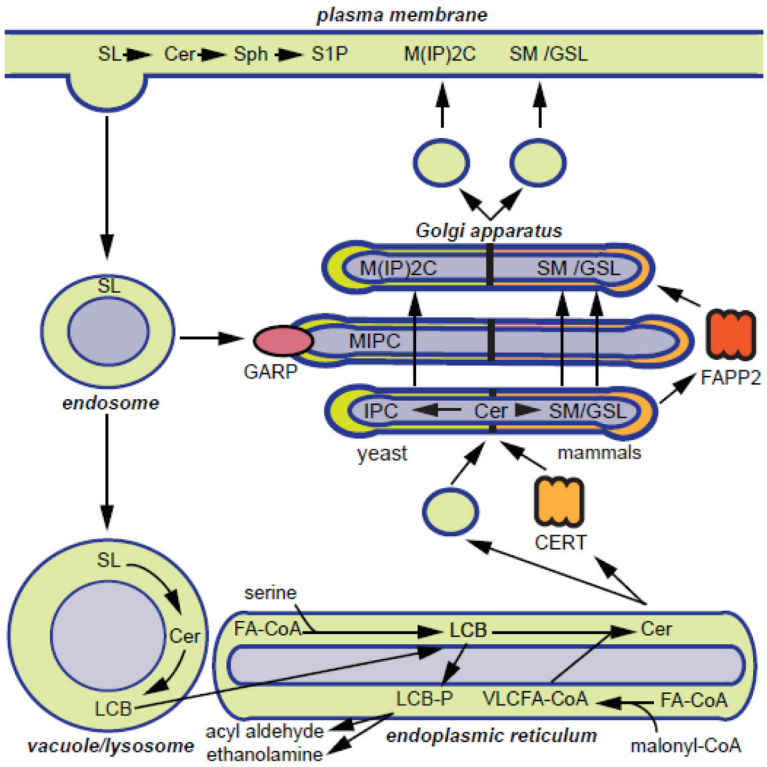

Figure 3.

Trafficking of sphingolipids. See text for details. Major routes of sphingolipid biosynthesis and degradation as they occur within the endomembrane system. Complex sphingolipid (SL), ceramide (Cer), sphingosine (Sph), sphingosine-1-phosphate (S1P), fatty acid (FA), coenzyme A (CoA), long-chain base (LCB), long-chain base phospohate (LCB-P) very long-chain fatty acid (VLCFA), ceramide transport protein (CERT), GlcCer-transfer protein (FAPP2), Golgi-associated retrograde protein (GARP)