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Current Topics

Biosynthesis and Trafficking of Sphingolipids in the Yeast *Saccharomyces cerevisiae*†

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1. BIOSYNTHETIC PATHWAY OF SPHINGOLIPIDS: GENES AND LOCALIZATION OF THE CORRESPONDING ENZYMES

The early steps in mammalian and *Saccharomyces cerevisiae* sphingolipid synthesis are similar, but they produce structurally and chemically different types of sphingoid bases, ceramides, and complex sphingolipids. The biosynthetic pathway of sphingolipids begins in the endoplasmic reticulum (ER) where it proceeds up to formation of ceramides. Complex sphingolipids [IPC, MIPC, and $M(IP)_2C$ in *S. cerevisiae*] are made in the Golgi apparatus. Exogenous sphingoid bases and sphingoid base phosphates can also enter into this pathway, but their incorporation requires a specific mechanism.

1.1. Endogenous Synthesis of Ceramide

Lcb1p, Lcb2p, and Tsc3p: Serine Palmitoyltransferase (SPT). The first committed step in the synthesis of ceramide is the condensation of L-serine with palmitoyl-CoA to generate the sphingoid base 3-ketodihydrosphingosine (3-KDS). This reaction is mediated by the pyridoxal 5′-phosphate-dependent serine palmitoyltransferase (SPT). SPT is membrane-associated and appears to be located on the cytoplasmic side of the ER in mouse liver (*1*) as well as in yeast (A. Kihara and Y. Igarashi, personal communication). In yeast, two genes, *LCB1* and *LCB2* (see Figure 1 and Table 1), are necessary for SPT activity and are thought to encode subunits of the enzyme (*2*−*4*). *LCB1* and *LCB2* encode homologous proteins of ∼560 amino acids, their sequences being 23% identical and 47% similar. Deletion of either of these genes is lethal, but lethality can be rescued by addition of sphingoid bases to the culture medium (*5*). Lcb1p interacts tightly with Lcb2p (*6*). Lcb1p and Lcb2p belong to a small family of pyridoxal 5′-phosphate-dependent enzymes (that catalyze the condensation of an amino acid and a carboxylic acid CoA thioester with concomitant decarboxylation of the amino acid) (*3*). Both protein sequences have a (D/E)XXXX-(S/T)XX**K**X(L/F)GXXGG(F/Y) motif that is related to a pyridoxal phosphate binding consensus sequence. In Lcb1p, the Lys is replaced with a Thr. The Lys has been shown to form a Schiff base with pyridoxal phosphate (*7*). Very recent data provide new insight into the roles of both proteins. Dominant point mutations in this motif in Lcb2p (Lys366 or His334) result in a drastic decrease in the SPT activity. Furthermore, other dominant point mutations in Lcb1p (Cys133 or Val144), deduced from a natural mutant form of the human homologue responsible for the hereditary sensory neuropathy type I disease, yield similar decreased SPT activity. These mutations do not abolish the interaction between Lcb1p and Lcb2p. Modeling studies suggest a possible explanation for these results. SPT is likely to have a single active site that lies at the Lcb1p−Lcb2p interface, and Lys366 and His334 of Lcb2p and Val144 and Cys133 of Lcb1p would belong to this active site (*8*).

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It has been shown recently that a third partner, Tsc3p, is necessary for optimal SPT activity. Tsc3p is a small membrane-associated protein of 80 amino acids, which interacts with both Lcb1p and Lcb2p. In its absence, the in vitro SPT activity is greatly reduced. It has been proposed that Tsc3p may bind palmitoyl-CoA and deliver it to the Lcb1p−Lcb2p complex, thus increasing SPT activity (*6*). Moreover, *TSC3* is only essential at elevated temperatures (*6*). Dominant mutations of Lcb2p (located outside of the catalytic site) can bypass the Tsc3p requirement at high temperatures by increasing Tsc3p-independent SPT activity (*9*).

Tsc10p: 3-KDS Reductase. In the second step of sphingolipid synthesis, 3-KDS is reduced to *erythro*-dihydrosphingosine (DHS, also called sphinganine) by the enzyme encoded by *TSC10.* NADPH is required for this reaction (*10*). *TSC10* is essential for viability, but a *tsc10*Δ mutant can grow if supplied with DHS or phytosphingosine (PHS) (*11*). Tsc10p is a membrane-bound protein having a single predicted membrane-spanning domain, its hydrophobic C-terminus. Tsc10p is a member of the short chain dehydrogenase/reductase protein family, and its sequence has the $\mathbf{S}X_{12}\mathbf{Y}X_3\mathbf{K}$ motif that is found in the catalytic site of these enzymes. The three conserved amino acids are thought to participate in the reduction of a ketone to a hydroxyl group by facilitating the transfer of a proton from the Tyr residue to the substrate. Tsc10p also contains the conserved sequence GX_3GXG, which forms a turn between a β-strand and an α-helix that borders the NADPH binding domain, known as the Rossman fold (*12, 13*). Tsc10p is necessary and sufficient for catalyzing this reaction step as its expression in *Escherichia coli* confers the NADPH-dependent reduction of 3-KDS to DHS activity. The C-terminal hydrophobic stretch is not required for this activity (*11*).

Elo1p, Elo2p, Elo3p, Tsc13p, Ybr159w, and Acb1p: Fatty Acid Elongation. A very long chain fatty acyl-CoA (VLCFA-CoA) is then linked to the sphingoid base to form ceramide. In yeast, VLCFAs (C20:0 to C26:0) are minor compounds, accounting for only 4% of the total fatty acid pool; however, they are crucial for ceramide formation. C26:0 is the most abundant VLCFA, and it is almost exclusively found in ceramide. Therefore, synthesis of VLCFA is critical for the sphingolipid pathway in yeast. *ELO1* was the first gene characterized for its role in membrane-bound fatty acid elongation, different from the soluble cytoplasmic fatty acid synthase (FAS) complex. Elo1p is involved in the elongation of 14:0 to 16:0 fatty acids (*14*). *ELO2* and *ELO3* were identified by their homology to *ELO1*. They are 76 and 72% similar and 56 and 52% identical to *ELO1*, respectively. The simultaneous disruption of both *ELO2* and *ELO3* is lethal, suggesting that their products have a high degree of overlapping function. However, the different pattern of accumulation of VLCFA intermediates in the Δ*elo2* and Δ*elo3* strains, and the inability of one gene to restore wild-type (WT) levels of VLCFA in a strain deleted for the other gene, suggest that Elo2p and Elo3p play different roles. Indeed, the primary activity of Elo2p is to elongate C20:0 fatty acids to C-22:0 fatty acids. This enzyme can also convert C22:0 to C24:0 fatty acids, but cannot convert C24:0 to C26:0 fatty acids. Elo3p is responsible for this last step, but it can also make C22:0 and C24:0 fatty acids with less efficiency than Elo2p (*15*).

Recently, another gene involved in fatty acid elongation, *TSC13*, has been characterized. Unlike *ELO2* and *ELO3*, *TSC13* is an essential gene. Tsc13p is likely to be required to catalyze a step in each cycle of fatty acid elongation for acyl-CoA substrates of all chain length, namely, the reduction of the enoyl intermediate (see Figure 1) (*16*). Tsc13p forms a complex with Elo2p and Elo3p, but it is not known if Elo2p and Elo3p interact with each other (*16*). Like Elo2p and Elo3p, Tsc13p is an integral protein localized to the ER. However, Tsc13p is also specifically enriched at sites of vacuolar−nuclear envelope interaction, but its role there is unclear. Some data have shown that disruptions of *ELO2*, *ELO3*, or *TSC13* reduce sphingolipid levels by reducing the level of ceramide synthesis due to low levels of VLCFAs (*15*, *16*).

Quite recently, the *YBR159*w gene has been implicated in fatty acid elongation. Ybr159p is an integral protein, most likely located in the ER, which appears to play a role in β-ketoacyl reduction. Only small amounts of the 3-hydroxy intermediate accumulate in a *ybr159w*Δ strain, along with a 3-keto intermediate, while the amount of fully elongated products is greatly diminished (*17*). *YBR159w* is not an essential gene, and in its absence, 3-hydroxy intermediates are still synthesized at a later stage. These data suggest that another β-ketoacyl reductase may exist in yeast (*17*).

Finally, it has been shown that the acyl-CoA binding protein, Acb1p, plays an important role in this pathway. Indeed, in a strain depleted for Acb1p, the synthesis of sphingolipids is reduced by 50−70%. Under these conditions, an accumulation of fatty acid synthase end product C18:0-CoA and a simultaneously reduced level of C26:0 were observed. These results suggest that Acb1p is required for the transport or delivery of acyl-CoA (C18:0-CoA) to the elongation system (Elo2p and Elo3p) (*18*).

LAC1 and LAG1: Ceramide Synthase. YPC1 and YDC1: Ceramidase. A very long chain fatty acyl-CoA (generally 26 carbons long, but sometimes 24) is linked by an amide bond to DHS or PHS to generate dihydroceramide (DH-Cer) or phytoceramide (PH-Cer). This step is catalyzed by the ceramide synthase, which has recently been identified. *LAC1* and *LAG1* are involved in this reaction, which is CoA-dependent and fumonisin B1-sensitive (*19*, *20*). *LAC1* and *LAG1* encode two homologous integral membrane proteins that are 72% identical and 81% similar localized in the ER (*21*). Lac1p and Lag1p seem to have a redundant function, as deletion of either gene individually is not lethal (*19*, *21*). It has not yet been determined whether these two proteins are the sole components of the acyl-CoA-dependent ceramide synthase.

Two other homologous enzymes, Ydc1p and Ypc1p, are alkaline ceramidases, Ydc1p having specificity for DH-Cer (*22*) and Ypc1p for PH-Cer (*23*). Ypc1p and Ydc1p are also integral proteins localized to the ER (*23*). In the presence of fumonisin B1, which inhibits Lac1p and Lag1p activity, in the absence of fatty acyl-CoA, or in a double deletion of *LAC1* and *LAG1*, a reverse activity of Ydc1p and Ypc1p becomes apparent and produces small amounts of ceramides (*19*, *22*, *23*). The reverse ceramidase activity of Ydc1p is minor compared to that of Ypc1p, suggesting that these two enzymes do not share exactly the same role despite their high level of homology (52% identical).

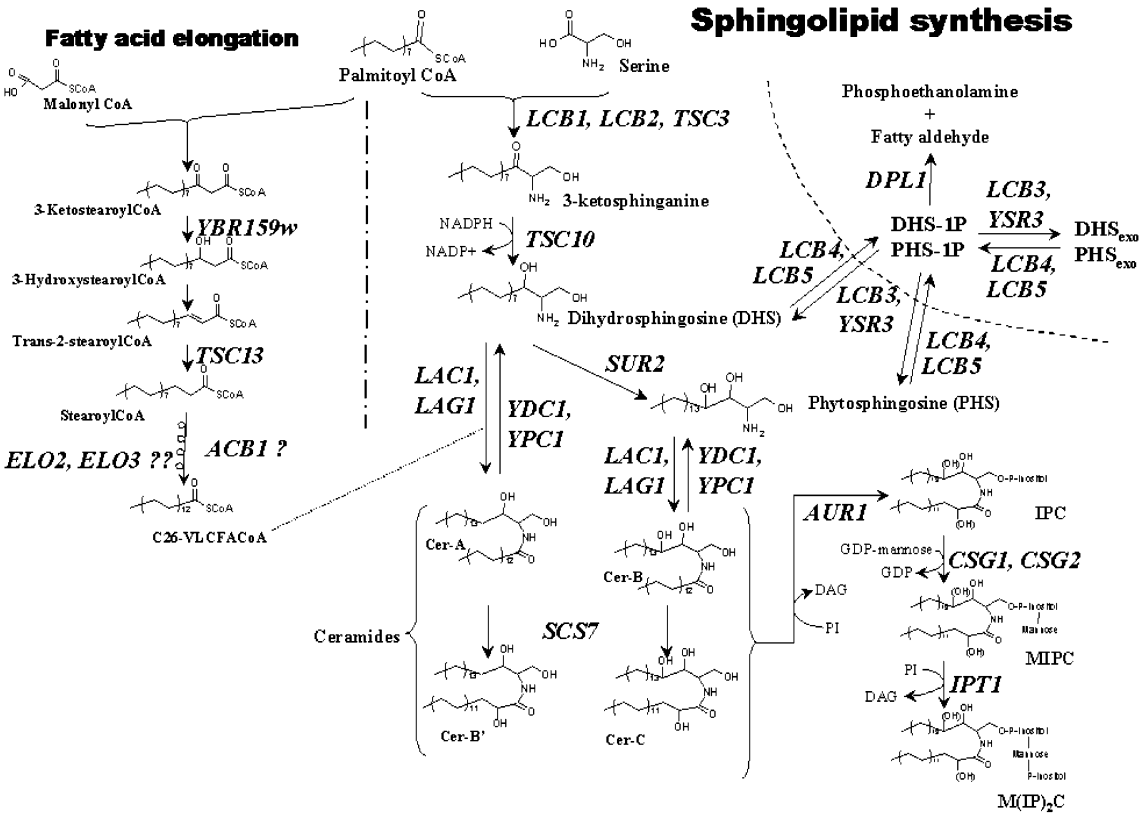

FIGURE 1: Sphingolipid biosynthetic pathway in the yeast *S. cerevisiae*. Gene names are shown in italics, and more details about their function can be found in Table 2. On the left side, the elongation machinery of fatty acyl-CoA is depicted. In the top right corner, the incorporation of exogenous sphingoid bases into the pathway is shown. 4-Hydroxylation of DHS probably occurs on both free sphingoid base and ceramide. Complex sphingolipids can be further hydroxylated on their fatty acid moiety (not shown in the diagram; see the text). The structure of the headgroup of $M(IP)_2C$ is inositol-1-P(6)mannose(α-1,2)inositol-1-P(1)ceramide (R. L. Lester et al., personal communication).

Sur2p: 4-Hydroxylation of DHS and DH-Cer. DHS is hydroxylated at C4 to yield PHS, which is the primary sphingoid base found in most fungal and plant ceramides (85% PHS vs 15% DHS in yeast) (*24*). This reaction is catalyzed by Sur2p, which can also use DH-Cer as a substrate to produce PH-Cer (*25*, *26*). It is not yet clear whether DHS or DH-Cer is the preferred substrate for C4 hydroxylation by Sur2p. PHS rapidly accumulates transiently after a 37 °C heat shock, suggesting that under these conditions DHS can be hydroxylated before incorporation into ceramide (*27*, *28*). Sur2p is an integral membrane protein and is localized at the ER (*29*). It contains a cytochrome b_5 domain and also three conserved general motifs, $HX_{(2-3)}(XH)H$, where the His residues coordinate a μ-oxo-bridged diiron cluster (Fe−O−Fe) that functions as part of the reaction center. *SUR2* is not essential for growth, and a null mutant strain shows no defect in vegetative growth or stress resistance.

Scs7p Ccc2p, Sur7p, Ynl194p, and Ydl222p: Hydroxylation of the Fatty Acid Moiety of Ceramides. In yeast, the fatty acid moiety of ceramide or complex sphingolipid is either non-, mono-, or dihydroxylated. The first hydroxylation occurs in the ER and is mediated by Scs7p (*30*). The second hydroxylation occurs in the Golgi apparatus and requires Cu^{2+} and the Golgi copper transporter encoded by *CCC2* (*31*, *32*).

Scs7p acts in the specific α-hydroxylation of very long chain fatty acids (C26:0) bound to sphingolipids (first hydroxylation). Scs7p has two hydrophobic domains, each capable of spanning the membrane twice. Scs7p also contains five conserved general motifs, $HX_{(2-3)}(XH)H$, where the His residues coordinate a μ-oxo-bridged diiron cluster (Fe−O−Fe) that functions as part of the reaction center. *SCS7* is not an essential gene (*30*). It is the hydroxylation enzyme or one of its components (*33*). PH-Cer seems to be preferred over DH-Cer, because in a *sur2*Δ mutant IPC-A and MIPC-A accumulate (*25*). Like Sur2p, Scs7p contains a cytochrome b_5 domain at its N-terminus. It may be retrieved to the ER because its C-terminal sequence (KMKYE) matches a consensus sequence for retrieval to the ER.

Ccc2p is required for further hydroxylation of the fatty acid chain. It is not yet known whether this hydroxylation occurs on the C26 fatty acyl-CoA prior to ceramide formation, on the ceramide prior to inositol phosphorylation, or on complex sphingolipids (*32*). As Ccc2p is localized to the late or post-Golgi (*34*), the hydroxylation most likely occurs on complex sphingolipids (see section 2).

Recently, a new family of proteins (Sur7p, Ynl194p, and Ydl222p) has been described to be involved in the sphingolipid biosynthetic pathway (*35*). They are homologous integral membrane proteins (*36*), localized to novel cortical patches of the plasma membrane (PM). Deletions of *SUR7*, *YNL194*, or/and *YDL222* alter the sphingolipid content of the PM by influencing the sphingoid base length and the hydroxylation rate of both the sphingoid base and fatty acid moieties of IPC. In particular, the composition of the PM of *sur7*Δ and *ydl222*Δ single mutants shows a decrease in the level of hydroxylation of IPC, while *ynl194*Δ and *ydl222*Δ *sur7*Δ *ynl194*Δ mutants show an increase in the level of IPC

hydroxylation and a relative decrease in the level of IPCs with longer (C20) sphingoid bases (*35*).

1.2. Metabolism of Exogenous Sphingoid Bases

Lcb4p and Lcb5p: Phosphorylation of Sphingoid Bases. The *LCB4* and *LCB5* genes encode lipid kinases that add phosphate to DHS and PHS to form DHS-1P and PHS-1P, respectively. They are the sole sphingoid base kinases, as an *lcb4*Δ*lcb5*Δ strain has no sphingoid base kinase activity (*37*). Lcb4p, and Lcb5p, encode sphingoid base kinase activity by themselves and are not regulatory units as expression of either of these genes in *E. coli* shows sphingoid base kinase activity (*37*). The cytosolic sphingoid base kinase activity appears to be stereospecific because the nonbiological *threo*-DHS isomer is poorly phosphorylated (*37*−*39*).

LCB4 and *LCB5* are not essential, and deletion of either or both of these genes causes no changes in vegetative growth. However, an *lcb4*Δ*lcb5*Δ strain has a reduced rate of incorporation of exogenous sphingoid bases into sphingolipids but to a far lesser extent than *lcb3*Δ (*40*). Lcb4p represents 97% of total cellular kinase activity, with the other 3% being due to Lcb5p. Two-thirds of the Lcb4p and one-third of the Lcb5p kinase activity are in the membrane fraction, a puzzling finding as neither protein contains a membrane localization signal. *LCB4* but not *LCB5* is required for incorporation of exogenous sphingoid bases into ceramide. Interestingly, it is the membrane-associated form of Lcb4p, which is required for this activity. These data raise the possibility that membrane-associated and cytosolic kinases may play distinct roles (K. Funato et al., manuscript submitted for publication).

Lcb3p and Ysr3p: Dephosphorylation of DHS-1P and PHS-1P. LCB3 and *YSR3* encode phosphatases that are able to dephosphorylate sphingoid base phosphates (*41*). They dephosphorylate sphingoid base phosphates, but not ceramide phosphate nor phospholipids. *LCB3* and *YSR3* encode integral membrane proteins with four to eight transmembrane domains predicted according to different algorithms. Each has a novel lipid phosphatase motif consisting of three conserved domains (*42*). The activity of Lcb3p purified from *S. cerevisiae* has been characterized (*43*). Neither of these genes is essential, and both of the encoded proteins are localized to the ER (*44*).

Although Lcb3p and Ysr3p are 53% identical, they differ in several aspects of their physiological functions (*45*). (i) *LCB3* has higher transcription levels, whereas mRNA of *YSR3* is barely detectable (*44*). (ii) An *lcb3*Δ strain, but not a *ysr3*Δ strain, is protected from heat shock in SD medium. In YPD medium, the opposite situation is observed, as the *ysr3*Δ mutant is more resistant to heat shock than is the *lcb3*Δ mutant. Northern blot analysis showed that the *LCB3* message predominates in log phase cells, and the strength of the *YSR3* message increases with heat stress. It seems that *YSR3* may play a major role under stress conditions (*46*). (iii) Lcb3p, but not Ysr3p, is critical for the incorporation of exogenous DHS into ceramide (*41*). An *lcb3*Δ strain accumulates sphingoid base phosphates, has a lower level of ceramide and sphingolipids, is hypersensitive to australifungin, a ceramide synthesis inhibitor, and is more resistant to growth inhibition by sphingoid bases (*43*). Overexpression of *LCB3* leads to increased levels of ceramide (*44*). The failure of an *lcb3*Δ strain to incorporate DHS into sphingolipids is due to the inability to dephosphorylate sphingoid base phosphates, suggesting a phosphorylation−dephosphorylation cycle for incorporation of exogenous sphingoid bases into sphingolipids (*43*, *46*).

Dpl1p: Degradation of Sphingoid Base Phosphate. DHS-1P and PHS-1P are degraded by a sphingoid base phosphate lyase activity encoded by *DPL1*. Dpl1p cleaves sphingoid base phosphates between C2 and C3 to produce ethanolamine phosphate and a fatty aldehyde. This reaction requires pyridoxal phosphate as a cofactor (*47*). Dpl1p activity is stereospecific for the D-*erythro*-sphingoid base, and prefers C16-DHS-1P as a substrate (*48*). Dpl1p is localized to the ER (*49*). One single transmembrane domain, close to the N-terminus, is predicted. *DPL1* is not essential for vegetative growth; however, a *dpl1*Δ strain is hypersensitive to growth inhibition by sphingoid base (*47*), and both exogenously added and endogenous sphingoid base phosphates accumulate (*48*, *50*, *51*).

1.3. From Ceramide to Complex Sphingolipids

Aur1p: IPC Synthase. Ceramide is further converted to inositol phosphorylceramide (IPC), the first of three complex sphingolipids, each of them containing inositol phosphate. The inositol phosphate group of phosphatidylinositol is transferred to the C1 OH group of ceramide by IPC synthase, a membrane-bound enzyme (*52*). The *AUR1* gene encodes the IPC synthase or a subunit of this enzyme (*53*). *AUR1* is an essential gene (*54*), suggesting that either the lack of complex sphingolipids or accumulation of ceramide or other precursors is lethal for the cells. Our recent findings strongly support the hypothesis that accumulation of ceramide rather than a lack of sphingolipid is responsible for lethality (*19*).

Levine et al. have shown that Aur1p is localized to the Golgi apparatus, primarily in the *medial* compartment (*55*). Aur1p contains several predicted transmembrane domains, with its N-terminus being in the lumen and its C-terminus in the cytosol (*55*). Analysis of Aur1p homologues revealed four conserved domains, which are similar to the motifs identified in a superfamily of integral membrane phosphatases and soluble haloperoxidases (*56*). The active site is located in the lumen of the Golgi (*55*). The first conserved domain is important for conferring sensitivity to aureobasidin A, an inhibitor of IPC synthase. Two independent aureobasidin A-resistant clones have point mutations in this region (Phe158Tyr and Leu137Phe, and His157Tyr) (*54*, *57*).

Csg1p, Csg2p, and Vrg4p: Mannosylation of IPC To Form MIPC. IPC is then mannosylated to yield mannose inositol-P-ceramide (MIPC). In this reaction, the mannosyl group is supplied by GDP-mannose, and Vrg4p has been shown to deliver GDP-mannose from the cytosol to the lumen of the Golgi. *VRG4* is essential for the formation of mannosylated sphingolipids, although it also affects N-linked and O-linked glycoprotein modifications (*58*). The IPC mannosylation reaction requires two genes: *CSG1* (*32*) and *CSG2* (*2*). Mutants in either gene are viable, but their deletion prevents MIPC formation and causes IPC accumulation. Csg1p is probably a mannosyltransferase, because it contains a region of 93 amino acids that is similar to two yeast α-1,6-mannosyltransferases, Och1p and Hoc1p. The function of Csg2p is not obvious. It has an EF-Ca^{2+}-binding domain and

9−10 predicted transmembrane domains, but it is unclear why this protein is necessary for mannosylation of IPC (*59*, *60*). Csg2p may play a role in the regulation of calcium ion homeostasis in the ER (*60*) where the protein has been localized (*61*); this role might be distinct from its role in MIPC synthesis, which takes place in the Golgi (*31*).

Strains deleted for *CSG1* and *CSG2* are hypersensitive to calcium. Different lines of evidence support the idea that the calcium sensitivity is due to accumulation or mislocalization of IPC (more specifically, IPC-C) rather than to the lack of MIPC (*32*). This phenotype has been very useful for identifying a number of genes involved in the sphingolipid biosynthetic pathway (*2*).

Ipt1p: Formation of M(IP)$_2$C. The final and most abundant sphingolipid, M(IP)$_2$C, is formed by transfer of an inositol phosphate group from phosphatidylinositol onto MIPC, a reaction very similar to the one requiring Aur1p. *IPT1* was first identified on the basis of its homology with *AUR1*; the two genes are 27% identical. It was then shown that *IPT1* encodes the enzyme necessary for this reaction. Because of its degree of homology with Aur1p, one can expect the active site of Ipt1p to be on the lumenal side of the Golgi membranes. *IPT1* is not an essential gene, and deletion mutants show no severe phenotype. An *ipt1*Δ strain lacks M(IP)$_2$C synthase activity, makes no detectable M(IP)$_2$C, and has increased levels of MIPC (*62*). M(IP)$_2$C normally accounts for 75% of the sphingolipids in wild-type *S. cerevisiae* cells, with the other 25% being equally divided between IPC and MIPC. It appears that *S. cerevisiae* is able to sense its total sphingolipid content and adjust the relative level of MIPC to compensate for the absence of M(IP)$_2$C. Sphingolipids are estimated to make up 7−8% of the mass of the PM (*62*).

Isc1p: Complex Sphingolipase. ISC1 encodes an enzyme that catalyzes the breakdown of the three complex sphingolipids into ceramide (*63*, *64*). Isc1p contains two putative transmembrane domains, but its subcellular localization remains to be elucidated. The activity of this enzyme is greatly enhanced by the presence of phosphatidylserine (*64*) and is *N*-ethylmaleimide-sensitive (J. Holthuis, personal communication). *isc1*Δ cells have strongly reduced sphingolipase activity, suggesting that *ISC1* is the major gene encoding a complex sphingolipase (*64*). These data suggest that ceramide can be synthesized both *de novo* and by catabolism of IPC, as is the case in mammalian cells by using sphingomyelinase.

2. TRAFFICKING OF SPHINGOLIPIDS: TRANSLOCATION, TRANSPORT, AND SORTING

While in the yeast *S. cerevisiae* most of the enzymes involved in the sphingolipid synthesis have been cloned and functionally characterized, much less is known about the distribution and topology of sphingolipids in cellular membranes, as well as their intracellular transport or sorting between organelles. The membranes of different intracellular organelles have different lipid compositions that are critical for their proper functions. Since the first step in the biosynthesis of most membrane lipids takes place in the ER, the synthesized lipids have to be transported to their final destination. The movement of lipids between organelles should be tightly regulated and carried out by specific mechanisms for each lipid. Generally, there are three possible mechanisms for lipid transport from one membrane to the other: (i) vesicular transport as for proteins, with budding of vesicles from a donor membrane, delivery, and fusion to the target membrane; (ii) transfer of lipid monomers through the cytosol (this can occur either by spontaneous diffusion or by protein-facilitated transport); and (iii) lateral diffusion of lipids between membranes at specific regions of direct membrane contact. Furthermore, lipids can translocate across membrane bilayers, a process termed flip-flop. This section focuses on the intracellular movement of sphingolipids in *S. cerevisiae*.

2.1. Sphingoid Base and Sphingoid Base Phosphate Travel to the Site of Ceramide Synthesis

The ER is the site for *de novo* synthesis of sphingoid bases, followed by further synthesis of ceramides in all eukaryotic cells. In mammalian cells, these early synthetic steps are believed to occur at the cytosolic surface of the ER. Treatment of intact membranes with proteases or impermeant inhibitors has been observed to inactivate mammalian ER synthetic enzymes (*65*, *66*). However, the topology of the active site of these enzymes has not been definitively established. According to this model, ceramide is probably translocated toward the lumen of ER, because the active site of ceramide galactosyltransferase, which transfers UDP-galactose to ceramide to form galactosylceramide, is localized at the lumenal side of the ER (*67*). This may also be the case in yeast, if ceramide is produced at the cytosolic surface of the ER (Figure 2, step 1), because it would need to be translocated to the lumen where it is used for lipid remodeling of GPI anchors (*68*).

However, new evidence raises the possibility that ceramide is synthesized on the lumenal side of the ER (Figure 2, step 2). In yeast, exogenously added sphingoid bases can be incorporated into ceramide, requiring a cycle of phosphorylation and dephosphorylation of the sphingoid bases for high efficiency (*43*). Deletion of *LCB3* or both *LCB3* and *YSR3* almost completely blocks the incorporation of the exogenous sphingoid base into ceramide (*44*). Since the active site of Lcb3p appears to be located in the lumen of the ER (A. Kihara and Y. Igarashi, personal communication), the resulting free sphingoid bases could be metabolized into ceramide in the lumen of the ER. Clearly, topological analysis of the active sites of ceramide synthases, Lag1p and Lac1p, as well as a 3-KDS reductase, Tsc10p, is needed to understand the possible movements of sphingoid base and ceramide across the ER membrane.

As already mentioned, yeast can incorporate exogenous sphingoid bases, as well as sphingoid base phosphates, into sphingolipids, so they must be delivered from the PM to the ER membrane. Recently, a member of the ATP binding cassette (ABC) family, the human cystic fibrosis transmembrane conductance regulator (CFTR), has been shown to stimulate uptake of sphingosine 1-phosphate and the related sphingoid base phosphate, DHS-1P, but not sphingosine (*69*). Similar cellular uptake of lipids mediated by such a translocator is thought to occur in yeast. A member of the ABC transporter superfamily encoded by *YOR1*, a *S. cerevisiae* oligomycin resistance gene, is highly homologous with human CFTR and is located at the PM (*70*). In contrast to

Table 1: Genes Involved in the Sphingolipid Biosynthetic Pathway in *S. cerevisiae*, Their Function, and Their Localization (P, predicted localization; E, experimentally determined)

gene	function	localization
ACB1	fatty acyl-CoA carrier	?
AUR1	synthesis of IPC, possible IPC synthase or a subunit of the enzyme	*medial*-Golgi (E)
CCC2	Golgi copper transporter, required for hydroxylation of the fatty acid moiety of sphingolipids	late- or post-Golgi (E)
CSG1 (*SUR1*)	necessary for α-mannosylation of IPC	?
CSG2	addition of mannose to IPC, function unclear	ER (E), ?
DPL1	breakdown of sphingoid base phosphates, possible sphingoid base phosphate lyase	ER (E)
ELO2 (*FEN1*)	synthesis of C_{24} fatty acids, possible component of fatty acid elongation	ER (E)
ELO3 (*SUR4*)	conversion of C_{24} to C_{26} fatty acids, possible component of fatty acid elongation	ER (E)
ICS1	complex sphingolipase	?
IPT1	synthesis of $M(IP)_2C$, possible $M(IP)_2C$ synthase	?
LAC1	synthesis of ceramide, possible ceramide synthase or a subunit of the enzyme	ER (E)
LAG1	synthesis of ceramide, possible ceramide synthase or a subunit of the enzyme	ER (E)
LCB1	synthesis of sphingoid bases, catalytic subunit of serine palmitoyltransferase	?
LCB2 (*SCS1*)	synthesis of sphingoid bases, catalytic subunit of serine palmitoyltransferase	?
LCB3 (*YSR2*)	sphingoid base 1-phosphate phosphatase	ER (E)
LCB4	sphingoid base kinase	cytosol and ER (E)
LCB5	sphingoid base kinase	cytosol and Golgi (E)
SCS7 (*FAH1*)	required for specific α-hydroxylation of the fatty acid moiety of sphingolipids	ER (P)
SUR2 (*SYR2*)	hydroxylation of DHS or dihydroceramide at position C4 to generate PHS or phytoceramide	ER (E)
SUR7	influences the sphingoid base length and the rate of hydroxylation of both sphingoid base and fatty acid moieties	cortical patches of the plasma membrane (E)
TSC3	subunit of SPT, necessary for optimal activity of LCB1 and LCB2	?
TSC10	3-ketosphinganine reductase	ER (E)
TSC13	required for fatty acid elongation, probable enoyl reductase	ER, and enriched at the sites of vacuole−nuclear contacts (E)
VRG4	required for the transport of GDP-mannose in the lumen of the Golgi	Golgi complex (E)
YBR159w	required for fatty acid elongation, putative β-ketoacyl reductase	?
YDC1	alkaline dihydroceramidase, with a reverse CoA-independent activity of ceramide synthase in the presence of FB1 or in a Δ*lac1*Δ*lag1* strain	ER (E)
YDL222w	influence the sphingoid base length and the rate of hydroxylation of both sphingoid base and fatty acid moieties	cortical patches of the plasma membrane (E)
YNL194w	influence the sphingoid base length and the rate of hydroxylation of both sphingoid base and fatty acid moieties	cortical patches of the plasma membrane (E)
YPC1	alkaline phytoceramidase, with a reverse CoA-independent activity of ceramide synthase in the presence of FB1 or in a Δ*lac1*Δ*lag1* strain	ER (E)
YSR3 (*LBP2*)	sphingoid base 1-phosphate phosphatase	ER (E)

the human CFTR, however, Yor1p appears to function in pumping sphingoid base phosphates out of yeast cells (Figure 2, step 3) and, consequently, preventing their accumulation and toxic effects, because expression of *YOR1* is required for resistance to fumonisin B1 and sphingosine (C. Mao and L. Obeid, personal communication). Protein-mediated sphingoid base efflux across the PM has recently been reported (*71*). This efflux seems to be mediated by Rsb1p, an integral plasma membrane protein (Figure 2, step 3). Rsb1p has an ATP binding motif, and Rsb1p-dependent efflux is ATP-dependent. Rsb1p is active on sphingoid bases but not on sphingoid base phosphates. Translocation to the cytosolic leaflet of the PM is expected to be important in mediating uptake of exogenous sphingoid bases and sphingoid base phosphates, but no transporters for this event have been identified (Figure 2, step 4).

2.2. Ceramide Travels to the Site of IPC Synthesis

After synthesis in the ER, ceramide must be transported to the Golgi for synthesis of IPC. Evidence has been presented that ceramide is transported from the ER to the Golgi apparatus by both vesicular (Figure 2, step 5) and nonvesicular (Figure 2, step 6) means in yeast (*72*). The existence of a nonvesicular transport mechanism for ceramide was suggested by in vivo studies with early secretory mutants (*31*, *68*). This process is apparently ATP-independent. Similarly, in mammalian cells, an energy-independent mechanism delivers ceramide to the Golgi membrane for both sphingomyelin (SM) and glucosylceramide (GlcCer) synthesis (*73*). Interestingly, studies using an in vitro assay revealed that nonvesicular transport of ceramide requires ER−Golgi membrane contacts that are likely to be specific and function to facilitate the transfer of ceramide from the ER to the Golgi (Figure 2, step 6) (*72*). Membrane contacts have been observed between the ER and trans-Golgi cisternae (*74*) in mammalian cells, and between the ER and PM (*75*), between the ER and mitochondria (*76*), and at the nuclear−vacuolar interface (*16*) in yeast. In addition, an important role of membrane−membrane contacts in intracellular lipid movements has been reported for ER-to-mitochondria trafficking of phosphatidylserine (*77*). In vitro studies have also shown that a cytosolic protein is required for a late step of ceramide transport after formation of ER−Golgi membrane contacts, probably for exchange of ceramide between the cytosolic leaflet of the ER and the Golgi membranes, since no direct fusion of the ER with Golgi membranes occurs during this in vitro assay (*72*). On the basis of the localization of Aur1p and the topology of its putative active site (*55*), ceramide on the cytosolic side of the Golgi must be translocated to the lumenal side for IPC synthesis. An energy-independent translocator probably mediates this translocation, because IPC synthesis via nonvesicular ceramide transport is energy-independent. In mammalian cells, the existence of an energy-independent translocator has been proposed for translocation of GlcCer to the lumenal side of the Golgi membrane (*78*). For SM synthesis, ceramide might also be translocated toward the lumenal side of Golgi membranes by an energy-independent translocator (*73*).

Both vesicular and nonvesicular mechanisms deliver ceramide to the site of IPC synthesis in vivo. So far, it is

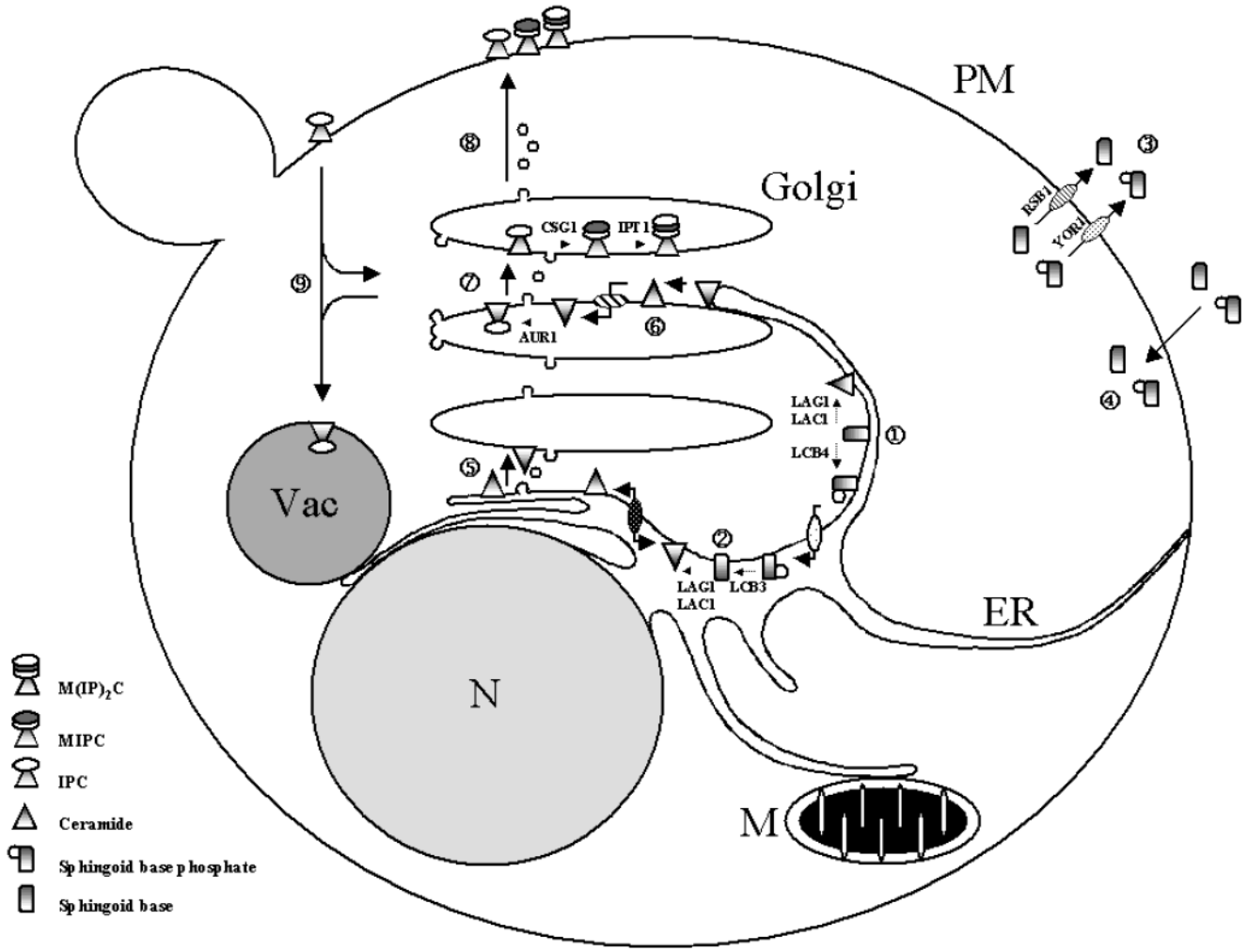

FIGURE 2: Schematic representation of sphingolipid trafficking in the yeast *S. cerevisiae*. Sphingoid base synthesized *de novo* in the ER is N-acylated by ceramide synthases, Lag1p and Lac1p, to yield ceramide. This process occurs either at the cytosolic surface (step 1) or at the lumenal side (step 2) of the ER. In both cases, ceramide can cross the ER membrane. A fraction of sphingoid base is phosphorylated by the major sphingoid base kinase, Lcb4p, and the resulting sphingoid base phosphate can translocate toward the lumenal leaflet of the ER, where it can be dephosphorylated by sphingoid base phosphate phosphatase, Lcb3p. Sphingoid base and sphingoid base phosphate accumulated intracellularly by enhanced metabolism or by exogenous supply are pumped out of yeast cells by Rsb1p and Yor1p, respectively (step 3). Exogenously added sphingoid base and sphingoid base phosphate can be delivered to the site of ceramide synthesis (step 4). After synthesis in the ER, ceramide is transported to the Golgi by a vesicular (step 5) or a nonvesicular pathway (step 6). The vesicular pathway delivers ceramide to the *cis*-Golgi, whereas the nonvesicular pathway delivers ceramide to the *medial*-Golgi, where IPC synthase, Aur1p, is located. This pathway, which depends on membrane−membrane contacts, most likely ER−*medial*-Golgi contacts, allows a rapid exchange of ceramide from the ER to the cytosolic surface of the Golgi. Then, ceramide is translocated to the lumenal side of the Golgi membrane, where it can be converted to IPC. The lumenal IPC may follow the vesicular transport route to reach the different subcompartments of the Golgi, where it can be hydroxylated to form a subclass of IPC (IPC/D). Subsequently, IPC is converted to MIPC by Csg1p and to $M(IP)_2C$ by Ipt1p (step 7). All complex sphingolipids are transported to the PM via the vesicular pathway (step 8). The preferential enrichment in IPC in the Golgi and in the vacuole occurs by uncertain routes or mechanisms (step 9).

not clear why these two pathways coexist. However, it has been shown that IPC synthase is located primarily in *medial*-Golgi but not in the *cis*-Golgi compartment (*55*), which means that ceramide transported to the *cis*-Golgi compartment via the vesicular pathway cannot be metabolized until it is delivered to the *medial*-Golgi compartment. Therefore, it is postulated that vesicular transport would function to provide free ceramide for regulation of cellular processes between ER and early Golgi compartments, such as protein transport or signal transduction events. In yeast, ceramide and/or sphingoid base is required specifically for transport of GPI-anchored proteins to the Golgi (*79*). In contrast, nonvesicular transport would provide ceramide directly for IPC synthesis.

Interestingly, in mammalian cells, the content of SM and cholesterol in COPI-coated vesicles is lower than in donor Golgi membranes, suggesting segregation of these lipids from retrograde transport vesicles (*80*). It will be interesting to know whether IPC, as well as ceramide, recycles back from the Golgi compartment to the ER, either via vesicular or nonvesicular pathways.

2.3. IPC/B and IPC/C Travel to the Site of IPC/D, MIPC, and $M(IP)_2C$ Synthesis

Previous studies using radiolabeled *myo*-inositol or DHS with early secretory mutants in yeast, namely, *sec12*, *-16*, *-17*, *-18*, *-22*, and *-23*, have shown that biosynthesis of a subclass of IPC (IPC/D), MIPC, and $M(IP)_2C$ is largely

dependent on genes that are required for the vesicular transport of proteins from the ER to the Golgi, even though synthesis of IPC/B and IPC/C still continues via nonvesicular transport (*31*). In addition, the biosynthesis of IPC/D, MIPC, and $M(IP)_2C$ clearly requires metabolic energy. In contrast, the synthesis of these lipids is not affected in cells with secretory blocks at late stages involving the budding (*sec14*) or fusion (*sec6*) of secretory vesicles between the Golgi and the PM, indicating that vesicular transport from the Golgi apparatus to the PM is not required for the biosynthesis of these lipids. Therefore, the sites of their synthesis are restricted to Golgi compartments in the secretory pathway. Thus, the defect in complex sphingolipid synthesis in early *sec* mutants is most simply interpreted in terms of a model in which IPC/B and IPC/C are synthesized in the Golgi compartment to which ceramide is delivered by nonvesicular transport. The biosynthesis of IPC/D, MIPC, and $M(IP)_2C$ would require a vesicular transport step, perhaps delivery of the sphingolipid to another Golgi compartment or retrieval of Golgi enzymes by vesicular traffic to the compartment where the sphingolipid precursors are located (Figure 2, step 7). Hydroxylation of IPC/C to form IPC/D requires *CCC2* (*32*), and mannosylation of IPC to form MIPC and $M(IP)_2C$ requires *VRG4* (*58*). *CCC2* encodes a Cu^{2+} transporter, and *VRG4* encodes a GDP-mannose transporter. They mediate the transport of ion and sugar from the cytoplasm to the lumen of the Golgi, respectively. Although Vrg4p is broadly distributed throughout the Golgi complex (*58*), Ccc2p appears to be restricted to a late or post-Golgi compartment (*34*). The restricted localization of Ccc2p is consistent with the vesicular transport model, since the IPC synthase, Aur1p, is mainly localized in a Mnt1p (a marker for *medial*-Golgi) positive compartment but not in a Sec7p or a Tlg1p (marker for the late Golgi and endosomes) positive compartment; therefore, Ccc2p is in a later Golgi compartment than is Aur1p. On the basis of the topology of Aur1p and the function of Vrg4p as a lumenal GDP-mannose transporter, the synthesis of all complex sphingolipids most likely occurs in the Golgi lumen. Since the bulk of complex sphingolipids would be in the outer leaflet of the PM with their polar headgroups pointed toward the yeast cell wall, the vesicular pathway might be used as a transport mechanism simply to maintain their asymmetric distribution. The effects of early *sec* mutations on biosynthesis of IPC/D, MIPC, and $M(IP)_2C$ could be explained by the fact that some of these mutations affect the localization of late Golgi proteins, or cause the dispersion and disappearance of late Golgi structure (*81*−*83*). It will be important to define the localization within the Golgi of the enzymes, Csg1p and Ipt1p, involved in the sphingolipid biosynthesis, as well as their topology in the Golgi.

2.4. Complex Sphingolipids Travel to the PM and Vacuoles

The fact that all complex sphingolipids are present in secretory vesicles isolated from late secretory yeast mutants, *sec1* or *sec6* (*84*), raises the possibility that Golgi-to-PM or Golgi-to-vacuole transport of sphingolipids occurs via the vesicular transport mechanism of secretory proteins. Indeed, a later study using the late secretory mutants *sec1*, *sec6*, and *sec14* has shown that inositol-containing sphingolipids do not reach the PM of these mutants under nonpermissive conditions (*85*). These observations indicate that complex sphingolipids are transported from the Golgi to the PM via the vesicular pathway of secretory proteins (Figure 2, step 8).

An earlier study has shown that IPC is highly enriched in the Golgi and in the vacuole, whereas the largest amounts of MIPC and $M(IP)_2C$ are found in the PM (*84*). Since the synthesis of these complex sphingolipids occurs in the Golgi apparatus, their different distribution in other membranes implies the existence of a sorting mechanism for complex sphingolipids. Although the physical role of sphingolipid sorting remains to be elucidated, several possible sorting mechanisms, especially for enriching IPC in the Golgi and in the vacuole, are illustrated in step 9 of Figure 2. First, IPC transported to the PM may preferentially recycle back to the Golgi through the endocytic/recycling pathway. At the same time, a fraction of IPC could reach the vacuole by the same mechanism. Second, the enrichment of IPC in the Golgi apparatus and in the vacuole might be due to the regulated retention of IPC in the Golgi apparatus and its specific transport from the Golgi compartments to the vacuole via transport vesicles. Third, a nonvesicular mechanism that delivers specifically IPC from the PM to the Golgi apparatus and to the vacuole is also possible, though unlikely. In this respect, it will be interesting to know whether and to what extent IPC, as well as the other complex sphingolipids, is present in the cytosolic leaflets of the PM, the Golgi, and the vacuole membrane.

3. FUTURE DIRECTIONS AND PERSPECTIVES

This review has focused on the current knowledge we have about the biosynthetic pathway and trafficking of sphingolipids in *S. cerevisiae*. Most of the genes encoding biosynthetic enzymes have been identified now, but new genes may still be found, because the enzymes have not been purified to homogeneity and, thus, their subunit composition remains unknown. One challenge in this field is to purify these enzymes and further characterize their activities. It would also be interesting to determine their three-dimensional structures, which could greatly help in understanding their physiological roles and the topology of the biosynthetic reactions. Another important issue to address is the regulation of the pathway, which can only be understood in the context of knowledge about the enzymes. It is also important to note that ceramide and the complex sphingolipids of *S. cerevisiae* [IPC, MIPC, and $M(IP)_2C$] are mixtures rather than unique molecular species. They differ in chain length and in the extent of hydroxylation of both sphingoid bases and fatty acid moieties. These differences are likely to be physiologically important, but supporting data are at present meager (*86*). Finally, many questions with regard to the trafficking of sphingolipids remain to be answered. The cell-free system used to study the ER-to-Golgi trafficking of ceramide could be a powerful tool for purifying and biochemically characterizing the proteins that are involved in sphingolipid transport. The creation of yeast mutants that are defective in sphingolipid transport would also give new insights into this field.

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SUPPORTING INFORMATION AVAILABLE

Diverse specific and useful inhibitors of different steps of the sphingolipid biosynthetic pathway (Table 2). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Mandon, E. C., Ehses, I., Rother, J., van Echten, G., and Sandhoff, K. (1992) *J. Biol. Chem. 267*, 11144−11148.
2. Zhao, C., Beeler, T., and Dunn, T. (1994) *J. Biol. Chem. 269*, 21480−21488.
3. Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L., and Dickson, R. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 7899−7902.
4. Buede, R., Rinker-Schaffer, C., Pinto, W. J., Lester, R. L., and Dickson, R. C. (1991) *J. Bacteriol. 173*, 4325−4332.
5. Pinto, W. J., Srinivasan, B., Shepherd, S., Schmidt, A., Dickson, R. C., and Lester, R. L. (1992) *J. Bacteriol. 174*, 2565−2574.
6. Gable, K., Slife, H., Bacikova, D., Monaghan, E., and Dunn, T. M. (2000) *J. Biol. Chem. 275*, 7597−7603.
7. Mukherjee, J. J., and Dekker, E. E. (1990) *Biochim. Biophys. Acta 1037*, 24−29.
8. Gable, K., Han, G., Monaghan, E., Bacikova, D., Natarajan, M., Williams, R., and Dunn, T. M. (2002) *J. Biol. Chem. 277*, 10194−10200.
9. Monaghan, E. P., Gable, K., and Dunn, T. M. (2002) *Yeast 19*, 659−670.
10. Pinto, W. J., Wells, G. W., and Lester, R. L. (1992) *J. Bacteriol. 174*, 2575−2581.
11. Beeler, T., Bacikova, D., Gable, K., Hopkins, L., Johnson, C., Slife, H., and Dunn, T. (1998) *J. Biol. Chem. 273*, 30688−30694.
12. Duax, W. L., Griffin, J. F., and Ghosh, D. (1996) *Curr. Opin. Struct. Biol. 6*, 813−823.
13. Ghosh, D., Wawrzak, Z., Weeks, C. M., Duax, W. L., and Erman, M. (1994) *Structure 2*, 629−640.
14. Toke, D. A., and Martin, C. E. (1996) *J. Biol. Chem. 271*, 18413−18422.
15. Oh, C. S., Toke, D. A., Mandala, S., and Martin, C. E. (1997) *J. Biol. Chem. 272*, 17376−17384.
16. Kohlwein, S. D., Eder, S., Oh, C. S., Martin, C. E., Gable, K., Bacikova, D., and Dunn, T. (2001) *Mol. Cell. Biol. 21*, 109−125.
17. Beaudoin, F., Gable, K., Sayanova, O., Dunn, T., and Napier, J. A. (2002) *J. Biol. Chem. 277*, 11481−11488.
18. Gaigg, B., Neergaard, T. B., Schneiter, R., Hansen, J. K., Faergeman, N. J., Jensen, N. A., Andersen, J. R., Friis, J., Sandhoff, R., Schroder, H. D., and Knudsen, J. (2001) *Mol. Biol. Cell 12*, 1147−1160.
19. Schorling, S., Vallee, B., Barz, W. P., Riezman, H., and Oesterhelt, D. (2001) *Mol. Biol. Cell 12*, 3417−3427.
20. Guillas, I., Kirchman, P. A., Chuard, R., Pfefferli, M., Jiang, J. C., Jazwinski, S. M., and Conzelmann, A. (2001) *EMBO J. 20*, 2655−2665.
21. Barz, W. P., and Walter, P. (1999) *Mol. Biol. Cell 10*, 1043−1059.
22. Mao, C., Xu, R., Bielawska, A., Szulc, Z. M., and Obeid, L. M. (2000) *J. Biol. Chem. 275*, 31369−31378.
23. Mao, C., Xu, R., Bielawska, A., and Obeid, L. M. (2000) *J. Biol. Chem. 275*, 6876−6884.
24. Grilley, M. M., Stock, S. D., Dickson, R. C., Lester, R. L., and Takemoto, J. Y. (1998) *J. Biol. Chem. 273*, 11062−11068.
25. Haak, D., Gable, K., Beeler, T., and Dunn, T. (1997) *J. Biol. Chem. 272*, 29704−29710.
26. Grilley, M. M., and Takemoto, J. Y. (2000) *Methods Enzymol. 311*, 9−14.
27. Dickson, R. C., Nagiec, E. E., Skrzypek, M., Tillman, P., Wells, G. B., and Lester, R. L. (1997) *J. Biol. Chem. 272*, 30196−30200.
28. Jenkins, G. M., Richards, A., Wahl, T., Mao, C., Obeid, L., and Hannun, Y. (1997) *J. Biol. Chem. 272*, 32566−32572.
29. Cliften, P., Wang, Y., Mochizuki, D., Miyakawa, T., Wangspa, R., Hughes, J., and Takemoto, J. Y. (1996) *Microbiology 142*, 477−484.
30. Dunn, T. M., Haak, D., Monaghan, E., and Beeler, T. J. (1998) *Yeast 14*, 311−321.
31. Puoti, A., Desponds, C., Fankhauser, C., and Conzelmann, A. (1991) *J. Biol. Chem. 266*, 21051−21059.
32. Beeler, T. J., Fu, D., Rivera, J., Monaghan, E., Gable, K., and Dunn, T. M. (1997) *Mol. Gen. Genet. 255*, 570−579.
33. Mitchell, A. G., and Martin, C. E. (1997) *J. Biol. Chem. 272*, 28281−28288.
34. Yuan, D. S., Dancis, A., and Klausner, R. D. (1997) *J. Biol. Chem. 272*, 25787−25793.
35. Young, M. E., Karpova, T. S., Brugger, B., Moschenross, D. M., Wang, G. K., Schneiter, R., Wieland, F. T., and Cooper, J. A. (2002) *Mol. Cell. Biol. 22*, 927−934.
36. Sivadon, P., Peypouquet, M. F., Doignon, F., Aigle, M., and Crouzet, M. (1997) *Yeast 13*, 747−761.
37. Nagiec, M. M., Skrzypek, M., Nagiec, E. E., Lester, R. L., and Dickson, R. C. (1998) *J. Biol. Chem. 273*, 19437−19442.
38. Skrzypek, M. S., Nagiec, M. M., Lester, R. L., and Dickson, R. C. (1998) *J. Biol. Chem. 273*, 2829−2834.
39. Lanterman, M. M., and Saba, J. D. (1998) *Biochem. J. 332*, 525−531.
40. Zanolari, B., Friant, S., Funato, K., Sutterlin, C., Stevenson, B. J., and Riezman, H. (2000) *EMBO J. 19*, 2824−2833.
41. Qie, L., Nagiec, M. M., Baltisberger, J. A., Lester, R. L., and Dickson, R. C. (1997) *J. Biol. Chem. 272*, 16110−16117.
42. Stukey, J., and Carman, G. M. (1997) *Protein Sci. 6*, 469−472.
43. Mao, C., Wadleigh, M., Jenkins, G. M., Hannun, Y. A., and Obeid, L. M. (1997) *J. Biol. Chem. 272*, 28690−28694.
44. Mao, C., Saba, J. D., and Obeid, L. M. (1999) *Biochem. J. 3*, 667−675.
45. Mao, C., and Obeid, L. M. (2000) *Methods Enzymol. 311*, 223−232.
46. Mandala, S. M., Thornton, R., Tu, Z., Kurtz, M. B., Nickels, J., Broach, J., Menzeleev, R., and Spiegel, S. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 150−155.
47. Saba, J. D., Nara, F., Bielawska, A., Garrett, S., and Hannun, Y. A. (1997) *J. Biol. Chem. 272*, 26087−26090.
48. Zhang, X., Skrzypek, M. S., Lester, R. L., and Dickson, R. C. (2001) *Curr. Genet. 40*, 221−233.
49. Grote, E., Vlacich, G., Pypaert, M., and Novick, P. J. (2000) *Mol. Biol. Cell 11*, 4051−4065.
50. Skrzypek, M. S., Nagiec, M. M., Lester, R. L., and Dickson, R. C. (1999) *J. Bacteriol. 181*, 1134−1140.
51. Kim, S., Fyrst, H., and Saba, J. (2000) *Genetics 156*, 1519−1529.
52. Becker, G. W., and Lester, R. L. (1980) *J. Bacteriol. 142*, 747−754.
53. Nagiec, M. M., Nagiec, E. E., Baltisberger, J. A., Wells, G. B., Lester, R. L., and Dickson, R. C. (1997) *J. Biol. Chem. 272*, 9809−9817.
54. Heidler, S. A., and Radding, J. A. (1995) *Antimicrob. Agents Chemother. 39*, 2765−2769.
55. Levine, T. P., Wiggins, C. A., and Munro, S. (2000) *Mol. Biol. Cell 11*, 2267−2281.
56. Heidler, S. A., and Radding, J. A. (2000) *Biochim. Biophys. Acta 1500*, 147−152.
57. Hashida-Okado, T., Ogawa, A., Endo, M., Yasumoto, R., Takesako, K., and Kato, I. (1996) *Mol. Gen. Genet. 251*, 236−244.
58. Dean, N., Zhang, Y. B., and Poster, J. B. (1997) *J. Biol. Chem. 272*, 31908−31914.
59. Beeler, T., Gable, K., Zhao, C., and Dunn, T. (1994) *J. Biol. Chem. 269*, 7279−7284.
60. Tanida, I., Takita, Y., Hasegawa, A., Ohya, Y., and Anraku, Y. (1996) *FEBS Lett. 379*, 38−42.
61. Takita, Y., Ohya, Y., and Anraku, Y. (1995) *Mol. Gen. Genet. 246*, 269−281.
62. Dickson, R. C., Nagiec, E. E., Wells, G. B., Nagiec, M. M., and Lester, R. L. (1997) *J. Biol. Chem. 272*, 29620−29625.
63. Wells, G. B., Dickson, R. C., and Lester, R. L. (1998) *J. Biol. Chem. 273*, 7235−7243.
64. Sawai, H., Okamoto, Y., Luberto, C., Mao, C., Bielawska, A., Domae, N., and Hannun, Y. A. (2000) *J. Biol. Chem. 275*, 39793−39798.
65. Michel, C., and van Echten-Deckert, G. (1997) *FEBS Lett. 416*, 153−155.
66. Hirschberg, K., Rodger, J., and Futerman, A. H. (1993) *Biochem. J. 290*, 751−757.
67. Sprong, H., Kruithof, B., Leijendekker, R., Slot, J. W., van Meer, G., and van der Sluijs, P. (1998) *J. Biol. Chem. 273*, 25880−25888.
68. Reggiori, F., Canivenc-Gansel, E., and Conzelmann, A. (1997) *EMBO J. 16*, 3506−3518.

69. Boujaoude, L. C., Bradshaw-Wilder, C., Mao, C., Cohn, J., Ogretmen, B., Hannun, Y. A., and Obeid, L. M. (2001) *J. Biol. Chem. 276*, 35258−35264.
70. Katzmann, D. J., Epping, E. A., and Moye-Rowley, W. S. (1999) *Mol. Cell. Biol. 19*, 2998−3009.
71. Kihara, A., and Igarashi, Y. (2002) *J. Biol. Chem. 28*, 28.
72. Funato, K., and Riezman, H. (2001) *J. Cell Biol. 155*, 949−959.
73. Fukasawa, M., Nishijima, M., and Hanada, K. (1999) *J. Cell Biol. 144*, 673−685.
74. Ladinsky, M. S., Mastronarde, D. N., McIntosh, J. R., Howell, K. E., and Staehelin, L. A. (1999) *J. Cell Biol. 144*, 1135−1149.
75. Preuss, D., Mulholland, J., Kaiser, C. A., Orlean, P., Albright, C., Rose, M. D., Robbins, P. W., and Botstein, D. (1991) *Yeast 7*, 891−911.
76. Achleitner, G., Gaigg, B., Krasser, A., Kainersdorfer, E., Kohlwein, S. D., Perktold, A., Zellnig, G., and Daum, G. (1999) *Eur. J. Biochem. 264*, 545−553.
77. Voelker, D. R. (2000) *Biochim. Biophys. Acta 1486*, 97−107.
78. Van Meer, G., and Lisman, Q. (2002) *J. Biol. Chem. 13*, 13.
79. Muniz, M., and Riezman, H. (2000) *EMBO J. 19*, 10−15.
80. Brugger, B., Sandhoff, R., Wegehingel, S., Gorgas, K., Malsam, J., Helms, J. B., Lehmann, W. D., Nickel, W., and Wieland, F. T. (2000) *J. Cell Biol. 151*, 507−518.
81. Ward, T. H., Polishchuk, R. S., Caplan, S., Hirschberg, K., and Lippincott-Schwartz, J. (2001) *J. Cell Biol. 155*, 557−570.
82. Wooding, S., and Pelham, H. R. (1998) *Mol. Biol. Cell 9*, 2667−2680.
83. Morin-Ganet, M. N., Rambourg, A., Deitz, S. B., Franzusoff, A., and Kepes, F. (2000) *Traffic 1*, 56−68.
84. Hechtberger, P., Zinser, E., Saf, R., Hummel, K., Paltauf, F., and Daum, G. (1994) *Eur. J. Biochem. 225*, 641−649.
85. Hechtberger, P., and Daum, G. (1995) *FEBS Lett. 367*, 201−204.
86. Swain, E., Baudry, K., Stukey, J., McDonough, V., Germann, M., and Nickels, J. T., Jr. (2002) *J. Biol. Chem. 10*, 10.

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