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The Yeast Sphingolipid Signaling Landscape

David J. Montefusco[1], **Nabil Matmati**[2], and **Yusuf A. Hannun**[2]

[1]Dept. Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina

[2]Stony Brook Cancer Center, Stony Brook University, Stony Brook, New York

Abstract

Sphingolipids are recognized as signaling mediators in a growing number of pathways, and represent potential targets to address many diseases. The study of sphingolipid signaling in yeast has created a number of breakthroughs in the field, and has the potential to lead future advances. The aim of this article is to provide an inclusive view of two major frontiers in yeast sphingolipid signaling. In the first section, several key studies in the field of sphingolipidomics are consolidated to create a yeast sphingolipidome that ranks nearly all known sphingolipid species by their level in a resting yeast cell. The second section presents an overview of most known phenotypes identified for sphingolipid gene mutants, presented with the intention of illuminating not yet discovered connections outside and inside of the field.

1. Introduction

Sphingolipids are a structurally diverse class of lipids implicated in a number of cell signaling functions. The development of a detailed understanding of sphingolipid signaling and sphingolipid-based treatments is growing in urgency as sphingolipids are implicated in an ever-increasing list of diseases (Kolter, 2011; Pralhada Rao et al., 2013), such as cancer (Canals and Hannun, 2013), Alzheimer's (Yuyama et al., 2013), and diabetes (Russo et al., 2013). Homology between the yeast and human pathways (Hannun et al., 2001), and the advantages of yeast as a model organism have made it an essential tool to address the most pressing problems in the field. The goal of this article is to invite investigators with a wide range of interests both inside and outside the sphingolipid field to examine yeast sphingolipid signaling and its potential connections to higher organisms.

The *Saccharomyces cerevisiae* model represents the vanguard of several key discoveries related to sphingolipid mediated pathways such as regulation of nutrient uptake (Chung et al., 2001; Skrzypek et al., 1998), transport of GPI-anchored proteins (Skrzypek et al., 1997), heat stress (Dickson et al., 1997a; Jenkins et al., 1997), and others (Dickson, 2008;

Correspondence to: Yusuf A. Hannun.

Schneiter, 1999). Moreover, budding yeast has greatly contributed to the molecular identification of several key enzymes of sphingolipid metabolism, which has been essential for modern day molecular approaches towards dissecting pathways of sphingolipid function in both yeast and mammals.

The wide diversity of signaling functions regulated by sphingolipids is mirrored by the structural diversity of sphingolipid species and metabolites. One of the core objectives of the field is to connect specific bioactive sphingolipids to specific signaling roles and target molecules. A vast array of yeast signaling functions have been found to rely on sphingolipids in general, but sorting out those functions specific to ceramide as a class of sphingolipid and moreover those specific to individual ceramide species, is hampered by the complexity of the sphingolipid metabolic pathway{Hannun, 2011 #1578}. This complexity makes it difficult to genetically alter production of a single target species without affecting many other species, which is one of the major challenges of the field. Ceramides as a class of sphingolipids have been placed upstream of many important functions in yeast such as nutrient transporter signaling (Guenther et al., 2008), and cell cycle regulation (Matmati et al., 2009), but to pinpoint downstream target molecules requires resolution at the level of individual ceramides. New methods are emerging to address this challenge that rely heavily on advances in lipidomics and bioinformatics {Alvarez-Vasquez, 2005 #1760}(Cowart et al., 2010b)(Montefusco et al in press).

The simultaneous quantitative analysis of multiple sphingolipid species from a single sample, known as sphingolipidomics, is playing an increasingly central role in the advancement of sphingolipid research. Yeast lipidomics has advanced tremendously, especially in the study of ceramide (Bielawski et al., 2010), requiring detailed separation and quantification of the large number of structurally similar species. A few laboratories have also advanced the quantification of the complex sphingolipids (Cerantola et al., 2009; Klose et al., 2012) and sphingoid bases (Bielawski et al., 2010); however, addressing the interplay of sphingolipids within the complete yeast lipidome would require consolidation of a patchwork of datasets involving the various classes and subclasses of lipids.

The first section of this article aims at integrating 14 outstanding datasets into a single model yeast lipidome encompassing all known species. The second section employs the advantages of the yeast model to identify and classify many of the known roles of sphingolipids. This section summarizes the vast majority of phenotypes resulting from mutation or overexpression of any gene that impacts the sphingolipidome, including genes on the periphery of sphingolipid synthesis such as those involved in fatty acid uptake, synthesis, or elongation. Phenotypes are categorized by specific effects or by connections to specific pathways, with the intention of identifying connections between behavior of the cell and alteration of the sphingolipidome.

2. The Yeast Sphingolipidome

The yeast sphingolipidome is far simpler than that of humans, but is highly homologous at the level of sphingoid bases and ceramides (Dickson, 1998; Hannun et al., 2001). The initiating step of sphingolipid synthesis in both species is the condensation of serine and a

fatty acyl-CoA, usually palmitoyl-CoA, to form ketodihydrosphingosine, by the action the of serine palmitoyl transferase (SPT) complex consisting of Lcb1, Lcb2 (Pinto et al., 1992) and Tsc3 (Gable et al., 2000). This enzyme is inhibited by the Orm proteins (Breslow et al., 2010) which are in turn regulated by phosphorylation (Roelants et al., 2011). Ketodihydrosphingosine is then converted to dihydrosphingosine (sphinganine) by the action of the reductase Tsc10 (Beeler et al., 1998). Dihydrosphingosine (DHS) undergoes acylation by the action of (dihydro) ceramide synthases Lag1, Lac1 and Lip1 (Guillas et al., 2001; Schorling et al., 2001) to form dihydroceramide (DHC). At this point, and subsequently yeast and humans diverge. The human DES1 desaturates DHC forming ceramide (Hannun et al., 2001), whereas in yeast Sur2 hydroxylates DHS/DHC to form phytosphingosine (PHS) and phytoceramide (PHC), respectively (Figure 2) (Grilley et al., 1998; Teresa M. Dunn, 1998). There are significant variations in the structure of ceramides introduced by the action of various ceramide synthases that introduce differing chain lengths at the amidelinked fatty acid, and hydroxylases that act on the either the sphingoid base (Sur2 in yeast) (Grilley et al., 1998; Haak et al., 1997) or the fatty acid (Scs7 in yeast)(Haak et al., 1997).

Subsequent to formation of ceramide, complex sphingolipids are generated primarily by modifications at the 1-OH position of ceramide. Mammals introduce various carbohydrate units at this position to create a rich repertoire of glycosphingolipids and gangliosides (Hannun and Bell, 1989; Kolter et al., 2002). In contrast, yeast incorporate only inositol and mannose to form the three classes of complex sphingolipids, inositolphosphoceramide (IPC), by the IPC synthase consisting of Aur1 and Kei1 (Sato et al., 2009), mannose inositolphosphoceramide (MIPC) by the MIPC synthases Csg1, Csg2 and Csh1 and mannosediinositolphosphoceramide ($M(IP)_2C$) by the inositolphosphotransferase Ipt1 (Figure 1)(Becker and Lester, 1980; Dickson and Lester, 1999b).

The yeast sphingolipidome can therefore be divided into three classes of molecule: the long chain sphingoid bases (DHS/PHS) and their phosphates, ceramides (DHC/PHC), and complex sphingolipids/inositol phosphoceramides (IPCs). Each class is then subdivided based on hydroxylation of the sphingoid backbone or the fatty acid, the length of the fatty acid, and the nature of the specific moieties on the headgroup. Additional complexity in the sphinoglipidome arises because in addition to C18 sphingoid bases, C16 and C20 sphingoid bases have been observed, synthesized by incorporation of C14 and C18 fatty acids myristate and stearate, respectively (Cowart and Hannun, 2007). Furthermore the presence of ceramide/complex sphingolipid species containing a fifth hydroxyl group have been reported within lipidomic datasets (Ejsing et al., 2009; Klose et al., 2012), but have not been studied further. A generic sphingolipid structure is shown in Figure 2 (bottom right), with the sphingoid backbone, acyl chain, hydroxyl groups and headgroups labeled.

2.1 Assembly of the Model Sphingolipidome

While the layout of the sphingolipid metabolic pathway suggests many structural variations, it gives no indication as to the quantity of each lipid found in a typical cell. A number of laboratories have quantified yeast sphingolipids by HPLC-MS/MS and/or HPLC methods, creating an ever more comprehensive picture. Data from a number of key references were

collected here to establish a 'model' yeast lipidome whereby all sphingolipid species for which data are available have been arranged in order of abundance (Figure 2). These data were consolidated from 14 published datasets which varied in their experimental goals, approaches, units of quantification, and lipid coverage. Some datasets such as (Ejsing et al., 2009) assessed nearly every known sphingolipid species at some cost to sensitivity. Others focused only on the complex sphingolipids (Cerantola et al., 2009), while others quantified the ceramides and sphingoid bases only, achieving high sensitivity and precision (Cowart and Hannun, 2007; Huang et al., 2012)(Montefusco *et al* in press)

These datasets were integrated in a systematic manner by organizing the model lipidome into sections: sphingoid bases, ceramides, IPCs, MIPCs and $M(IP)_2Cs$. This step was necessary for integration of datasets because as mentioned previously, most references covered only a portion of the lipidome. The dataset of (Ejsing et al., 2009) was first inserted into the model lipidome, due to its breadth of coverage it provided a number of marker lipids within each group that assisted in ranking lipid groups relative to one another, and provided reference points for integration of subsequent datasets. Next the datasets of (Cerantola et al., 2009; Cowart et al., 2010b)(Montefusco et al in press), being the next widest in coverage, were integrated by ranking lipids within each dataset and inserting them into the model lipidome where there was agreement between preselected reference lipids. The reference lipids are: C26-alpha hydroxy phytoceramide-$M(IP)_2C$ (C26-αOH-$M(IP)_2C$), C26-αOH-PHC, C18-DHS, C16-DHC and C16-PHC; these were used to establish the order of magnitude into which each lipid falls. Then the datasets of (Cerantola et al., 2007; Xue Li Guan, 2006) and (Klose et al., 2012) were integrated within the ceramide and sphingoid bases, and finally the datasets from (Aronova et al., 2008; Cowart and Hannun, 2007; Huang et al., 2012; Ponnusamy et al., 2008; Vionnet et al., 2010; Zhang et al., 2001) were integrated. The model data are plotted in the top of Figure 2. The model sphingolipidome places each lipid into one of 6 groups spanning 5 orders of magnitude.

Lipid ratios were consistent enough within each dataset that there were no inconsistencies at the modest resolution level presented in the model lipidome. Comparing lipid ratios among datasets that employed mass spectrometry (MS) was straightforward despite differences among units used to express lipid levels and differences in the normalization method. MS datasets have expressed lipid levels in moles, grams, or ion counts, and normalized to total phospholipid or number of cells extracted. Two of the datasets that contributed detailed data on complex sphingolipids (Cerantola et al., 2007) and (Cerantola et al., 2009) employed radiolabeling and thin layer chromatography (TLC). For these studies TLC spots were normalized to total labeled lipid, and samples were extracted from the same number of labeled cells.

2.2 Insights from the Model Sphingolipidome

It has been known for some time that by far the dominant sphingolipid species in yeast is αOH-C26-$M(IP)_2C$, followed by αOH-C26-MIPC then αOH-C26-IPC (Cerantola et al., 2007; Ejsing et al., 2009; Klose et al., 2012; Xue Li Guan, 2006), and as is apparent in Figure 2, αOH-C26-PHC is the next most abundant (Cowart et al., 2010b)(Montefusco *et al* in press). However the latter is in group 5 of the model lipidome, and is therefore on par

with 13 other lipids. Sphingolipids with C26 acyl chains constitute over 90 % of the model lipidome, and species containing αOH-PHC constitute 80 – 90 % of the model lipidome. When sorted by acyl chain length, C24 species are the next most abundant. The three complex αOH-C24 species are most abundant followed by the C24-PHC species one order of magnitude lower (Figure 2). The abundance of species with acyl chain lengths shorter than C24 drop off sharply, with C22 species constituting only about 0.1 % of the sphingolipidome. Thus in overall character, the yeast sphingolipids are often thought of as C26-αOH mannoseinositol lipids. Much of the early work on yeast sphingolipids has focused on the predominantly very-long-chain character of the sphingolipidome, emphasizing its role in structural integrity of the membrane (van der Rest et al., 1995). At that time, it was assumed that the less abundant species played only a supporting role(Gunther Daum, 1998).

While αOH-PHC-containing lipids dominate the lipidome by mass, individual αOH-ceramide species exist over a very wide range of concentrations. They are the only lipid class found in all six levels of the model lipidome. Interestingly all of the αOH species with acyl chains shorter than C24 are found at the lowest level of the model lipidome (groups 1 and 2). In contrast, the less hydroxylated PHC and DHC-based species have a more even distribution in lipid level with respect to acyl chain length. For example C26-MIPC-PHC is found in the fifth group, while C26-$M(IP)_2C$-PHC is found in the first group. Another interesting trend is that with only two exceptions, the middle three levels of the model lipidome are exclusively occupied by ceramides, while complex sphingolipids occupy the first, fifth and sixth levels.

Ceramides, with the exception of αOH-C26-PHC, occupy the bottom 4 levels of the model lipidome. As with the overall trend in αOH species, the majority of αOH-ceramides are found in the lower two levels. Nearly all of the DHC and PHC species are found in the middle three levels of the model lipidome, and with only few exceptions the DHCs are more abundant than the PHCs. A very interesting contrast between DHC and PHC is that with PHC, the trend of increasing abundance with increasing acyl chain length seen with αOH-PHC is more or less preserved, while with DHC this trend is completely absent; for example the most abundant DHC species are C18 and C20.

Another notable observation is the presence of the C16 and C20 sphingoid bases in the model lipidome (Cowart and Hannun, 2007; Huang et al., 2012). These molecules are not well studied in any organism, but they may play a role in the yeast heat stress response (Cowart and Hannun, 2007; Ferguson-Yankey et al., 2002; Jenkins et al., 1997), and as discussed in the following section, the SPT subunit Tsc3 is thought to be essential for their production (Cowart and Hannun, 2007). These species are not very abundant overall, but surprisingly C16-DHS and C16-DHS-1-phosphate (C16DHS1P) were reported to be on par with C18PHS/DHS (level 4 of the model lipidome).

An additional class of sphingolipids has been discovered in mammals arising from the promiscuity of the SPT complex for the amino acid substrate, whereby glycine or alanine are substituted for serine (Rotthier et al., 2010). The resulting products are deoxysphingoid bases and demethyloxysphingoid bases respectively, which have been connected to

hereditary neuropathy in humans (Penno et al., 2010). The budding yeast model has been used to help demonstrate that a human mutant SPT subunit is necessary and sufficient for production of these lipids (Gable et al., 2010). These lipids do not appear in the model lipidome because they have not yet been detected in wild type yeast, probably due to sensitivity of detection.

It should be noted that growth conditions can have a profound impact on the sphingolipidome. Addition of key nutrients and metabolites to the media has been shown to dramatically alter the lipid profile. For example addition of serine or palmitate was shown to alter the sphingolipidome within minutes (Alvarez-Vasquez et al., 2005; Brice et al., 2009; Montefusco et al., 2012), and starvation followed by addition of inositol altered levels of complex sphingolipids within 2 hours {Jesch, 2010 #1179}. Furthermore addition of myristate was shown to specifically alter the level of C-14 phytoceramide (Montefusco et al in press), however less is known about adaptation of the sphingolipidome over longer time periods. Datasets used to assemble the model lipidome were collected using either rich media or synthetic complete media, and differences between the lipid profiles of these two media are not apparent at the resolution level of the model lipidome.

3. Phenotypes of the Yeast Sphingolipid Pathway

A number of excellent reviews have discussed the enzymes of the sphingolipid pathway in detail, including the reactions of the enzymes, and the downstream functions of some of the well-characterized lipid groups (Dickson, 2008; Dickson and Lester, 1999b; Obeid et al., 2002). This article will build upon that work with two approaches. First, Table 1 shows a list of enzymes whose activity impacts sphingolipid levels, including genes involved in fatty acid synthesis, uptake, and elongation. Second, the known phenotypes resulting from disruption of each gene are categorized by the method described below, and categories represented are listed by enzyme in Table 1, and discussed in detail below. This approach is appropriate for the goals of this article because it highlights the largest possible number of sphingolipid functional roles, and can be attractive to investigators with a wide range of interests to seek out connections to yeast sphingolipids.

A tremendous amount of yeast sphingolipid metabolism has been deciphered using classical yeast genetics (Dickson, 2008). Several of the reactions carried out by each enzyme were identified by gene deletion and overexpression, and these methods allow a degree of control over the sphingolipid composition of the cell, but as mentioned previously a major obstacle to pinpointing functions of specific sphingolipid species is the lack of precise surgical control of the sphingolipidome using genetic methods. Many phenotypes have been associated with deletion or overexpression of sphingolipid genes, which in some cases has provided important clues as to the functions of specific lipid groups. Furthermore the non-uniform nature of phenotypes makes them difficult to search systematically. The phenotype inventory collected here helps to overcome that difficulty for the yeast sphingolipid investigator.

Phenotypes were selected from the literature focusing on identifying downstream effects of each gene mutation. To focus on downstream effects, obvious phenotypes that are direct

consequences of altered enzyme activity, such as increased substrate or decreased product of a deleted enzyme, were excluded. Each phenotype was then placed into one of 15 categories based on a pathway or set of pathways that appear to be involved. Examining the combination of genes that fall into each category offers a clue as to the lipid or set of lipids that may mediate the phenotypes. There are a number of interesting patterns that may be valuable for generating new hypotheses and experimental designs. Abbreviated category names are given in Table 1, and the abbreviations are given in the table caption.

Phenotype categories do not necessarily represent *bona fide* pathways, for example the 'ER stress' category represents a pathway, while the 'autophagy/apoptosis' category consolidates closely related pathways. Categories were selected based on recurring themes in the phenotype list, and the non-uniform nature of the phenotypes required some subjectivity in classification. For example a pharmacological agent known to target a specific pathway such as FK506 targeting calcinurin, would place an FK506 sensitivity or resistance phenotype into the 'calcium homeostasis/signaling' category. Phenotypes listed were identified using deletion mutants, temperature sensitive mutants, reduced function mutants or protein overproduction, and all were weighted equally.

3.1 Temperature Tolerance

The response to heat stress encompasses multiple pathways leading to a range of chemical and physical changes (Verghese et al., 2012). The 'temperature tolerance' category includes heat stress, thermotolerance and other temperature-dependent growth effects. Sphingolipid-dependent regulation of heat stress signals are well studied in yeast, and multiple effects were identified such as nutrient-related switching (Cowart et al., 2010b; Hearn et al., 2003; Montefusco et al., 2012), translation arrest/resumption (Meier et al., 2006), and mRNA sequestration (Cowart et al., 2010a). In the case of the sphingoid bases (Montefusco et al., 2012) and sphingoid base phosphates (Cowart et al., 2010b) downstream targets have been defined such as the HAP transcriptional regulators involved in mitochondrial respiration, and PKH/YPK in eisosome formation (Luo et al., 2008). These lipids are likely to have a direct involvement in the temperature sensitivity phenotypes of the *lcb4*Δ/*lcb5*Δ (Cowart et al., 2010b) *lcb3*Δ (Ferguson-Yankey et al., 2002; Mao et al., 1999; Skrzypek et al., 1999), *lcb5*Δ (Jenkins and Hannun, 2001) and *dpl1*Δ (Ferguson-Yankey et al., 2002; Gadde et al., 1998; Jenkins and Hannun, 2001) strains, as well as hypersensitivity to the Hsp90 inhibitor macbecin II (Franzosa et al., 2011).

The involvement of the sphingoid bases is not likely to be the only sphingolipid dependent effect related to heat stress. The *elo2*Δ (Franzosa et al., 2011), *ifa38*Δ (Han et al., 2002), and *fat1*Δ (deleted in the fatty acid importer/activator FAT1) (Watkins et al., 1998) strains all reveal temperature-related growth effects. These enzymes are among many upstream of sphingoid base synthesis, but they are distinguished by their specific phenotypes. For example *elo2*Δ and *lcb5*Δ are both sensitive to the *Hsp90* inhibitor macbecin II (Franzosa et al., 2011), this observation does not implicate a specific lipid, but it raises the intriguing question of how neither *elo1*Δ, *elo3*Δ nor for that matter *lcb4*Δ share the phenotype, since all mutants have disrupted sphingoid base production.

3.2 ER Stress/Unfolded Protein Response

The ER stress response pathway is a part of the response to a number of stressors in both yeast and human cells, and yeast share many features of their ER stress response with human cells (Kimata and Kohno, 2011). The second category in Table 1 combines ER stress and the unfolded protein response (UPR) because these are essentially the same pathway (Gardner et al., 2013), DTT treatment was shown to rescue the myricion sensitivity of *lcb1–100* (which is defective in de novo synthesis) by increasing sphingolipid synthesis via the UPR response gene HAC1 (Epstein et al., 2012). Strains deleted in the ORM genes *orm1Δ orm2*Δ, potent regulators of SPT activity, show elevated UPR as well as sensitivity to tunicamycin, a drug that stimulates ER stress (Gururaj et al., 2013; Han et al., 2010; Hjelmqvist et al., 2002), and *isc1*Δ shows elevated expression of orm2 (Gururaj et al., 2013). The *csg2*Δ strain is sensitive to DTT (Tan et al., 2009), which activates the unfolded protein response (UPR) (Jonikas et al., 2009), as are *elo2*Δ, *elo3*Δ, and *fat1*Δ (Jonikas et al., 2009). Because *elo2*Δ, *elo3*Δ and *fat1*Δ contribute only to the supply of fatty acyl-CoA longer than C14, together these results imply that Csg2-generated very long (acyl) chain (VLC) complex sphingolipids are necessary to maintain the UPR, and that the UPR in turn can regulate sphingolipid synthesis.

3.3 Cell Cycle

A number of features of yeast cell cycle regulation make it an excellent model for the study of cell cycle regulation, and it shares human regulatory growth factors that make it a valuable model for the study of tumorigenesis (Foster et al., 2010). The third category, “cell cycle” consists largely of phenotypes related to blocked progression between phases of the cell cycle, or failure to arrest cell cycle normally with stress. It is not implied that these defects share a common mechanism, but these clues may lead to novel mechanisms. Among other heat stress-related phenotypes, the *lcb1–100* temperature sensitive mutant has defective cell cycle arrest with heat stress (Jenkins and Hannun, 2001). The *scs7*Δ strain has defective sister-chromatid cohesion during anaphase (Mayer et al., 2004), as well as sensitivity to hydroxyurea (HU)(Parsons et al., 2004). The latter is not a cell cycle defect *per se*, however a well studied HU-sensitivity phenotype in the *isc1*Δ strain, results from altered phosphorylation of Cdc28 leading to a G2/M block (Matmati et al., 2009), thus the *scs7*Δ sensitivity should be considered in this context, and also may be cell-cycle-related. Similarly HU sensitivity of the *csg2*Δ strain (Liu et al., 2011) was classified as cell-cycle related, and *csg2*Δ also showed defective arrest of G1 phase under the stress of linoleic acid and hydroperoxide treatment (Fong et al., 2008). A more recent study (Matmati et al., 2013) showed *sur4*Δ, *double lag1*Δ,*lac1*Δ made cells more sensitive to HU. Moreover, depletion of phytoceramide by overexpressing YPC1 rendered wild type cells sensitive to HU. Together these phenotypes imply a role for ceramides, complex sphingolipids, or both in regulation of cell cycle.

Another set of closely related enzyme deletions showing cell cycle related defects are *lcb3*Δ, *lcb5*Δ and *dpl1*Δ. The *lcb5*Δ and *dpl1*Δ strains both show defects in arrest and recovery of cell cycle progression with heat stress (Jenkins and Hannun, 2001), and *lcb5*Δ is also sensitive to benomyl, a drug known to target cell cycle-related pathways (Pan et al., 2004). The *lcb3*Δ strain is especially interesting in that its expression is required for normal cell

cycle progression (Mao et al., 1999), and in wild type cells LCB3 expression is sensitive to cell cycle phase (Dickson and Lester, 1999a; Spellman et al., 1998), implying either that the sphingoid bases, or the sphingoid base phosphates are involved in a feedback mechanism related to cell cycle progression.

A third subgroup within the cell cycle category is the *elo3*Δ (Desfarges et al., 1993; Ni and Snyder, 2001), and *ole1*Δ (Stukey et al., 1989; Zhang et al., 1999) phenotypes related to budding. Ole1 is a key fatty acid desaturase (Stukey et al., 1990), and Elo3 activity affects the ratio of saturated to unsaturated lipids (Choi and Martin, 1999). Interestingly these genes which are closely related in function are also connected to a specific aspect of cell cycle regulation. It may be postulated that sphingolipid sidechain saturation plays a role in bud site selection. Overall, the cellcycle-related phenotypes imply that multiple sphingolipid species are involved in multiple elements of cell cycle regulation.

3.4 Actin/Morphology

The yeast cytoskeleton is essential for maintaining normal cell size and morphology, as well as organizing essential processes such as endocytosis (Robertson et al., 2009) and budding (Giblin et al., 2011). The next category is “actin/morphology”, which is the most populous category implying an intimate connection between sphingolipids and cytoskeletal organization. With the exception of the *lcb5*Δ decreased cell size phenotype (Jorgensen et al., 2002), entries in this category are required for ceramide and complex sphingolipid synthesis, implying that these species have a dominant role. Interestingly the sphingoid bases were previously implicated in activation of the PKH/YPK yeast kinases (Friant et al., 2001), which are mainly connected to cell integrity, but also known to regulate cytoskeleton dynamics (Roelants et al., 2002; Schmelzle et al., 2002), however no direct link has been made between sphingoid bases and cytoskeleton dynamics. These data imply that both sphingoid bases and complex sphingolipids may have a role in cytoskeletal regulation.

Many of the actin/morphology phenotypes in Table 1 are sensitivity to the drug dihydromotiporamine C (dhMotC). It was demonstrated that this drug inhibits growth of both yeast and mammalian cells by disrupting actin organization. Importantly, dhMotC growth inhibition is rescued by addition of DHS to yeast cells, and ceramide to human cells (Baetz et al., 2004). It was also confirmed that dhMotC targets the sphingolipid pathway, decreasing ceramide synthesis (Baetz et al., 2004). Five mutant strains *lcb1*Δ, *lcb2*Δ, *tsc10*Δ, *sur2*Δ and *csg1*Δ were reported as sensitive to dhMotC, and one, *csg2*Δ, was reported as resistant (Baetz et al., 2004). This combination implies that the sphingoid bases, ceramides, and/or Csg1-generated complex sphingolipids, are required for proper actin organization, but that Csg2-generated lipids are not required. Not enough is known about Csg2 specificity to speculate on a specific target lipid.

Additional actin-related phenotypes are reported for a number of sphingolipid pathway mutants. The dhMotC results imply that sphingolipids play vital and central roles in actin organization, thus it is likely that other phenotypes arise from disruption of similar downstream pathways. A few of the phenotypes listed here are not explicitly caused by an actin defect, but must heavily involve actin regulation, the most pertinent example is the endocytosis defect of temperature sensitive lcb1–100 mutant attributed to actin defects

(Zanolari et al., 2000). Additionally the *csg1*Δ, and *elo3*Δ show dumbbelllike morphology (Desfarges et al., 1993) resulting from incomplete cell division, *ifa38*Δ (Rose et al., 1995) shows defective pseudohyphal growth, while *faa3*Δ shows increased cell size (Jorgensen et al., 2002). The strains *csg1*Δ, *ipt1*Δ and *isc1*Δ were clearly shown to have disrupted actin organization. Both *csg1*Δ and *ipt1*Δ show defective actin depolymerization following salt stress (Balguerie et al., 2002), and *isc1*Δ shows defective actin polarization (Tripathi et al., 2011). Several sphingolipid pathway mutants *elo2*Δ, *elo3*Δ, *sur2*Δ, *csg1*Δ, and *ipt1*Δ were shown to suppress defective actin repolarization in the *rvs161*Δ and *act1-1* mutants (Balguerie et al., 2002).

3.5 Aging/Oxidative Stress

The study of aging and oxidative stress in yeast has led to discoveries with implications for aging diseases in humans (Longo et al., 2012). A highly influential discovery in the yeast sphingolipid field is the finding that deletion of the ceramide synthase gene LAG1, or treatment with the SPT inhibitor myriocin (Huang et al., 2012), led to a dramatic increase in replicative lifespan (D'mello et al., 1994), which is the maximum number of times a single cell can bud. Additionally it was found that LAG1 overexpression decreased replicative lifespan (Jiang et al., 2004). Overexpression of *YDC1* decreases chronological lifespan, the period of time individual cells remain viable, by triggering organelle fragmentation and apoptosis (Aerts et al., 2008). An intriguing wrinkle however is that lipidomic analysis has not revealed any significant difference in the lipid composition resulting from altered Lag1 or Ydc1 activity. Both enzymes are members of redundant pairs consisting of Lac1 and Ypc1 respectively, but both *lac1*Δ and *ypc1*Δ strains have unaltered lifespans. The *lag1*Δ and *ydc1*Δ phenotypes imply that either a ceramide or a sphingoid base mediate a signal that modulates lifespan. However an additional clue is that the complex sphingolipid synthase *csg1*Δ displayed reduced chronological lifespan in a screen for aging factors (Matecic et al., 2010), favoring ceramide over sphingoid bases in mediating the signal. Another intriguing link between sphingolipid production and aging is the involvement of the fatty acid elongase Elo3 in regulating telomere length (Askree et al., 2004; Ponnusamy et al., 2008), leading to shortened lifespan by way of a mechanism involving inositol phosphorylation. Together these phenotypes imply that a VLC ceramide regulates chronological lifespan.

Oxidative stress was placed with aging in Table 1, since the two are often linked, and accumulation of reactive oxygen species (ROS) is a key factor in both (Ikner and Shiozaki, 2005). Phenotypes listed in Table 1 as oxidative stress-related consist largely of sensitivity to an oxidizing agent such as peroxide, in the case of *ypc1*Δ (Higgins et al., 2002), *sur2*Δ (Berry et al., 2011), *csg1*Δ (Berry et al., 2011), *isc1*Δ (Almeida et al., 2008; Berry et al., 2011; Chang et al., 2002), and *elo2*Δ (Berry et al., 2011), or MMS as in *ypc1*Δ (Begley et al., 2002), *scs7*Δ (Begley et al., 2002), and *isc1*Δ (Chang et al., 2002; Matmati et al., 2009). There are also cases of resistance phenotypes such as the *fas1*Δ strain (deleted in the fatty acid synthetase gene FAS1) resistance to hydrogen peroxide (Matias et al., 2007), and the reported increased genomic stability of *lac1*Δ when challenged with the non-proteogenic amino acid canavanine (Alabrudzinska et al., 2011). The *elo1*Δ and *elo2*Δ strains also show ROS accumulation and oxidative stress sensitivity, when transformed with the human α-synuclein protein as part of a Parkinson's disease model (Lee et al., 2011). Taken together,

these phenotypes imply that a VLC PHC or αOH-PHC species is involved in aging and oxidative stress.

3.6 Apoptosis/Autophagy

The study of apoptosis and autophagy has widespread implications for future treatments of cancer (Long and Ryan, 2012) and heart disease (Dutta et al., 2012). A wide body of work has developed detailing the role of sphingolipids in apoptosis and autophagy of human and plant cells. The yeast model has provided insight into potential roles of sphingolipids in some of the autophagic processes, most notably implicating the DHS/PHS in downregulating nutrient transporters in yeast (Chung et al., 2001) and ceramide in human cells, leading to autophagy and eventually cell death (Edinger, 2009). One of the autophagic processes triggered by nutrient deprivation is piecemeal microautophogy of the nucleus (PMN, selective vacuolar degradation of the nucleus). A number of sphingolipid metabolic gene deletions were identified PMN defects (Dawaliby and Mayer, 2010). Specifically, *ypc1*Δ (Dawaliby and Mayer, 2010), *lag1*Δ (Dawaliby and Mayer, 2010; Guillas et al., 2003), *lcb3*Δ, *elo2*Δ and *elo3*Δ (Dawaliby and Mayer, 2010) were reported to have diminished PMN activity, and *tsc13*Δ was reported to have small sized PMN vesicles (Kvam et al., 2005). Overproduction of YPC1 was also shown to increase apoptosis, mitochondrial fragmentation and vacuolar defects (Aerts et al., 2008). The defects associated with the fatty acid elongation pathway (*elo2*Δ, *elo3*Δ, and *tsc13*Δ) imply that VLC fatty acids are involved, while the *ypc1*Δ, *lag1*Δ and *lcb3*Δ defects imply involvement of either a ceramide, sphingoid base or sphingoid base phosphate. Only the complex sphingolipids are disfavored as potential mediating lipids.

3.7 Protein Stability and Localization

Much of the mystery surrounding sphingolipid signaling mechanisms arises from the complexity of the intermolecular interactions in a native lipid membrane (Sezgin et al., 2012). Unless the challenges of studying these complex interactions are overcome, they place a limit on mechanistic understanding of lipid-based signaling mechanisms. The next phenotype heading is protein stability/localization, which is one of the broadest in terms of biological function because phenotypes were selected by possible similarities among their intermolecular mechanisms rather than their probable function in native yeast. Furthermore, localization and stability were grouped together because there is often a connection, although there is evidence of a link in only some of the instances cited here. Interactions described in this section include both native and nonnative proteins, because one goal is to stimulate hypotheses encompassing sphingolipid-protein interactions employing the yeast model.

A fairly large subgroup of proteins whose localization is altered by activity of sphingolipid enzymes shares two properties, first is association with detergent-insoluble plasma membrane (PM)-associated lipid raft domains, and second is dependence on the secretory pathway for localization. Proteins that fall into the former group are the proton pumping H^+-ATPase Pma1, the H^+/arginine anitporter Can1, the GPI-anchored protein Gas1, and the Na^+/H^+-antiporter Nha1. The *tsc3*Δ, *elo2*Δ, and *elo3*Δ mutants show defective Pma1 localization (Gaigg et al., 2005), while the *lcb1–100* mutant shows defective Can1

(Malínská et al., 2003), and Nha1 (Mitsui et al., 2009) localization, and *elo3*Δ shows defective Gas1 localization (Eisenkolb et al., 2002). The *elo2*Δ and *elo3*Δ strains also appeared in a screen for sensitivity to the drug monesin (Gustavsson et al., 2008), which inhibits post-Golgi trafficking.

Closely related to the defective PM localization by the secretory pathway are several protein stability phenotypes. Pma1 (Bagnat et al., 2001) as well as the uracil permease Fur4 (Chung et al., 2000), show loss of stability in the *lcb1–100* strain as well as increased stability in the *elo2*Δ strain (Balguerie et al., 2002; Gaigg et al., 2005) all of which are likely to be related to the PM mislocalization phenotypes. Additionally the tryptophan permease Tat2 shows a similar loss of stability in the *dpl1*Δ strain (Johnson et al., 2010). The human α-synuclein protein was transformed into yeast as a Parkinson's disease model, and it was found that in the *elo1*Δ, *elo2*Δ and *elo3*Δ strains, the protein was more toxic than in the wild type (Lee et al., 2011), likely resulting from altered trafficking of protein to the vacuole. The assortment of implicated sphingolipid genes does not point toward a specific lipid type, but implies a broad sphingolipid requirement for proper protein trafficking.

Another defect related to the secretory pathway is defective secretion of the signaling mucin Msb2. This defect appears in the *tsc13*Δ and *faa3*Δ strains, and interestingly *isc1*Δ showed Msb2 hypersecretion, and ELO2 overproduction gave the same effect (Chavel et al., 2010). This combination of phenotypes indicates that fatty acid transport and elongation are required for proper regulation of the secretion pathway, interestingly *tsc3*Δ is the only gene included that is directly involved in sphingolipid synthesis. Tsc3 is important because it was shown to be essential for production of C20 sphingoid bases, leading to the intriguing hypothesis that C20 sphingoid bases or C20 base-containing ceramides are involved in regulating secretion.

Additional localization defects include mislocalization of phosphoinositide (PI) signaling proteins, nuclear pore complex formation, defective actin organization, and non-selective ER egress. The PI signaling proteins Rom2 and Mss4 fail to localize properly to the PM with SPT inhibition (by myriocin), as well as in the *csg2*Δ and *isc1*Δ strains (Kobayashi et al., 2005). Additional phenotypes include defective nuclear pore complex formation resulting from downregulation of LCB2 and FAS2 using a doxycycline-supressable plasmid (Titus et al., 2010). The protein Rvs161 was mentioned previously, associated with actin, it is added here that Lcb1, Elo2 and Elo3 activity are required for proper localization of Rvs161 (Balguerie et al., 2002). Furthermore, *elo3*Δ shows defective localization of Rvs167 (Germann et al., 2005). Finally the *fat1*Δ strain showed defective selection of protein egress from the ER demonstrated by secretion of the chaperone protein Kar2 (Čopič et al., 2009).

The final phenotype sub-group detailed in this section is that in which sphingolipid mutants alter lipid-peptide interactions. It was found that several sphingolipid mutants show resistance to the specific pore-forming fungicidal peptides syringomycin, PM02734, and K1 killer toxin. Interestingly, the assortments of sphingolipid strains that confer resistance are specific to each peptide. The *sur2*Δ (Kaulin et al., 2005), *csg1*Δ, *csg2*Δ, *ipt1*Δ (Stock et al., 2000), and *elo2*Δ (Soustre et al., 2000) strains are resistant to syringomycin, while *scs7*Δ alone shows resistance to PM02734 (Herrero et al., 2008), and *elo3*Δ (Pagé et al., 2003),

*tsc10*Δ (Pagé et al., 2003), *lcb3*Δ (Pagé et al., 2003), and *ipt1*Δ (Tomishige et al., 2003) strains show resistance K1 killer toxin. The *csg1*Δ, *skn1*Δ and *ipt1*Δ strains are also resistant to the pore forming fungicide *Dahlia merckii* antimicrobial peptide 1 (Thevissen et al., 2000). It should be noted that K1 killer toxin attacks in two steps, first binding the cell wall and then inserting into the PM, and since *elo3*Δ and *tsc10*Δ also have altered cell wall properties (Pagé et al., 2003) (see 'cell wall' section below), the gene deletion may be affecting either or both step(s) in the process. The specificity of mutant combinations matched to each peptide implies that a specific lipid composition corresponds to a specific peptide sequence and/or structure. This observation may have implications for viral or toxin susceptibility, but also for signaling if native proteins bind and activate only when membranes display a specific lipid profile.

3.8 Mating and Sporulation

Mating and sporulation in yeast have largely been studied as genetic tools (Elrod et al., 2009) and in the context of beer and wine production (Maráz, 2002). This category pertains to yeast mating and sporulation pathways, including defective pheromone uptake, defective mating, and defective sporulation. The *lcb1–100* mutant shows blocked uptake of α-factor pheromone by endocytosis (Zanolari et al., 2000). Calcium uptake, which usually accompanies pheromone stimulus, is defective in the *ydc1*Δ, *isc1*Δ, and *lcb5*Δ strains (Martin et al., 2011). Although affecting the same aspect of mating, these phenotypes are mediated through unrelated pathways (endocytosis versus high affinity calcium influx signaling). Several sphingolipid mutants show defective sporulation including *skn1*Δ (Marston et al., 2004), *isc1*Δ (Enyenihi and Saunders, 2003), *elo2*Δ (el-Sherbeini and Clemas, 1995; Enyenihi and Saunders, 2003), *elo3*Δ (Enyenihi and Saunders, 2003), *ifa38*Δ (Enyenihi and Saunders, 2003), and *fat1*Δ (Deutschbauer et al., 2002; Enyenihi and Saunders, 2003). The collection of mutants associated with mating implies that ceramide is essential for regulation of these mating functions.

3.9 Cell Wall

As with mating and sporulation, cell wall functions are specific to yeast, but much of the data connecting sphingolipids to cell wall integrity implicate sphingolipids in more fundamental processes. The *tsc10*Δ and *elo2*Δ strains were shown to be sensitive to cell wall insults such as calcofluor white (Pagé et al., 2003; Ragni et al., 2011) and SDS (Pagé et al., 2003). In some cases more mechanistic detail has been gleaned, such as increased activity of the MAP kinase Slt2 in *elo3*Δ strain indicating that Elo3 is involved in cell wall stress not in cell wall assembly (Ragni et al., 2011). Specific alterations in cell wall properties have been observed such as altered mannose/glucose ratio in *ipt1*Δ, altered levels of beta-1,3-glucans in *elo2*Δ (el-Sherbeini and Clemas, 1995), and *elo3*Δ (Pagé et al., 2003). Also *ypc1*Δ shows high levels of the cell wall component Pir2 in the growth medium (de Groot et al., 2001), implying that activity of the ceramidase is required for proper linkage of this protein to the β-1,3-glucan network. Overall these phenotypes imply that the role of these sphingolipid genes in fundamental pathways, such as endocytosis, vesicular trafficking and GPI-anchored proteins, required for cell wall synthesis, is the basis for these cell wall-related phenotypes. Thus disruption of the yeast cell wall may provide an accessible model for studying the role of sphingolipids in a number of key processes.

3.10 Mitochondria

Yeast is an excellent model for recognizing mitochondrial defects, which are often manifested as poor growth on non-fermentable carbon sources such as ethanol or glycerol, or the appearance of petite colonies (Baile and Claypool, 2013). The involvement of sphingolipids in mitochondrial processes especially respiration has been the focus of a number of studies employing yeast. The *lcb5*Δ (Steinmetz et al., 2002), *isc1*Δ (Kitagaki et al., 2009; Kitagaki et al., 2007; Vaena de Avalos et al., 2005) and *elo3*Δ (Chung et al., 2003) strains all show low growth on non-fermentable carbon sources. A closely related phenotype is the formation of petite colonies resulting from halted growth upon consumption of fermentable carbon sources. All yeast produce a fraction of petite cells, but the *ydc1*Δ (Hess et al., 2009), *lip1*Δ (Tzagoloff and Dieckmann, 1990) and *elo2*Δ (Bianchi et al., 1999) strains produce petites at a higher frequency, implying that these enzymes are required for proper reproduction or maintenance of mitochondria. The *isc1*Δ phenotype was studied in greater detail, and it was found that the ISC1 gene was required for proper regulation of a number of genes required for mitochondrial function (Kitagaki et al., 2009; Vaena de Avalos et al., 2005). Lipid profiles of purified mitochondria showed dramatically diminished ceramide levels in *isc1*Δ, especially in the abundant VLC αOH-PHC species, however this knowledge did not lead to definitive identification of a specific species regulating mitochondrial function (Kitagaki et al., 2007).

Other mitochondrial phenotypes reported include increased expression of the iron regulon in *isc1*Δ (Almeida et al., 2008; Begley et al., 2002), sensitivity of *lcb2*Δ to indomethacin, a drug that targets the mitochondrial cytochrome *Cox1* (Lum et al., 2004), and sensitivity to the respiration-disrupting pathogen *Eutypa lata* (Kim et al., 2004). Additionally *faa2*Δ and *faa4*Δ, deleted in the fatty acid synthetases FAA2 and FAA4, show impaired mitochondrial fatty acid beta oxidation, although this observation alone is not strong evidence that sphingolipids are involved (Faergeman et al., 1997; van Roermund et al., 2001).

The critical dependence of Ypc1, Lip1 and Isc1 activity for proper mitochondrial function implies that ceramide is a key regulator. The *lcb5*Δ phenotype however implies that sphingoid bases or their phosphates play a role in mitochondrial viability as well. As mentioned above, bioinformatics approaches led to identification of a key role for PHS-1 phosphate in regulating the HAP complex which is required for proper mitochondrial function. Furthermore ρ_o cells, lacking their mitochondrial genome, were shown to be highly tolerant to toxicity of exogenous DHS/PHS (Panwar and Moye-Rowley, 2006), an effect shown to be mediated by the retrograde signaling pathway.

3.11 Nutrient Response/Uptake

The study of nutrient responses in yeast has greatly contributed to understanding of nutrient responses in all eukaryotes, and has been applied to the study of nutrient supply in cancer cells (Broach, 2012). This section is focused on nutrient response phenotypes that appear to arise upstream or independent of autophagy. As noted earlier, sphingoid bases were already shown to downregulate expression of nutrient transporters (Chung et al., 2001; Cowart and Obeid, 2007; Guenther et al., 2008), and some of the phenotypes described here are consistent with this observation. Meaning that if the mutation is expected to increase or not

change ceramide, than the phenotype is consistent with lower nutrient transporter expression, *e.g.* more sensitivity to nutrient deprivation. Some phenotypes consistent with transporter downregulation are that the *dpl1*Δ strain shows a 20 fold decrease in Can1-mediated arginine uptake (Robl et al., 2001), *elo3*Δ shows defective glucose uptake by Hxt3 and Snf3 (García-Arranz et al., 1994), and *lcb4*Δ shows sensitivity (weak) to rapamycin (Parsons et al., 2004).

Phenotypes not consistent with this pattern are the *scs7*Δ, *lcb3*Δ and *elo1*Δ strains, which most likely inhibit ceramide synthesis. All show reduced fitness on minimal media, a condition that would be consistent with less, not more, transporter expression. The *elo2*Δ strain shows reduced growth on synthetic complete media without lysine or without tryptophan (Giaever et al., 2002), and *isc1*Δ shows defective growth on minimal media without lysine and without tryptophan (Giaever et al., 2002). In both conditions, reduced ceramide synthesis coincides with increased susceptibility to nutrient deprivation. Finally the *lcb1–100* mutant shows defective uracil uptake after heat shock (Hearn et al., 2003), implying that ceramide synthesis is not required to induce nutrient transport.

These observations reveal that sphingolipids are essential regulators of nutrient responses, but that the mechanism may be quite complex, involving several lipid species. This conclusion is not as surprising since it was previously shown that PHS-1-phosphate (PHS1P) was implicated in activation of the transcription factor Hap4, involved in regulating respiration (Cowart et al., 2010b), and that PHS was implicated in a response to high serine levels (Montefusco et al., 2012).

3.12 Sterol Metabolism

Sphingolipids and sterols (ergosterol in yeast) have been known to physically associate in the membrane, to stabilize the highly asymmetrical ceramide structure (Jacquier and Schneiter, 2012; Simons and Vaz, 2004). Phenotypes that imply regulation of sterol metabolism by sphingolipids are discussed in this section. Several phenotypes consist of altered sensitivity to inhibitors of sterol metabolism, specifically *elo2*Δ and *elo3*Δ are resistant to fenpropimorph (Revardel et al., 1995; Soustre et al., 2000; Toulmay and Prinz, 2012), a fungicide which causes ignosterol accumulation inhibiting uracil uptake (Crowley et al., 1994).The elongase mutants are also resistant to SR31747 (Revardel et al., 1995; Tvrdik et al., 2000), an inhibitor of sterol isomerase activity (Silve et al., 1996). The *skn1*Δ and *ipt1*Δ strains are resistant to miconazole (François et al., 2009), which inhibits ergosterol synthesis by blocking lanosterol demethylation (Kelly et al., 1997). Because Skn1 and Ipt1 are both involved in $M(IP)_2C$ synthesis, this combination of phenotypes strongly implies that VLC-$M(IP)_2C$ is involved in sensitivity to sterol synthase inhibitors.

The phenotypes described above imply that sphingolipids in some way upregulate sterol synthesis; however, *isc1*Δ is sensitive to flucanazole (Parsons et al., 2004), implying the opposite. An additional phenotype that may yield a hypothesis as to a mechanism is the altered non-vesicular sterol transport in the *lcb1–100* strain causing increased free sterols (Baumann et al., 2005). If a fixed sterol/sphingolipid ratio is required for proper function, a loss of sterols might be compensated for by a proportional loss in sphingolipids.

The relationships between sterols and sphingolipids was investigated in detail in {Guan, 2009 #764}, using phenotypic effects of double and triple deletion mutants of the ergosterol and sphingolipid pathways. For example it was found that deletion of *ISC1* increased a *erg3*Δ *erg6*Δ double mutant temperature tolerance defect, as well as the erg3Δ and erg4Δ sorbic acid sensitivity phenotypes, and deletion of SUR2 or SCS7 suppressed slow growth of erg2 a low temperature. It was determined using fluorescent anisotropy that these and other phenotypes were mediated by altered membrane properties. Although this study did not directly identify mechanisms of regulation among the ergosterol and sphingolipid pathways, it illustrates the importance of balanced regulation between the two pathways.

3.13 Calcium Homeostasis/Signaling

Calcium signaling and homeostasis is an essential function of all eurkaryotic cells, and much of the knowledge gained by the study of calcium in yeast is immediately transferable to the study of human disease (Cui et al., 2009). A link between complex sphingolipid synthesis and calcium sequestration was discovered early on with the observation that Csg2 activity is required for growth in high calcium. In the *csg2*Δ strain, a non-exchangable pool of calcium accumulates (Beeler et al., 1994; Takita et al., 1995; Tanida et al., 1996), and this condition is associated with reduced calcinurin activity leading to reduced phosphorylation of phosphoinositide binding protein Slm2 (Tabuchi et al., 2006). A phenotype that is most likely related is a resistance to calcium of the $M(IP)_2C$ synthase mutant *ipt1*Δ (Dickson et al., 1997b), all of which clearly points to MIPC as essential for proper calcium regulation. Although the mechanism for these effects is not understood in detail, this appears to be a rare opportunity to pinpoint a specific lipid, MIPC, regulating a specific protein, the calcium channels.

Additional phenotypes indicate a critical role of sphingolipids in regulating uptake and maintaining calcium homeostasis. The *elo3*Δ strain is sensitive to the calcinurin inhibitor FK506 caused by increased calcium accumulation (Martin et al., 2011; Parsons et al., 2004), and *elo3*Δ releases a greater amount of vacuolar calcium than wild type in response to hyperosmotic shock (Loukin et al., 2008), and these phenotypes corroborate a role for sphingolipids in regulating calcium channels. However, it was also shown that *elo2*Δ and *elo3*Δ have reduced activity of the vacuolar ATPase, a proton pump necessary for vacuolar ion transport (Chung et al., 2003), hinting at the possibility of a more fundamental role for sphingolipids in ion homeostasis.

Finally it was found that in the *dpl1*Δ strain, calcium uptake was increased with exogenous sphingosine (Birchwood et al., 2001; Saba et al., 1997). This observation led to the finding that PHS1P strongly stimulated calcium uptake, possibly through a novel calcium channel. This mechanism emphasizes the multi-faceted nature of calcium regulation by the sphingolipids.

3.14 Salt Stress

Salt stress is closely related to calcium homeostasis since it is mitigated by calmodulin/calcinerin activation of P-type ATPases (Giaever et al., 2002). A number of screens have identified sphingolipid mutants only with increased sensitivity to salt stress, and none of the

strains showed decrease sensitivity. The *lag1*Δ, *lcb4*Δ, *csg1*Δ, *csh1*Δ, *elo2*Δ, *ifa38*Δ, *faa3*Δ (Kallay et al., 2011), *faa1*Δ (deleted in the fatty acid importers/activators FAA3 and FAA1), *ipt1*Δ (Giaever et al., 2002; Kallay et al., 2011), *scs7*Δ (Giaever et al., 2002), *isc1*Δ (Betz et al., 2002; Giaever et al., 2002), *fas1*Δ (de Jesus Ferreira et al., 2001), *sur2*Δ (Barreto et al., 2011) show sensitivity to NaCl, KCl and/or LiCl.

Tolerance to salt stress also requires osmotolerance mediated by the HOG pathway (Brewster et al., 1993), which was shown to be regulated by sphingolipids (Tanigawa et al., 2012). The *elo2*Δ and *elo3*Δ strains had elevated Hog1 activation by phosphorylation leading to increased transcription of target genes, while the *sur2*Δ, *csg1*Δ, *csh1*Δ, and *ipt1*Δ strains did not. The authors additionally used the complex sphingolipid synthesis inhibitor aureobasidin to conclude that DHC-IPC is the specific lipid mediating activation of Hog1. In the case of the latter four deletion strains, sensitivity to salt stress is not likely to be mediated through this pathway.

3.15 Drug Sensitivity

The study of drug sensitivity in yeast advances two major areas, first is the possibility of identifying fundamental relationships between membrane composition and permeability to drugs either by diffusion or by regulation of membrane channels, and second is the identification of novel sphingolipid-dependent signaling pathways. Deletion of the phosphoryltransferase gene IPT1, required for synthesis of $M(IP)_2C$, causes a multi-drug sensitivity phenotype (Hallstrom et al., 2001) in both *S. cerevisiae* and *Candida albicans* (*Prasad et al., 2005)*. Additionally deletion strains of the equivalent genes of IPT1, ELO2 and ELO3 in *C. albicans* showed multidrug sensitivity phenotypes attributed to improper localization of the membrane channel Cdr1 (Pasrija et al., 2008). In these cases it appears that the loss of long chain and very long chain $M(IP)_2C$ led to increased permeability most likely through a combination of diffusion and loss of membrane channel activity.

The drug sensitivity category also highlights altered sensitivity to drugs or treatments that have not been pinned to specific pathways or molecular targets. Thus the importance of these phenotypes may emerge, as targets for each drug are characterized in greater detail. This category is very general due to the variety of different drugs represented, thus these phenotypes are not discussed in detail, but are summarized in Table 2.

3.16 Unclassified Phenotypes

The final category is the miscellaneous phenotypes (Table 3) largely consisting of general phenotypes that are difficult to link to specific pathways. This includes phenotypes related to osmotic stress (Giaever et al., 2002; Mira et al., 2010; Tomishige et al., 2003), reduced tolerance to altered pH (Giaever et al., 2002; Mira et al., 2010; Tomishige et al., 2003), vacuolar defects (Aerts et al., 2008; Chung et al., 2003; Marash and Gerst, 2001), and ethanol tolerance (Kajiwara et al., 2000; Kim et al., 2011). These phenotypes may be of value to researchers attempting to link distant pathways with a common physiological output.

4. Conclusions

Tremendous progress has been made in pinning downstream functions to specific lipid groups, and in the case of the sphingoid bases and base phosphates, the level of mechanistic detail is quite advanced (Cowart and Obeid, 2007; Cowart et al., 2010b). Sphingolipid regulation of some of the functions discussed here such as protein trafficking, cell cycle regulation, and calcium homeostasis are fairly well studied (Cowart and Obeid, 2007; Dickson, 2008). However the multitude of lipid species and their hydrophobicity are challenges to pinpointing functions for individual ceramides and complex sphingolipids.

Some novel high-throughput methodologies show promise in overcoming these challenges. Bioinformatics methods have been employed that can resolve connections between lipid behavior and gene expression when the cell is challenged (Cowart et al., 2010b)(Montefusco et al in press). These methods have led to identification of a novel role for PHS1P (Cowart et al., 2010b) as well as novel and distinct functions for long chain (LC) and VLC DHCs (Montefusco et al in press). Novel high throughput methodology also demonstrated binding of the known sphingolipid effector Ypk1 to C18-PHC and DHC (Gallego et al., 2010). Continued development of these types of methodologies is critical to overcoming the challenges of sphingolipid signaling.

The goal of this article is to create a landscape overview of sphingolipid signaling in yeast. The two major sections of the article represent the two ends of the landscape, first is a detailed biochemist's view of the yeast sphingolipidome, and second is the biologist's view of the cell reacting to changes in the sphingolipidome. The aim of the sphingolipid signaling field is to connect these two views and eventually deduce the signaling function of each species.

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Highlights

The S. *cerevisiae* model and how changes in sphingolipid metabolism affect other critical signaling pathways.

A complete view of the yeast lipidome requires integration of data produced by several research groups.

A model sphingolipidome was assembled here by integrating several datasets focused on different lipid subgroups.

It is difficult to pinpoint effects of individual lipid metabolites through observation of individual gene deletion phenotypes.

A nearly comprehensive survey of single gene deletion phenotypes of the sphingolipid metabolic pathway are analyzed here.

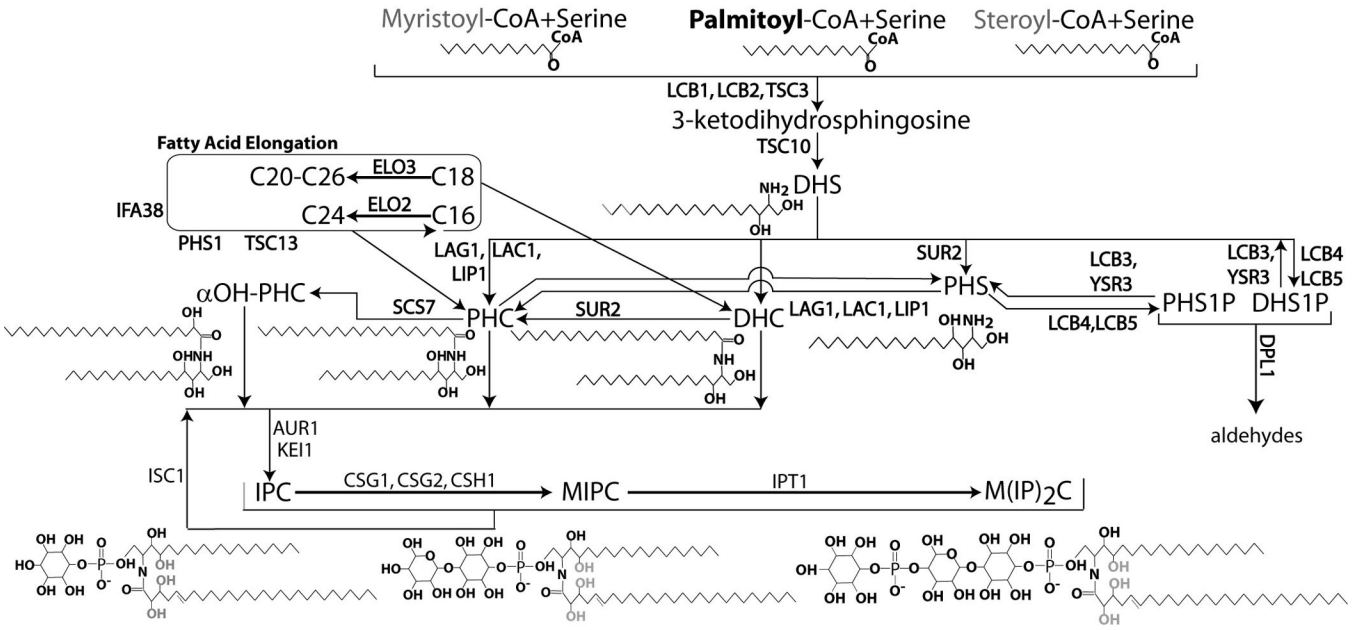

Figure 1.

The Yeast Sphingolipid Metabolic Pathway. This pathway schematic includes incorporation of myristate and stearate to make C16 and C20 sphingoid bases.

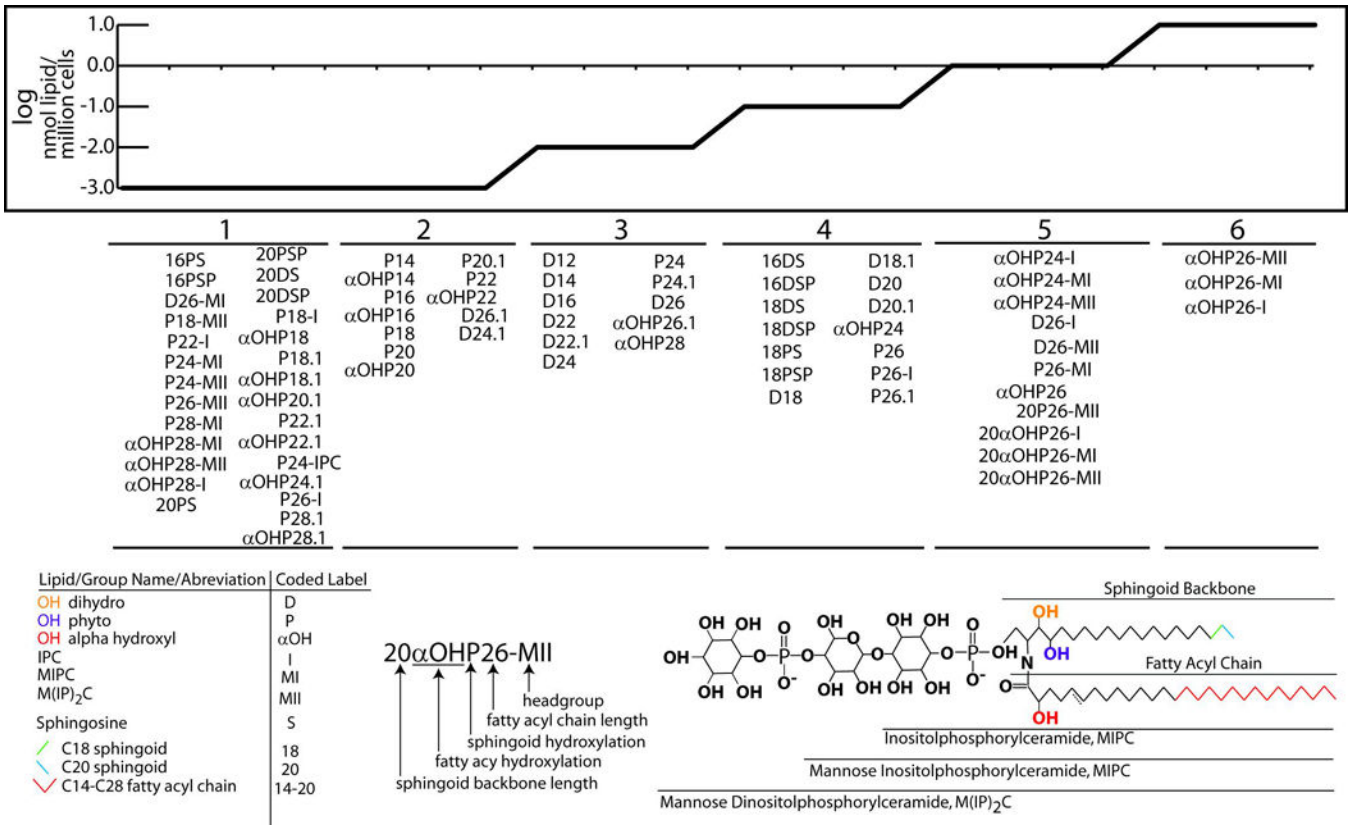

Figure 2.

The model yeast sphingolipidome. The bottom left shows the structure of a model yeast sphingolipid to assist in visualization of the model lipidome. The bottom left gives the trivial lipid name, the abbreviated names used in this figure, and specifies the corresponding portion of the model sphingolipid. Lipids belonging to each of the six lipid groups are listed together.

Table 1

Quick reference table to identify categories of phenotypes represented in mutants of the specified gene.

Gene Mutated	Phenotype Category
LCB1	temp. tol., ER st./UPR, c. cyc., act./morph., ag./ox. str., pr. stab./loc., mat./spor., nu. res./up., ster., act./morph.
LCB2	act./morph., pr. stab./loc., mito., Ca^{2+}
TSC3	temp. tol., pr. stab./loc.
TSC10	act./morph., pr. stab./loc., c. wall
YPC1	ag./ox. str., aut./apop., c. wall, mito.
YDC1	temp. tol., ag./ox. str., aut./apop., mat./spor., mito.
LAG1	ag./ox. str., aut./apop., salt st.
LAC1	ag./ox. str., aut./apop.
LIP1	mito.
SUR2 (SYR2)	act./morph., ag./ox. str., pr. stab./loc., salt st.
SCS7	c. cyc., act./morph., ag./ox. str., pr. stab./loc., nu. res./up, salt st.
YSR3	temp. tol., salt st.
LCB3(YSR2)	temp. tol., c. cyc., aut./apop., pr. stab./loc., nu. res./up.
LCB4	nu. res./up., salt st.
LCB5	temp. tol., c. cyc., act./morph., mat./spor., mito.
DPL1	temp. tol., c. cyc., pr. stab./loc., nu. res./up., Ca^{2+}
ORM1	ER st./UPR
ORM2	ER st./UPR
AUR1	act./morph., c. wall
KEI1	pr. stab./loc.
CSG1 (SUR1)	c. cyc., act./morph., ag./ox. str., pr. stab./loc., salt st.
CSG2	ER st./UPR, C. cyc., act./morph., pr. stab./loc., Ca^{2+}
CSH1	salt st.
SKN1	pr. stab./loc., mat./spor., ster.
IPT1	act./morph., pr. stab./loc., c. wall, ster., Ca^{2+}, salt st.
ISC1	ER st./UPR, c. cyc., act./morph., ag./ox. str., pr. stab./loc., mat./spor., mito., nu. res./up., ster., salt st.
FAS1	ag./ox. str., salt st.
FAS2	pr. stab./loc.
ELO1	ag./ox. str., pr. stab./loc., nu. res./up.
ELO2	temp. tol., ER st./UPR, act./morph., ag./ox. str., aut./apop., pr. stab./loc., mat./spor., c.
(FEN1)	wall, mito., nu. res./up, ster., Ca^{2+}, salt st.
ELO3	ER st./UPR,. c. cyc., act./morph., ag./ox. str., aut./apop., pr. stab./loc., mat./spor., c.
(SUR4)	wall, mito., nu. res./upl., ster., Ca^{2+}
IFA38	temp. tol., ag./ox. str., mat./spor., salt st.
TSC13	aut./apop., pr. stab./loc.
OLE1	temp. tol., c. cyc

Gene Mutated	Phenotype Category
FAA1	salt st.
FAA2	mito.
FAA3	ag./ox. str., pr. stab./loc., Ca^{2+}, salt st.
FAA4	mito., Ca^{2+}
FAT1	temp. tol., ER st./UPR., pr. stab./loc., mat./spor., ster.

Abbreviated names for phenotype categories: temp. tol. – temperature tolerance, ER st./UPR – ER stress/unfolded protein response, c. cyc. – cell cycle, act./morph. – actin/morphology, ag./ox. str. – aging/oxidative stress, aut./apop. – autophagy/apopotosis, pr. Stab./loc. – protein stability/localization, mat./spor. – mating/sporulation, c. wall – cell wall, mito. – mitochondria, nut. Res./up. – nutrient resonse/uptake, ster. – sterol metabolism, Ca^{2+} - calcium homeostasis/signaling, salt st. – salt stress

Table 2

Drug resistance phenotypes.

Mutant	Drug	Effect	Reference
lcb1+/−	cymoxanil	S	(Baetz et al., 2004)
	hygromycin B, spermine, tetramethylammonium	S	(Barreto et al., 2011)
*sur2*Δ	canavanine, sodarin	R	(Botet et al., 2008; Shi et al., 2011)
*scs7*Δ	mycophenolic acid, cycloheximide, camptothecin, tunicamycin	S	(Parsons et al., 2004; Riles et al., 2004)
SCS7 o/x	edelfosine	S	(Zaremberg et al., 2005)
*lcb3*Δ	australifungin	S	(Kobayashi and Nagiec, 2003)
	canavanine, hygromycin B	R	(Qie et al., 1997)
*lcb5*Δ	cordycepin, benomyl	S	(Holbein et al., 2009)
*orm1*Δ	tunicamycin	S	(Han et al., 2010)
*orm2*Δ	tunicamycin	S	(Han et al., 2010; Hjelmqvist et al., 2002)
*csg1*Δ	caffeine	S	(Ragni et al., 2011)
*csg2*Δ	arsenite, duramycin, sulfometuron methyl	S	(Parsons et al., 2004; Roelants et al., 2009; Thorsen et al., 2009)
*ipt1*Δ	canavanine	R	(Shi et al., 2011)
*isc1*Δ	hydroquinine, cycloheximide, flucanazole	S	(North et al., 2011; Parsons et al., 2004)
*fas1*Δ	indomethacin, sulfinpyrazone	S	(Lum et al., 2004)
*elo2*Δ	edelfosine, staurosporine, P. aeruginosa pyocyanin toxin, monensin, australofungin, fumonisin B, wortmanin, multidrug*	S	(Bianchi et al., 1999; Gustavsson et al., 2008; Kobayashi and Nagiec, 2003; Parsons et al., 2004; Ran et al., 2003; Zaremberg et al., 2005)
	echinocandins, caffeine, vanadate, canavanine, K28 A/B Killer Toxin	R	(Bianchi et al., 1999; Carroll et al., 2009; Dickson and Lester, 1999a; Shi et al., 2011)
*elo3*Δ	edelfosine, australofungin, sulfometuron methyl	S	(Kobayashi and Nagiec, 2003; Parsons et al., 2004; Zaremberg et al., 2005)
	canavanine, fenpropimorph, K28 A/B Killer Toxin	R	(Carroll et al., 2009; Shi et al., 2011; Toulmay and Prinz, 2012)
*ifa38*Δ	gliotoxin	R	(Chamilos et al., 2008)
*tsc13*Δ	K28 A/B killer toxin	R	(Carroll et al., 2009)
*fat1*Δ	cisplatin	R	(Lanthaler et al., 2011)

The mutant listed is either more sensitive (S) or resistant (R) to the drug than the wild type strain.

Table 3

Uncharacterized phenotypes.

Mutant	Effect	Reference
*ydc1*Δ	defective vacuoles	(Aerts et al., 2008)
*lip1*Δ	lipoic acid deficient	(Tzagoloff and Dieckmann, 1990)
*sur2*Δ	reduced fitness at pH 8 1.5 M sorbitol sensitive, acetic acid	(Giaever et al., 2002)
*scs7*Δ	sensitive	(Giaever et al., 2002; Mira et al., 2010)
*lcb4*Δ	1.5 M sorbitol sensitive	(Giaever et al., 2002)
*lcb5*Δ	$NiSO_4$ sensitive acetic acid sensitive, increase Cwp1	(Arita et al., 2009)
*csg1*Δ	protein with sorbitol	(Tomishige et al., 2003)
*csg2*Δ	increase Cup1 protein with sorbitol, decreased Ypk1,Fpk1 phosphorylation, organic acid sensitive	(Roelants et al., 2009; Schuller et al., 2004; Tomishige et al., 2003)
*isc1*Δ	acetic acid sensitive	(Mira et al., 2010)
*elo1*Δ	1.5 M sorbitol sensitive	(Giaever et al., 2002)
*elo2*Δ	pH 7.5 growth defect, defective vacuolar function, low Vma1,Vma2,Vma3 in vacuolar membranes, Sso2 not phosphorylated	(Chung et al., 2003; Marash and Gerst, 2001)
*elo3*Δ	growth defect high dextrose, acetic acid sensitive, low pH sensitive, less Pma1 protein, low Vma1,Vma2,Vma3 in vacuolar membranes	(Dickson and Lester, 1999a; Mira et al., 2010; Pitoniak et al., 2009)
*ole1*Δ	ethanol tolerant at low temperature, increased ethanol production	(Kajiwara et al., 2000; Kim et al., 2011)
*faa2*Δ	elevated pH when grown on glycerol and oleate	(Ashrafi et al., 1998)

Phenotypes that did not fit into the selected phenotype categories.