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Yeast sphingolipid metabolism: clues and connections[1,2]

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Abstract: This review of sphingolipid metabolism in the budding yeast *Saccharomyces cerevisiae* contains information on the enzymes and the genes that encode them, as well as connections to other metabolic pathways. Particular attention is given to yeast homologs, domains, and motifs in the sequence, cellular localization of enzymes, and possible protein–protein interactions. Also included are genetic interactions of special interest that provide clues to the cellular biological roles of particular sphingolipid metabolic pathways and specific sphingolipids.

Key words : yeast, sphingolipid metabolism, subcellular localization, protein–protein interactions, stress response, aging.

Résumé : Cette revue du métabolisme des sphingolipides chez la levure *Saccharomyces cerevisiae* en bourgeonnement contient des informations sur les enzymes et les gènes les codant, ainsi que sur les liens avec d'autres voies métaboliques. Une attention particulière est portée aux protéines homologues chez la levure, aux domaines et motifs dans la séquence, à la localisation cellulaire des enzymes et aux interactions potentielles entre protéines. De plus, nous incluons également les interactions génétiques ayant un intérêt particulier parce qu'elles donnent des indices sur les rôles biologiques de sphingolipides spécifiques et de voies métaboliques particulières des sphingolipides dans ces cellules.

Mots clés : levure, métabolisme des sphingolipides, localisation intracellulaire, interactions entre protéines, réponse à un stress, vieillissement.

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Introduction

Sphingolipids are complex membrane lipids known to be important in cellular structures and are now recognized to have equally important roles in cellular function and regulation (Funato et al. 2002; Hannun and Obeid 2002; Jenkins 2003; Obeid et al. 2002; Spiegel and Milstien 2003). To understand regulation and function of sphingolipids, it is imperative to study sphingolipid metabolism. Sphingolipids usually consist of three structural elements, the sphingoid backbone, or long chain base (LCB), which may have an additional very long chain fatty acid (VLCFA) amide linked to the base, and may have a polar head group linked at the C1 carbon. The particular length of the fatty acid, hydroxylation and saturation sites, and head group substituents provide considerable variety in the specific sphingolipids found within a given organism as well as across species. The chemical details may vary, but the metabolic pathways and respective enzymes are highly conserved across plant, fungi, and animal kingdoms. The yeast *Saccharomyces cerevisiae* has served as a unique model to uncover sphingolipid metabolic pathways and has emerged as a scaffold upon which sphingolipid metabolism and function can be elucidated.

The sphingolipid pathway is directly connected to several other metabolic pathways, including fatty acid synthesis and elongation, sterol metabolism, serine utilization, and phospholipid synthesis and breakdown. To understand the response to environmental stimuli, one must explore those connections and how various environmental changes affect the interrelationships between pathways. Another critical aspect of the functioning of sphingolipids as cellular signal transducers is their localization. It is known that some sphin-

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golipid enzymes are anchored in membranes and that sphingolipids are transported among different cellular compartments. Many details of this differential localization are still to be explored. Along with the localization information, it is necessary to investigate which other proteins may interact with those of the sphingolipid pathway.

During the past few years, several methods have been developed for the purpose of shedding light on each of these areas (Bader et al. 2003). Since the sequencing of the yeast genome, high-throughput large-scale analyses have been conducted to determine subcellular localization of the proteome (Huh et al. 2003; Kumar et al. 2002), protein interaction networks (Gavin et al. 2002; Ho et al. 2002), transcriptomes for numerous experimental conditions (Boer et al. 2003; Brown et al. 2001; Causton et al. 2001; Cho et al. 1998; Horak et al. 2002; Posas et al. 2000; Tadi et al. 1999), regulatory modules (Jelinsky et al. 2000; Tavazoie et al. 1999), and transcript and protein levels (Ghaemmaghami et al. 2003). Additionally, novel bioinformatics based approaches have provided clues for finding homologs, conserved motifs, common promoter elements, and regulatory modules. It can be difficult to compare results from these diverse analyses, and each has advantages and biases that must be considered (Bader and Hogue 2002; von Mering et al. 2002). For instance, two-hybrid interaction analysis can have a 50% false positive rate but is unbiased towards low abundance proteins, whereas mass-spectrometry-based methods such as tandem affinity purification (TAP) can identify multimember complexes but are biased towards high-abundance proteins. Online databases now such as the Comprehensive Yeast Genome Database (CYGD), the *Saccharomyces* Genome Database (SGD), and the Yeast Protein Database (YPD) are immensely helpful in teasing out the details of a metabolic pathway in yeast (Garrels 2002). These yeast databases and several global analysis websites are easily accessed from yeast Microarray Global Viewer (yMGV), which provides a visualization and data mining tool for genome-wide yeast expression data (Le Crom et al. 2002; Marc et al. 2001).

This review summarizes information on sphingolipid metabolism in yeast, with emphasis on the enzymes of sphingolipid metabolism, their cellular localization, and their interactions as a means to shed light on their regulation. It contains pertinent information retrieved from the above-mentioned databases and describes some of the more interesting mutant phenotypes as well as connections to other metabolic pathways. The function, localization, and biological processes for the gene products mentioned in this review will follow the Gene Ontology nomenclature as used by YPD and SGD. Unless specifically referenced, information about a given gene was taken from SGD, YPD, and (or) MIPS (Garrels 2002). For clarity of organization, the review divides the sphingolipid pathway into three units: de novo synthesis, complex lipids, and the LCB-phosphates.

Components of sphingolipid metabolism

De novo synthesis

Serine palmitoyltransferase in yeast

The first committed step of de novo synthesis produces the sphingoid backbone 3-ketosphinganine (3-ketodihydrosphingosine) from the condensation of serine and palmitoyl-CoA by the serine palmitoyltransferase complex, releasing CO_2 and CoA. The complex has two homologous subunits encoded by *LCB*1 and *LCB*2 (long chain base) (EC 2.3.1.50) (Pinto et al. 1992) and a post-translational activator encoded by *TSC*3 (temperature-sensitive suppressor of calcium sensitivity) (Gable et al. 2000). The reaction requires a pyridoxal phosphate cofactor (Weiss and Stoffel 1997) and both subunits contain a sequence motif, (D/E)XXXX(S/T)XXKX-(L/F)GXXGG(F/Y), which is similar to the consensus pyridoxal phosphate binding sequence (Funato et al. 2002).

Evidence suggests Lcb1p and Lcb2p are localized to the endoplasmic reticulum (ER). They were found to be integral membrane proteins and to precipitate with membrane fractions (Gable et al. 2000; Nagiec et al. 1994). In addition, Lcb1p belongs to the ER, as determined in a recent study using high-throughput immunolocalization of epitope-tagged gene products and subsequent extrapolation using a Bayesian based algorithm (Kumar et al. 2002). Lcb1p and Lcb2p were determined to be heterodimers by coimmunoprecipitation and size exclusion chromatography (Gable et al. 2002, 2000). Lcb1p has no predicted transmembrane domains, but Lcb2p has two.

Tcs3p was also found to be an integral membrane protein and to localize with the serine palmitoyltransferase complex at the ER membrane as it coimmunoprecipitates with Lcb1p and Lcb2p at low salt concentration (0.3 M NaCl), but not at high concentration (0.6 M NaCl). Tsc3p is associated with membranes as determined by cofractionation with microsomes and detergent solubilization (Gable et al. 2000). No known homologs of Tsc3p are found in other organisms, but there are fungal orthologs. Tsc3p has one predicted 23-amino-acid transmembrane domain, and PROSITE indicates one ATP–GTP binding site motif A (P-loop), AKRRNGKS.

The serine–palmitoyl-CoA condensation reaction is optimized by the *TSC*3 product possibly by interacting with palmitoyl-CoA. In microsomes the reaction is inhibited by palmitoyl-CoA in excess of the optimal concentration of 0.2 mM (Gable et al. 2000). Deletion of *TSC*3 is lethal at high temperatures, possibly because increased de novo sphingolipid synthesis is needed for cell viability at higher temperatures. Recently, *lcb*2 dominant suppressor mutants were characterized that negated the need for Tsc3p for cell viability at elevated temperatures. These mutants showed increased Tsc3p-independent SPT activity (Monaghan et al. 2002).

The SPT complex appears to be regulated at multiple levels. *LCB*2 may be regulated transcriptionally, as transcripts decrease 2.5-fold late post the diauxic shift (DeRisi et al. 1997). The *LCB*2 promoter contains two copies of an 11-bp presumptive sterol regulatory sequence element, and the *LCB*1 promoter contains one (Vik and Rine 2001). Recent work has suggested sterol regulation of some parts of the sphingolipid pathway, particularly the hydroxylation states of LCBs and the complex sphingolipids (Swain et al. 2002).

Emerging evidence indicates that components of the SPT complex are involved in protein–protein interactions. Gavin et al. (2002) performed a high-throughput protein complex study by affinity precipitation, and the results showed that Lcb2p associates with a complex known as Kap104p that functions in protein and RNA transport. This 20-member

complex also includes Lcb1p. Other complex members of particular interest are Bmh1p and Bmh2p, two proteins that exhibit DNA binding function and are involved in the processes of Ras protein signal transduction, mitogen-activated protein kinase (MAPK) pseudohyphal growth, and sporulation. Other proteins in the Kap104p complex include an ER-to-Golgi transporter, a phosphate transporter, and a protein involved with cell wall biogenesis. A recent high-throughput two-hybrid study shows that Lcb1p associates with Shq1p and YDR365c, proteins of small nucleolar ribonucleoprotein particle (snoRNP) metabolism and of unknown function, respectively (Ito et al. 2001).

Long chain base formation

Dihydrosphingosine is rapidly formed from 3-ketosphinganine by the NADPH-dependent oxidoreductase (EC 1.1.1.102) encoded by *TSC*10 (Beeler et al. 1998). This protein is a member of the short-chain dehydrogenase and reductase family designated by adh_short in the Protein families database (Pfam). Null mutants of *TSC*10 are inviable without exogenous supply of long chain bases (Beeler et al. 1998). Tsc10p has one predicted transmembrane domain that is 23 amino acids in length (Krogh et al. 2001), is predicted to be integral to the ER membrane, and cofractionates with microsomes (Beeler et al. 1998). YMR226c, an oxidoreductase involved in serine metabolism, has 25% identity to *TSC*10.

*TSC*10 mRNA had reduced abundance in null mutants of the RNA polymerase II transcription factor Paf1p, indicating possible regulation (Chang et al. 1999). In the two-hybrid study by Ito et al. (2001), Tsc10p was found to interact with Aar3p, a putative aryl alcohol dehydrogenase involved in aldehyde metabolism. Affinity precipitation studies indicate that Tsc10p belongs to the 16-member complex of Tps1p, an α,α-trehalose-phosphate synthase (Gavin et al. 2002). This complex functions in carbohydrate metabolism and stress response, and is a probable regulator of glucose influx into the cell and into the glycolytic pathway (Voit 2003). Of note is that yeast become thermotolerant by accumulating trehalose, and this may be mediated at least in part by de novo synthesis of sphingolipids in response to heat stress. Tsc10p complexing with Tps1p may explain this dual regulation.

Long chain base hydroxylation

Sur2p/Syr2p catalyzes hydroxylation of dihydrosphingosine or dihydroceramide at the C4 position to produce phytosphingosine or phytoceramide (Grilley et al. 1998; Haak et al. 1997). This protein is named for suppression of *RVS*161 (reduced viability upon starvation), as well as for syringomycin response protein (Syr2p). Sur2p has four predicted transmembrane domains and is integral to the ER membrane. It contains a sterol desaturase domain and an eight-histidine motif grouped into three characteristic clusters that may bind a catalytically active di-iron cluster (Cliften et al. 1996). Sur2p has 33% identity to Erg3p and 52% identity to Erg125p (Grilley et al. 1998), a C-5 sterol desaturase and C-4 sterol methyl oxidase, respectively.

*SUR*2 expression may be regulated by the cell cycle, as its mRNA abundance peaks during the G1 phase (Spellman et al. 1998). *SUR*2 shows synthetic lethality with *CSG*2 (Haak et al. 1997) and *RVS*161 (Desfarges et al. 1993). The null mutant is viable, but has only dihydrosphingosine, whereas wild type cells mostly contain phytosphingosine (Haak et al. 1997). Additionally, null mutants exhibit a 10-fold increase in DHS-1-P (Kim et al. 2000) and have abnormal morphology that includes dumbbell-like forms in stationary phase (Desfarges et al. 1993; Takita et al. 1995).

Long chain base acylation

*LAG*1 was first identified as a longevity assurance gene involved in cell aging (D'Mello et al. 1994), and since has been implicated in C26 CoA-dependent yeast ceramide synthesis (Guillas et al. 2001) and ER-to-Golgi transport of glycosylphosphatidylinositol (GPI) anchored proteins (Barz and Walter 1999). The 73% identical *LAC*1 (longevity-assurance gene 1 cognate) has also been identified as essential for acyl-CoA-dependent ceramide synthesis (Guillas et al. 2001; Schorling et al. 2001) and transport of GPI-anchored proteins to the Golgi from the ER (Barz and Walter 1999). As yet, the specific molecular function for both proteins is unknown but is thought to be ceramide synthesis or regulation thereof. Both are located in the endoplasmic reticulum and contain consensus ER retention signal sequences KKXX or KXKXX (Barz and Walter 1999), both contain the lag1p domain (Jiang et al. 1998) found in ~40 other proteins (Venkataraman and Futerman 2002), and both have seven predicted transmembrane domains. The synthesis of ceramide mediated by *LAG*1/*LAC*1 requires the availability of C26 fatty acids which are formed in a series of fatty acid synthesis and elongation steps by *ACB*1, *ACC*1, *FAS*1, *FAS*2, *FEN*1/*ELO*1, *SUR4/ELO2*, and *TSC*13/*ELO*3 (Gaigg et al. 2001; Han et al. 2002; Kobayashi and Nagiec 2003; Kohlwein et al. 2001; Oh et al. 1997; Trotter 2001).

Single null mutants of *LAG*1 and *LAC*1 are viable and display wild type growth. Two background strains have been used for the double null mutants with different results. The W303 strain used by Barz et al. (1999) produces double nulls that exhibit poor growth and have defects in the cell wall, whereas the YPK9 strain used by Jiang et al. (1998) produces inviable double nulls (Barz and Walter 1999; D'Mello et al. 1994; Jiang et al. 1998) Deletion of *LAG*1 increases the mean and maximum lifespan of *S. cerevisiae* by ~50%, and *LAG*1 steady-state mRNA abundance decreases with replicative age (D'Mello et al. 1994). *LAC*1 is one of 71 genes with peak expression in S phase (Spellman et al. 1998) and one of 40 genes induced in a *ROX*1 null mutant (Kwast et al. 2002) that misexpresses several heme-regulated genes. This is not surprising, as *ROX*1 is a heme-dependent transcriptional repressor of hypoxic genes, thus implying that ceramide synthesis could be regulated under hypoxic conditions.

High-throughput two-hybrid analysis shows that Lac1p interacts with Lag1p and Yaf9p, the latter being a yeast chromatin modifying complex (Ito et al. 2001). Another two-hybrid study showed Lac1p interaction with Tem1p, a GTP-binding protein of the *Ras* superfamily involved in termination of M phase. Tem1p associates with over a hundred proteins, including some heat shock proteins (Uetz et al. 2000). Additionally, Lac1p was shown by affinity precipitation to associate with *ESA*1 and *EPL*1, two histone acyltransferase (Gavin et al. 2002). Lag1p associates with Nup116p, a structural molecule that is part of a nuclear pore involved with nucleocytoplasmic transport (Ito et al. 2001).

Both Lac1p and Lag1p associate with the product of YMR298w, which is uncharacterized but also associates with Cdc24p and Nup4p, a calcium binding protein involved in budding and shmooing and a structural protein of the nuclear pore complex, respectively (Ito et al. 2001). All of these associations are of great interest considering the role *LAG*1 plays in yeast aging and ceramide in mammalian senescence.

Ceramidases

Two homologous yeast alkaline ceramidases, Ypc1p and Ydc1p, have been identified that preferentially deacylate phytoceramide and dihydroceramide, respectively, releasing free fatty acid from the sphingosine backbone. Each enzyme has reduced activity towards the others' substrate, and neither hydrolyzes unsaturated mammalian-type ceramide. Both enzymes exhibit highest activity at pH > 9. Ypc1p has major reverse activity that forms phytoceramide or dihydroceramide from palmitic acid and phytosphingosine or dihydrosphingosine independently of the CoA required in the *lag*1/*lac*1-dependent ceramide synthase step described above. Ydc1p has minor reverse activity in vitro only with dihydrosphingosine (Mao et al. 2000*a*, 2000*b*).

*YPC*1 and *YDC*1 have 52% identity with several highly conserved domains. Both sequences contain the ER retention sequence, KKXX, at their carboxyl termini and have several predicted transmembrane domains, suggesting localization to the ER membrane. GFP tagging confirmed ER localization for both proteins. Ydc1p and Ypc1p have putative cAMP and PKC phosphorylation sites, and Ydc1p has a tyrosine kinase phosphorylation site (Mao et al. 2000*b*). Both proteins contain an alkaline ceramidase domain (Pfam domain: aPHC) at amino acids 13–313 for *YPC1* and amino acids 14–313 for *YDC*1.

Both single-deletion mutants as well as the double mutant are viable under normal conditions; however, Δ*ydc*1 shows greater sensitivity to heat stress than either Δ*ypc*1 or the wild type, while the double mutant shows intermediate sensitivity to heat stress (Mao et al. 2000*b*). The quadruple mutant Δ*lag*1Δ*lac*1Δ*ypc*1Δ*ydc*1 is viable in the W303 background strain with no detectable levels of ceramides or complex sphingolipids, but has two unknown lipids that are alkali stable and are also found in the *lag*1Δ*lac*1Δ mutant (Schorling et al. 2001).

Transcription under acidic conditions of both *YDC*1 and *YPC*1 depends on the stress response transcription factors Msn2p and Msn4p, as demonstrated by microarray analysis (Causton et al. 2001). *YDC*1 is one of 273 genes possibly regulated transcriptionally by Ndd1p, a transcription activator specific to the G2–M phase of the mitotic cell cycle, as determined by large-scale chromatin immunoprecipitation (Horak et al. 2002). The mRNA level of *YPC*1 is altered by environmental conditions, as demonstrated in a systematic Northern analysis of more than 1000 genes. The mRNA abundance increased during ammonium starvation, heat shock, hyperosmolarity, and stationary phase, but decreased during an upshift of glucose (Brown et al. 2001).

Complex sphingolipids

Inositol phosphorylceramide synthase

The first complex sphingolipid, inositol phosphorylceramide (IPC), is formed by transferring a *myo*-inositol phosphate group from phosphatidylinositol to the C_1-hydroxyl of ceramide with the concomitant release of diacylglycerol (DAG). *AUR*1 encodes the IPC synthase or a subunit of the enzyme that catalyses this reaction. *AUR*1 is an essential gene (Winzeler et al. 1999) and mutants accumulate ceramide in the presence of phytosphingosine, leading to cell death (Nagiec et al. 1997). Null mutants have altered morphology, including loss of microtubules and tubulin (Hashida-Okado et al. 1996). *AUR*1 is not required for GPI-anchor remodeling (Reggiori and Conzelmann 1998).

Aur1p contains a domain that belongs to the *PAP*2 superfamily (i.e., type 2 phosphaditic acid phosphatases) (Levine et al. 2000), and this is one of four conserved domains shared by several pathogenic fungal homologs, as well as by Ipt1p (Heidler and Radding 2000). There are several predicted transmembrane segments that overlap with these conserved domains.

Levine et al. (2000) conducted several studies that indicate Aur1p is integral to the Golgi membrane with the N-terminal region within the lumen and the C-terminal region facing the cytosol. These studies included cofractionation with Golgi markers, medial Golgi marker immunofluorescence, and C6-NBD-ceramide and C6-NBD-IPC localization to the ER and Golgi, respectively, with the latter dependent on Aur1p.

Activity is induced twofold by addition of inositol to the growth medium and requires the inositol regulatory proteins Opi1p, a negative regulator of phospholipid biosynthesis, as well as Ino2p and Ino4p, transcription factors required for derepression of inositol-choline-regulated genes involved in phospholipid synthesis (Ko et al. 1994). Enzyme activity is highest during mid to late exponential growth and is reduced sevenfold in stationary phase from the maximum (Ko et al. 1994). Messenger RNA abundance peaks during the G2 phase of the cell cycle, suggesting that *AUR*1 expression may be cell cycle regulated (Spellman et al. 1998).

IPC synthase activity was reduced in vitro in a dose-dependent manner by phytosphingosine and sphinganine (dihydrosphingosine), with IC_{50} values of 4.3 and 3 mol%, respectively (Wu et al. 1995). This inhibition of IPC synthesis by elevated sphingoid bases could explain the reduction of complex sphingolipid synthesis and steady-state values observed in vivo under fumonisin B1 inhibition of ceramide synthase (Wu et al. 1995). Also, the enzyme activity is inhibited by the fungicide aureobasidin A with an IC_{50} of ~0.2 nM (Nagiec et al. 1997) and by khafrefungin with an IC_{50} of 0.5 μg/mL (Mandala et al. 1997).

Mannosylation

The second class of complex sphingolipids, mannosylinositolphosphorylceramide (MIPC), contain a mannose attached to the inositol. This mannose is provided by the nucleotide sugar GDP-mannose and is transported into the Golgi lumen by the product of the essential gene *VRG*4 whose deletion mutant has blocked biosynthesis of MIPC (Dean et al. 1997).

The mannosylation of IPC involves two proteins encoded by *SUR*1/*CSG*1 and *CSG*2, which are named for supressor of *RVS*161 and calcium-sensitive growth, respectively. Mu-

tants of *SUR*1 and *CSG*2 fail to grow in medium containing 100 mM Ca^{2+} (Beeler et al. 1994, 1997). Another mutation study demonstrated the ability of the *Sur* family of proteins to suppress various phenotypes of *RVS*161 (Desfarges et al. 1993).

Recent work by Uemara et al. (Uemura et al. 2003) has elucidated a second partner for Csg2p that functions as IPC mannosyltransferase, namely Csh1p. Substrate specificity was demonstrated for the two complexes, with Csh1p–Csg2p showing less activity for IPC-B and especially IPC-C. Both Csg1p and Csh1p, but not Csg2p, contain a 93-amino-acid stretch homologous to Och1p and Hoc1p, which are α-1,6-mannosyltransferases (Beeler et al. 1997). From this and other results, the authors propose that Csg2p is a regulatory unit with either Sur1p/Csg1p or Csh1p as the catalytic unit (Uemura et al. 2003).

Csg2p contains the calcium binding domain DNNNSGSVTNEDV at position 95–107, has nine or ten predicted transmembrane segments, and is integral to the ER membrane (Beeler et al. 1994; Takita et al. 1995). Sur1p has a glycosyltransferase sugar-binding region containing a DXD motif (Pfam: Gly_transf_sug) at position 86–168. Additionally, Sur1p has three predicted transmembrane domains and contains a secretory pathway signal sequence, but is of unknown localization.

The *SUR*1 promoter contains the STRE stress response element CCCCT beginning at position –302. *SUR*1 is one of 16 genes that have repressed transcription in the presence of cAMP. Most of these metabolic enzymes are induced by nutritional limitations, nitrogen starvation in the case of *SUR*1. However, cAMP does not repress *SUR*1 transcription post-diauxic transition. Additionally, *SUR*1 is one of 10 genes that show reduced transcription in the double null mutant of stress response transcription factors Msn2p–Msn4p (Tadi et al. 1999). Abundance of *SUR*1 mRNA is maximal post-exponential growth phase (Tadi et al. 1999) and during the cell cycle, mRNA abundance fluctuates and peaks during late G1 phase (Cho et al. 1998).

*SUR*1 shows synthetic lethality with *CSG*2, *SCS*7, and *RVS*161. The first two are not surprising, as *CSG*2 is part of the mannosyl transferase complex with *SUR*1, and *SCS*7 is required for hydroxylation of the VLFCA of complex sphingolipids (Haak et al. 1997). The third gene with *SUR*1 synthetic lethality, *RVS*161, is required for cell viability after N, C, or S starvation. *RSV*161 and *RVS*167 encode Bin/amphiphysin/Rvs (BAR) adaptor proteins that form a complex that regulates actin, endocytosis, and viability following starvation or osmotic stress. This complex is localized to the actin cortical patch and lipid rafts (Lester et al. 1993). Rvs161p and sphingolipids were recently shown to be required for actin repolarization after salt stress (Balguerie et al. 2002).

Two-hybrid analysis shows associations between Sur1p and three other proteins. The first is Bzz1p, which is involved in actin patch polarization and is a component of the Myo3p–Las17p–Vrp1p actin assembly complex. Bzz1p is also part of the cell's salinity response and is localized in the actin cortical patch or the cytoplasm. Sur1p also associates with the product of *PAM*1, a coiled-coil protein involved with pseudohyphal growth that is a multicopy suppressor of loss of protein phosphatase 2A activity encoded by *PPH*21, *PPH*22, and *PPH*3. The third Sur1p association by two-hybrid is with Rif2p, a Rap1p-interacting factor involved with Rap1p in transcriptional silencing and telomere length regulation (Ito et al. 2001).

The above information on regulation and associations of *SUR*1 and *CSG*2 further corroborates a role for sphingolipids in regulation of various cellular stress responses.

Hydroxylation of VLCFA of complex sphingolipids

The α-hydroxylation of the C-26 fatty acyl chain of dihydroceramide or phytoceramide requires *SCS7*/*FAH*1 gene product, named for suppressor of choline sensitivity/fatty acid hydroxylase (Haak et al. 1997; Mitchell and Martin 1997). Additional hydroxylation of the VLCFA at an unspecified carbon requires Cu^{2+} and the copper transporter Ccc2p (Beeler et al. 1997).

Scs7p is homologous to three other yeast proteins, namely Cyb2p with 49% identity, Cyb5p with 38% identity and Ole1p with 30% identity. Cyb2p is an L-lactate dehydrogenase with cytochrome activity and is localized to the mitochondrial intermembrane space. Cyb5p is involved in fatty acid desaturation and sterol biosynthesis. Ole1p is a stearoyl-CoA desaturase required for synthesis of unsaturated fatty acids.

Scs7p contains a cytochrome b5 family, heme-binding domain signature, FLSEHPGG, at residues 41–48 and a fatty acid hydroxylase domain (Pfam: FA_hydroxylase) at amino acids 228–374. Scs7p contains consensus ER retention signal sequences in the C-terminus, KMKYE and VKKEK, as does the LCB hydroxylation protein Sur2p (as do Lag1p and Lac1p and ceramidases). Scs7p is one of five proteins in the oxo-diiron family that are localized to the ER, including Sur2p, Ole1p (Δ9 fatty acid desaturase), Erg25p (C-4 methyl sterol oxidase), and Erg3p (C-5 sterol desaturase).

Scs7p has synthetic lethality with the mannosylation proteins Csg2p (Haak et al. 1997) and Sur1p (Dunn et al. 1998). Interestingly, a triple null mutant in *scs*7, *csg*2, and *sur*2 is viable and synthesizes only the hydrophobic IPC-A (Haak et al. 1997).

Inositolphosphotransferase

The final step of the complex sphingolipid pathway was found by Dickson et al. (1997) to require *IPT*1 (inositolphosphotransferase) for synthesis of mannosyl diphosphorylinositol ceramide ($M(IP)_2C$), the most abundant complex sphingolipid in yeast, which is found primarily in the plasma membrane. This reaction occurs with the transfer of an inositol phosphate group from phosphatidyl-inositol (PI) onto MIPC, releasing DAG. As this is similar to the reaction that takes ceramide to IPC, it is not surprising that *IPT*1 and *AUR*1 are homologs with 27% identity. Null mutants are viable with increased levels of MIPC but no detectable $M(IP)_2C$ and are resistant to syringomycin E. Recently however, *IPT*1-independent synthesis of small amounts of $M(IP)_2C$ was reported when Δ*ipt*1 was grown on potato dextrose broth, suggesting the existence of another mechanism of $M(IP)_2C$ formation. Interestingly, even this small amount of $M(IP)_2C$ was enough to confer susceptibility to syringomycin E, as well as DmAMP1, an antifungal plant defensin. Researchers speculate that $M(IP)_2C$ may assist

Table 1. Enzymes involved in yeast sphingolipid metabolism.

Enzyme name	Enzymatic reaction (product)	Genes involved	Regulation	Drug inhibitors	Mammalian homologues
Serine palmitoyltransferase (SPT)	Synthesis of 3KDHS	*LCB*1 and *LCB*2 are subunits of SPT *TSC*3 is a post-translational activator of SPT	Pyridoxsal phosphate cofactor is required for the reaction Excess of palmitoyl-CoA (over 0.2 mM) inhibits the reaction in vitro	Myriocin, Sphingofungin B, C	*Lcb*1, *Lcb*2
NADPH-dependant oxidoreductase	Reduction of 3KDHS to DHS	*TSC*10	Paf1p possible positive regulator	—	—
LCB C4 hydroxylase	Hydroxylation of DHS or dihydroceramide to PHS or phytoceramide	*SUR*2	Possible cell-cycle regulation	—	—
Sphinganine *N*-acyltransferase (ceramide synthase)	Synthesis of dihydroceramide or phytoceramide	*LAG*1, *LAC*1	—	Fumonisin B1 AAL-toxins, australofungins	*Uog*-1, *trh*1(AY029531), *trh*4(AK010241), *clone*1(BC010032), *clone*4(AK022151)
Alkaline phyto-ceramidase	Preferentially hydrolyses PHS over DHS. Reverse CoA-independent ceramide synthase activity utilizing PHS and DHS.	*YPC*1	Induction of transcription in response to acidic conditions mRNA levels increased upon ammonium starvation, heat shock, and hyperosmolarity, stationary phase (systematic Northern analysis)	—	*haPHC*, *haCER*1, *maCER*1
Alkaline dihydro-ceramidase	Preferentially hydrolyses DHS over PHS. Minor reverse CoA-independent ceramide synthase activity utilizing DHS.	*YDC*1	Induction of transcription in response to acidic conditions Possible transcription regulation by Ndd1p	—	*haPHC*, *haCER1*, *maCER1*
Inositol phosphorylceramide (IPC) synthase	Synthesis of IPC	*AUR*1	Upregulated during the G2 phase of the cell cycle Upregulation during logarithmic growth phase and down regulation during stationary growth phase Activated by inositol	Khafrefungin Aureobasedin A	*SM synthase* (contra part)
C26 hydroxylase	Hydroxylation of C26 long-chain fatty acyl moiety of IPC	*SCS*7	—	Null mutant is resistant to syringomycin E	—

Table 1 (*concluded*).

Enzyme name	Enzymatic reaction (product)	Genes involved	Regulation	Drug inhibitors	Mammalian homologues
IPC mannosyltransferase	Synthesis of mannosylated sphingolipids (MIPC)	*SUR*1 is a possible catalytic unit of IPC mannosyltranferase	mRNA is up regulated during G1 phase of cell cycle Transcription is induced upon nitrogen starvation, and repressed by cAMP STRE stress response element in the promotor region	—	—
		*CSH*1 is a possible catalytic unit of IPC mannosyltranferase	—	—	—
		*CSG*2 is a putative yeast-specific Ca^{2+} membrane transporter; possible regulatory unit of IPC mannosyltranferase	Possible transcriptional regulation by Yap5p	—	—
Inositolphosphotransferase 1 (IPT1)	Synthesis of mannosyl diphosphorylinositol ceramide ($M(IP)_2C$)	*IPT*1	Induced by transcription factors Pdr1p and Pdr3p mRNA levels of *IPT*1 are increased in mutants that have lost the mitochondrial genome	Aureobasedin A	—
ISC1 has phospholipase C activity	Sphingolipid hydrolysis (dihydroceramide, phytoceramide)	*ISC*1	*ISC*1 is induced by alpha factor	—	*nSMase*2 (contra part)
Long-chain base (LCB) kinases	Sphingoid LCB phosphorylation (DHS-1-phosphate, PHS-1-phosphate)	*LCB*4	Possible regulation via proteosome	—	*SK*1, *SK*2
		*LCB*5	Regulated under cell damaging conditions	—	—
LCB-1-phosphatelyase	Breakdown of sphingoid long-chain phosphates (ethanolamine-P, hexadecanal or 4-OH-hexadecenal)	*DPL*1	—	—	*S1P lyase*
LCB-1-phosphate phosphatases	Sphingoid long-chain base-phosphates dephosphorilation (DHS, PHS, phosphate)	*LCB*3	Up regulation during G2 phase of the cell cycle Regulated under cell damaging conditions	Inhibition by low concentration of Triton X-100 (0.05%)	*S1P phosphatase*1
		*YSR*3	Very low levels of mRNA under normal conditions Induced by SR31747A, an immunosuppressant	—	*S1P phosphatase*2

Table 2. Genes involved in yeast sphingolipid metabolism.

Gene symbol	Synonyms	Phenotypes	Localization	Important protein domains or motifs	Protein abundance* (molecules/cell)	Interaction with other proteins
LCB1	*END8*, *TSC2*, *YMR296C*	The *LCB1* null mutant is lethal, requires sphingolipid long-chain base for sphingolipid synthesis and growth *lcb1*-100 is a temperature sensitive mutant	ER	Similar to pyridoxsal phosphate binding sequence	22 400	Lcb2p; Tsc3p determined by coimmunoprecipitation
LCB2	TSC1, *SCS1*, *YD9609.16*, *D4246*, *YDR062W*	Null mutant is lethal, requires sphingolipid long-chain base for sphingolipid synthesis and for growth	—	Alpha-oxoamine synthase conserved catalytic core domain Similar to pyridoxsal phosphate binding sequence Two potential transmembrane segments	54 500	Lcb1p; Tsc1p determined by coimmunoprecipitation
TSC3	*NORF21*, *YBR058C-A*	Null mutants are temperature sensitive and display reduced SPT activity	Membrane associated	—	—	Interaction with Lcb1p and Lcb2p, determined by coimmunoprecipitation
TSC10	*YBR1734*, *YBR265W*	Null mutant is lethal, requires exogenous PHS or DHS	ER or cytoplasm	Rossman fold, borders NADPH-binding domain Putative transmembrane domain	7 700	Two-hybrid interaction with Aar3p and Ylr255p
SUR2	*TSC7*, *SYR2*, *D9740.8*, *YDR297W*	Null mutant produces LCBs without C4 hydroxyl group	ER	Four predicted transmembrane domains Cytochrome b5 family heme-binding domain Eight histidine motifs	54 300	—
LAG1	*YHL003C*	The null mutant increases life span by 50% Double deletion mutant *lag1*Δ/*lac1*Δ is lethal or has a slow growth phenotype depending on the genetic background	ER	Lag1p motif TLC domain Seven predicted transmembrane segments	—	Interacts with Nup116p, Lac1p, and Ymr298p in two-hybrid assay
LAC1	*DGT1*, *YKL156*, *YKL008C*	The null mutant is viable Double deletion mutant *lag1*Δ/*lac1*Δ is lethal or has a slow growth phenotype depending on the genetic background	ER	Lag1p motif TLC domain Seven predicted transmembrane segments	2 840	Interacts with Tem1p, Lag1p, Yaf9p, and Ymr298p in two-hybrid assays
YDC1	*LPG21*, *YPL087W*	The null mutant is viable and sensitive to heat stress	ER	Several predicted transmembrane domains	—	—
YPC1	*YBR1305*, *YBR183W*	Null mutant shows elevated heat resistance and sensitivity to peroxides	ER	Several predicted transmembrane domains	—	—

Table 2 (*continued*).

Gene symbol	Synonyms	Phenotypes	Localization	Important protein domains or motifs	Protein abundance* (molecules/cell)	Interaction with other proteins
*AUR*1	*IPC*1, *ABR*1, *YKL*004*W*	Null mutant is lethal, accumulates ceramide in the presence of PHS, which leads to cell death	Golgi	Several predicted transmembrane domains Contains lipid phosphatase sequence motif (PAP2 superfamily)	4 170	—
*SCS*7	*FAH*1, *YM*8156.14, *YMR*272*C*	Null mutant is viable but lacks IPC-C	ER	Cytochrome b5 family heme-binding domain Histidine motifs domain, which binds oxo-diiron Four putative transmembrane segments	3 290	—
*SUR*1	*BCL*21, *CSG*1, *LPE*15, *YPL*057*C*	Null mutant is viable, accumulates IPC but not MIPC or $M(IP)_2C$ and has abnormal morphology	Membrane localization or secreted protein	Glycosyltransferase sugar-binding region containing DXD motif	—	Possible interaction with Csg2p. Interacts with Pam1p, Rif2p, Bzz1p in two-hybrid assay.
*CSH*1	*YBR*161*W*, *YBR*1212	—	—	Glycosyltransferase sugar-binding region containing DXD motif	195	—
*CSG*2	*CLS*2, *YBR*0404, *YBR*036*C*	Null mutant is viable, accumulates *IPC*, reduced amounts of $M(IP)_2C$ but does not accumulate MIPC and accumulates higher levels of Ca^{2+} in compartments exchangeable with extracellular Ca^{2+}	Membrane localization or secreted proteins	EF-hand calcium-binding domain Nine to ten potential transmembrane segments	—	—
*IPT*1	*SYR*4, *YD*8554.05, *D*4405, *YDR*072*C*	Null mutant is viable with increased levels of MIPC with no detectable $M(IP)_2C$	ER	Two PEST motifs At least eight putative transmembrane segments Conserved lipid phosphatase sequence motif	606	Interacts with Ydr107p in two-hybrid assay
*ISC*1	*YER*019*W*	—	Membrane fraction	Two predicted transmembrane segments P-loop (ATP, GTP binding site)	—	Interacts with Cvt17p, Rox1p, Rtt105p, and Srb4p in two-hybrid assay
*LCB*4	*O*3615, *YOR*171*C*	Null mutant is viable but does not have detectable long-chain base phosphates	Golgi, endosomes or ER	Predicted diacylglycerol kinase catalytic domain	2 840	Interacts with Akr2p, Gsp2p, Yer071p, and Stb5p in two-hybrid assays
*LCB*5	*L*8479.7, *YLR*260*W*	Null mutant is viable with increased levels of LCB phosphates	Golgi	Predicted diacylglycerol kinase catalytic domain	1 760	—
*DPL*1	*BST*1, *D*9819.5, *YDR*294*C*	Null mutant is viable and displays increased levels of PHS-1 phosphate	ER, cytoplasm	Possible N-terminal acetylation One possible transmembrane segment	13 100	—

Table 2 (*concluded*).

Gene symbol	Synonyms	Phenotypes	Localization	Important protein domains or motifs	Protein abundance* (molecules/cell)	Interaction with other proteins
LCB3	*YSR2*, *LBP1*, *J0671*, *YJL134W*	Null mutant is viable and has increased resistance to growth inhibition by sphingoid long chain bases	Membrane fraction; ER based on Lcb3p-GFP	Contains lipid phosphatase sequence motif (PAP2 superfamily) Four to eight putative transmembrane segments, Putative GPI anchor attachment signal	—	Interaction with Prm8p in two-hybrid assay
YSR3	*LBP2*, *YSR2–1*, *YKR053C*	Null mutant is viable	Membrane fraction	Contains lipid phosphatase sequence motif (PAP2 superfamily) At least five putative transmembrane segments, Putative GPI anchor attachment signal	—	Interacts with Dit1p in two-hybrid assay

*Protein abundances from the Yeast GFP Fusion Localization Database (at http://yeastgfp.ucsf.edu) (Ghaemmaghami et al. 2003; Huh et al. 2003).

with pore formation and stability that allows toxin entry into the cell (Im et al. 2003).

The Ipt1p sequence contains two PEST motifs, which are hydrophilic regions enriched in proline (P), glutamic acid (E), serine (S), and threonine (T) that are thought to target proteins for rapid degradation (Rechsteiner and Rogers 1996). Ipt1p has at least eight putative transmembrane segments. Like its homolog Aur1p, Ipt1p contains a domain belonging to the *PAP*2 superfamily and four conserved domains found in homologous pathogenic fungi (Heidler and Radding 2000). Large scale analysis of the subcellular localization of the yeast proteome indicates Ipt1p localizes to the ER and to the cytoplasm (Kumar et al. 2002).

*IPT*1 is induced by the pleiotropic drug resistance transcription factors PDR1p and PDR3p and contains a single response element in its promoter (Hallstrom et al. 2001; Kolaczkowska et al. 2002). *IPT1* is not required for mating or sporulation (Dickson et al. 1997), nor is it involved in remodeling of GPI anchors (Reggiori and Conzelmann 1998). Ipt1p activity is inhibited by aureobasidin A in the micromolar range (Dickson et al. 1997). Mutations in *IPT*1, as well as in other genes of sphingolipid synthesis (*SUR*1, *SUR*2, *FEN*1, and *SUR*4), suppressed the salt-sensitive actin repolarization defect of *rvs*161Δ mutants (Balguerie et al. 2002).

Ipt1p has one interaction with YDR107cp as determined by two-hybrid analysis (Ito et al. 2001). This protein is not yet characterized but has 74% identity and 86% similarity to Emp70p, a putative transporter found in the endosome.

Breakdown of complex sphingolipids

*ISC*1 encodes a protein with phospholipase-C-type activity that hydrolyzes complex sphingolipids in vitro. Higher V_{max} values and lower K_m values for MIPC and $M(IP)_2C$ as compared with IPC suggest they are the preferred substrates. This activity requires the presence of phosphatidylserine or other acidic phospholipids and Mg^{2+} and is inhibited by Mn^{2+} (Sawai et al. 2000).

Isc1p belongs to the magnesium-dependent exonuclease III - apurinic endonuclease (ExoIII-APE) nuclease family, has two putative transmembrane domains (Okamoto et al. 2002), and localizes to membrane fractions (Sawai et al. 2000). Isc1p also contains a novel "P-loop-like" motif, identified based on the presence of an ATP-GTP binding site, that plays a role in either substrate recognition and (or) catalysis (Okamoto et al. 2003). Isc1p plays a role in cellular growth. The enzyme localizes to the mitochondria and is activated in a growth-dependent manner. Isc1p modulates the levels of yeast ceramides during growth (Vaena de Avalos et al. In press).

*ISC*1 is induced by the mating pheromone alpha-factor, and this induction requires Ste12p, as demonstrated by chromatin immunoprecipitation (Ren et al. 2000). *ISC*1 mRNA was shown to be bound by Mex67p, a nuclear export protein, but not by Yra1p, a yeast RNA annealing protein (Hieronymus and Silver 2003).

*ISC*1 is required for yeast to develop halotolerance to sodium and lithium ions by the induction of *ENA*1 which encodes a cation-extrusion pump of the P-type ATPase family (Betz et al. 2002).

Isc1p interactions via two-hybrid analysis were reported for Cvt17p, Rox1p, Rtt105p, and Srb4p (Ito et al. 2001). Cvt17p is a lipase that functions in autophagy and membrane degradation. Rtt105p negatively regulates DNA transposition. Srb4p is a subunit of the RNA polymerase II holoenzyme–mediator complex. Rox1p is a heme-induced transcription factor that represses hypoxic genes. Recent genomic analysis of anaerobically induced genes showed that *ROX1* controls approximately one third of those genes, including several involved in sphingolipid metabolism (*TSC*10, *SUR*2, *LAC*1, *SCS*7, *YSR*3, and *LCB*3) (Kwast et al. 2002).

Phosphorylated LCBs

LCB kinases

*LCB*4 and *LCB*5 encode two paralogous kinases with 53% amino acid identity that phosphorylate DHS and PHS to DHS-1-P and PHS-1-P, respectively. *LCB*4 provides 97% of the total LCB kinase activity, and two thirds of that activity is membrane associated, although the protein contains no known membrane localization signal or predicted transmembrane domain. One third of the *LCB*5 activity is similarly membrane associated (Nagiec et al. 1998). Recently, Hait et al. (2002) showed the localization of Lcb4p to the trans-Golgi network and late endosomes by indirect immunoflurescent microscopy and subcellular fractionation. Additionally, Lcb4p was shown to be required for the incorporation of exogenous LCB into ceramide and is localized to the ER membrane (Funato et al. 2003). Interestingly, Ysr2p/Lcb3p and Ysr3p, which dephosphorylate LCB-P and Dpl1p, which in turn degrades LCB-P, are found in the ER and are also required for incorporation of exogenous LCB into ceramide. This suggests a role for phosphorylation and subsequent dephosphorylation of LCBs in their transport to the site of sphingolipid biosynthesis (ER).

Both *LCB*4 and *LCB*5 contain a presumed DAG kinase catalytic domain (Pfam: DAGK_cat). Single null mutants are viable and have no detectable phenotype. The double null mutant shows no LCB kinase activity and has wild type growth (Nagiec et al. 1998). *LCB*4 is one of 61 genes co-repressed by carbon limitation when grown on glucose in an aerobic chemostat (Boer et al. 2003).

Lcb4p interacts with Gsp2p, Akr2p, Stb5p, YER071c (Ito et al. 2001) and Rad1p (Uetz et al. 2000) via high-throughput two-hybrid analysis. Akr2p is involved in the constitutive endocytosis of the mating pheromone receptor Ste3p. Gsp2p is a GTP-binding protein involved in nuclear organization. Stb5p exhibits transcription factor activity in response to xenobiotic stimulus. Rad1p belongs to the nucleotide excision repair factor 1 complex. YER071c is an uncharacterized protein that belongs to a putative cell polarity protein complex that also includes the F-actin capping subunits *CAP*1 and *CAP*2 (Gavin et al. 2002).

Phosphatases YSR2/LCB3 and YSR3

Three groups working independently to discover additional enzymes in the sphingolipid pathway found a phosphatase of the long chain base phosphates (Mandala et al. 1998; Mao et al. 1997; Qie et al. 1997). This gene product has three domains that comprise a lipid phosphatase motif belonging to the *PAP*2 superfamily of phosphoesterases. The enzyme is predicted to have four or more transmembrane domains and a C-terminus GPI site, and was localized to the ER by GFP fusion protein. Recent work suggests that Lcb3p has eight transmembrane domains with the conserved domains in the ER lumen (Kihara et al. 2003). Lcb3p/Ysr2p has over 50% identity at the amino acid level to Ysr3p, which shares the above domains of Lcb3p, but does not have the same physiological role.

The mRNA levels of *LCB*3 is four times greater than *YSR*3 as determined by RT–PCR of total RNA extracted from exponentially growing cells under normal conditions (Mao et al. 1999), and peaks in G2 of the cell cycle (Spellman et al. 1998). Ysr3p mRNA shows an increase during heat shock when grown on YPD as determined by Northern blot (Mandala et al. 1998). Additionally, the Ysr3p message is bound by two nuclear export proteins, Mex67p and Yra1p, as determined by immunoprecipitation and microarray (Hieronymus and Silver 2003). The immunosuppressant drug SR31747A induces *YSR*3 (Cinato et al. 2002).

Neither *LCB*3 nor *YSR*3 are essential, but null mutants and overexpression mutants exhibit interesting phenotypes that demonstrate the different roles of the two proteins. Deletion of either gene results in accumulation of DHS-1-P, and the double mutant has higher levels of DHS-1-P than either single mutant (Mao et al. 1999). *LCB*3, but not *YSR*3, is needed for incorporation of exogenous sphingoid bases into complex sphingolipids (Qie et al. 1997).

*LCB*3 has synthetic lethality with *DPL*1 (Kim et al. 2000; Zhang et al. 2001), the lyase that clears the phosphorylated sphingoid bases towards the glycerolipid pathway; with *CKA*2 (Kobayashi and Nagiec 2003), the α′ subunit of casein kinase; and with *SUR*4/*ELO*3 (Kobayashi and Nagiec 2003), a fatty acid elongase required for synthesis of the C26-CoA needed for sphingolipid biosynthesis.

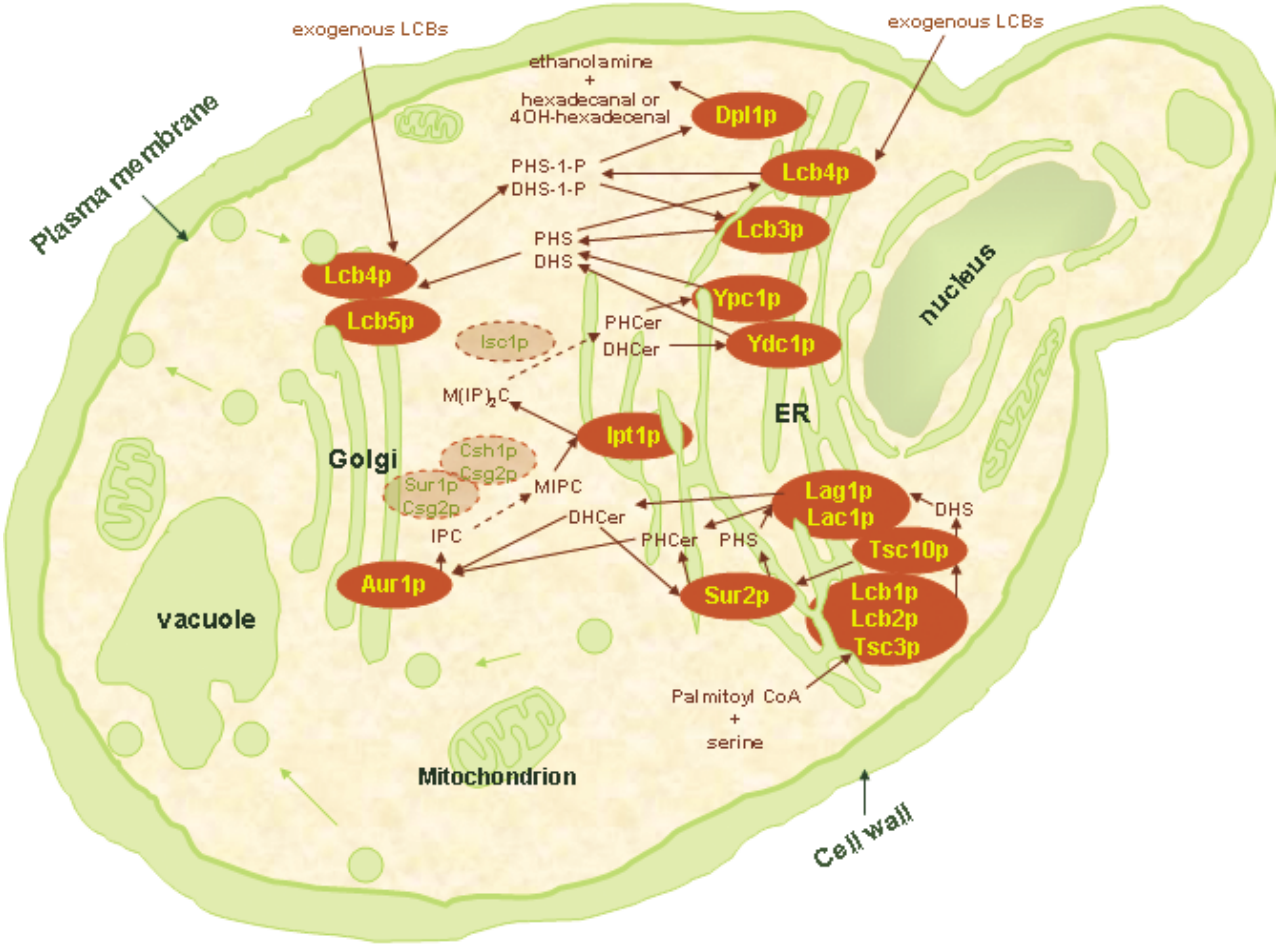
Lcb3p was shown by high-throughput two-hybrid to associate with Prm8p (Ito et al. 2001), a pheromone regulated membrane protein of unknown function that is involved with cell fusion during mating. Ysr3p associates with Dit1p in high-throughput two-hybrid analysis (Ito et al. 2001), and this gene product converts L-tyrosine to *N*-formyl-L-tyrosine in spore wall synthesis.

LCB phosphates breakdown

*DPL*1 encodes the dihydrophingosine-1-phosphate lyase (EC:4.1.2.27), which was first named as bestower of sphingosine tolerance (*BST*1), since it was identified from a clone demonstrating resistance to sphingosine (Saba et al. 1997). In a rate-limiting elimination reaction that depends on pyridoxal 5′-phosphate, the C-2,3 carbon bond of a phosphorylated sphingoid long chain base is cleaved, releasing phosphoethanolamine and palmitaldehyde, both of which may participate in glycerolipid metabolism (Saba et al. 1997). The preferred substrate is C-16 DHS-1-P rather than C-18 or C-20 (Zhang et al. 2001).

The sequence contains a pyridoxal-dependent decarboxylase conserved domain (Pfam: pyridoxal_deC). The protein was found to localize to the ER and cytoplasm by high throughput immunolocalization of tagged gene products (Kumar et al. 2002).

Fig. 1. Subcellular localization of yeast sphingolipid metabolism. A proposed model for the localization of proteins involved in yeast sphingolipid metabolism. The model is based on data from recent studies, addressing individual proteins or data summarized from YPD database. The localization of Isc1p, Csh1p, Csg2p, Sur1p, and Scg2p (ovals with dashed lines and arrows) is not conclusively established, and this figure shows only their biochemical position in the sphingolipid pathway. ER, endoplasmic reticulum; DHS, dihydrosphingosine; DHS-1-P, dihydrosphingosine 1-phosphate; PHS, phytosphingosine; PHS-1-P, phytosphingosine 1-phosphate; DHCer, dihydroceramide; PHCer, phytoceramide; IPC, inositolphosphorylceramide; MIPC, mannosylinositolphosphorylceramide; $M(IP)_2C$, mannose-(inositol-P)2-ceramide.



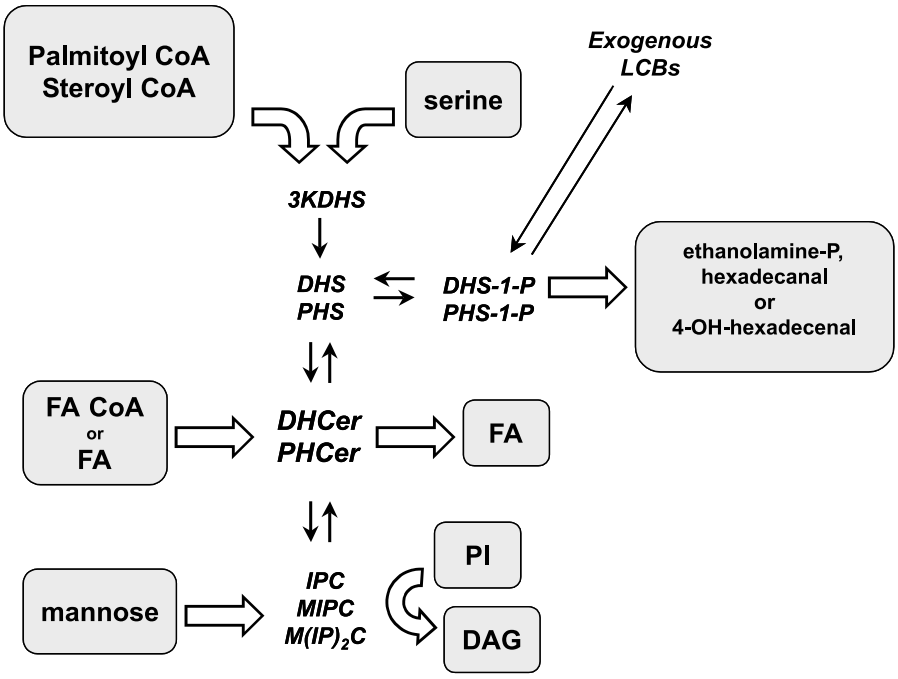
The null mutant is viable but sensitive to exogenous sphingoid bases. This sensitivity is suppressed by overproduction of Rsb1p, a putative transporter or flippase named for resistance to sphingoid long chain base (Kihara and Igarashi 2002). Dpl1p has synthetic lethality with Lcb3p as previously mentioned (Kim et al. 2000; Zhang et al. 2001), but this is suppressed by a *LCB*4 mutant. With almost 800 other genes, *DPL*1 mRNA is bound by Mex67p, a nuclear export protein, but not by Yra1p (Hieronymus and Silver 2003).

Discussion

Although the metabolism of sphingolipids in yeast constitutes a relatively small and contained system, it is able to coordinate responses to different conditions and exhibits complex relationships to other metabolic pathways. No doubt this is related to the dual nature of sphingolipids as both structural members of membranes and their role as second messengers. This review has brought together diverse information about each step of sphingolipid metabolism as a preliminary step to a better understanding of this complexity with which cells coordinate their responses to stimuli. Of course, there are still numerous gaps in our knowledge, and even for some the information provided here, a word of caution is in order. While many of the interactions mentioned are intriguing, they must be recognized as deductions from large-scale studies and require experimental verification on an individual level as well as determination of physiologic occurrence and significance. Also, this review does not discuss all mutant phenotypes or regulatory mechanisms, as these have been regularly addressed in several reviews (Dickson and Lester 2002; Hannun et al. 2001; Hannun and Obeid 2002; Obeid et al. 2002).

To assist the reader in consolidating and visualizing this information, two tables and two figures are provided. Table 1 summarizes information about the enzymes involved in each step of sphingolipid synthesis or breakdown. This includes the enzyme name, product of the reaction, and genes involved. Regulation, drug inhibitors, and mammalian

Fig. 2. Metabolites connecting sphingolipid metabolism to other metabolic pathways in yeast. For abbreviations, see the legend of Fig. 1.



homologs are also included. Table 2 provides details for each gene including aliases, some phenotypes, protein localization, and protein abundance. Additionally, important domains or motifs are listed as well as possible protein–protein interactions. Figure 1 illustrates the localization of enzymes and possible routes of sphingolipid metabolism in the cell. Finally, Figure 2 illustrates the connections of the sphingolipid pathway to other metabolic pathways.

Over the past two decades, our knowledge of details and relationships between sphingolipids has been growing with increasing speed. It is as though some pieces of a jigsaw puzzle had been turned face up and now must be fit together. To some degree, this matching of pieces is possible by comparing their shapes and colors, but this simple "intuitive" (Latin, *intueri*: to look at, contemplate) approach has its limitations, especially if we not only consider biochemical conversions between sphingolipids but attempt to understand the regulation that coordinates the pathway itself and sends out signals that control fundamental processes in the cell, such as differentiation, cell cycle arrest, and various stress responses. Such functional relationships are often subtler and require correspondingly sharper tools of analysis. The best candidates for these analyses may be mathematical models that quantify the dynamics of individual enzyme-catalyzed steps and allow the integration of these steps into a functioning entity that can be tested and queried.

Many options exist for setting up mathematical models (for example, see de Jong 2002). Among them, biochemical systems theory (Savageau 1969*a*, 1969*b*; Voit 2000) has proven particularly valuable for integrative analyses of metabolic and genetic networks. Using this approach, it was shown recently how dynamical modeling can be brought to bear on questions of sphingolipid metabolism (Alvarez-Vasquez et al. 2004). While preliminary, the model already showed the dynamics of perturbations in the metabolic network, allowed for checking the consistency of the available experimental data, and was used for simulated experiments that may provide insight into unknown regulatory signals in the sphingolipid pathway.

In conclusion, a wealth of information on sphingolipid metabolism and function has recently been gleaned from studies in yeast and mammalian systems. It is evident from reviewing the information in the literature and online data warehouses such as SGD, YPD, and CYGD that we have uncovered just the tip of the iceberg. The next level of research will begin to uncover sphingolipid modifying enzymes in protein–lipid and protein–protein interactions, input of different metabolic pathways, models of cell regulation and trafficking, and potentially to comprehend system behavior through future mathematical modeling. Stay tuned.

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