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Review

Yeast sphingolipids

Robert C. Dickson *, Robert L. Lester

Department of Biochemistry and the Lucille P. Markey Cancer Center, University of Kentucky Medical Center, Lexington, KY 40536-0084, USA

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Abstract

Many advances in our understanding of fungal sphingolipids have been made in recent years. This review focuses on the types of sphingolipids that have been found in fungi and upon the genes in *Saccharomyces cerevisiae*, the common baker's yeast, that are necessary for sphingolipid metabolism. While only a small number of fungi have been examined, most contain sphingolipids composed of ceramide derivatized at carbon-1 with inositol phosphate. Further additions include mannose and then other carbohydrates. The second major class of fungal sphingolipids is the glycosylceramides, having either glucose or galactose attached to ceramide rather than inositol phosphate. The glycosylceramides sometimes contain additional carbohydrates. Knowledge of the genome sequence has expedited identification of *S. cerevisiae* genes necessary for sphingolipid metabolism. At least one gene is known for most steps in *S. cerevisiae* sphingolipid metabolism, but more are likely to be identified so that the 13 known genes are likely to grow in number. The *AUR1* gene is necessary for addition of inositol phosphate to ceramide and has been identified as a target of several potent antifungal compounds. This essential step in yeast sphingolipid synthesis, which is not found in humans, appears to be an excellent target for the development of more effective antifungal compounds, both for human and for agricultural use.

Keywords: Ceramide; *AUR1*; Antifungal drug; Glycosylceramide; Sphingosine; (*Saccharomyces cerevisiae*)

Contents

* Corresponding author. Fax: +1-606-257-8940; E-mail: bobd@pop.uky.edu

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1. Introduction

The rapid, notable, and unexpected advancements in our knowledge of fungal sphingolipid synthesis and function, made since the field was last reviewed [1], require a timely summary and an indication of future research directions. Although the field of fungal sphingolipid research is still in its infancy, there is already more information available than can be summarized in this short review. Because of the space limitation and because this special issue focuses on glycosylation, we will emphasize the later steps in sphingolipid synthesis in which polar head groups are added to the 1-OH of ceramide (Fig. 1). *Saccharomyces cerevisiae* will be highlighted because more is known about its sphingolipids and because it is very likely to be the first eucaryotic organism for which all genes necessary for sphingolipid metabolism are identified. This repertoire of genes will provide an unprecedented and exciting opportunity to biochemically characterize sphingolipid metabolic enzymes, and to identify and characterize new functions for sphingolipids.

Our knowledge of the functions of sphingolipids and their precursors in *S. cerevisiae* is advancing rapidly and this information has been reviewed recently [2], so we will only summarize current information. Sphingolipids have been shown to be necessary for resistance to 37°C, low pH and osmotic stress [3], and for survival in stationary phase (unpublished results). It is not clear if sphingolipids are acting as signaling molecules, are playing some other role in these complex defense processes, or are characters in both roles. The intermediates dihydrosphingosine, phytosphingosine and ceramide (Fig. 1) have been implicated as second messengers during the heat stress response because their concentration increases after a heat shock [4,5]. One or more of them may be acting as a signal to regulate accumulation of trehalose and induction of transcription of *TPS2*, which encodes a subunit of trehalose synthase [4]. Data obtained from a strain lacking the long-chain base phosphate phosphatase gene, *LBP2*, suggest that phosphorylated long-chain bases are involved in resisting elevated temperature [6]. There are indications that sphingolipids regulate calcium homeostasis or components in calcium-mediated signaling pathways [7–10], but the mechanisms await characterization. Other data suggest the existence of a ceramide-activated protein phosphatase [11] composed of three subunits encoded by *SIT4*, *TPD3* and *CYC55* [12]. Ceramide synthesis is known to be necessary for trafficking of secretory vesicles from the endoplasmic reticulum (ER) to the Golgi apparatus [13–15]. Ceramide often replaces the diacylglycerol moiety in glycosylphosphatidylinositol-anchored proteins [16–20]. We anticipate many additions to this list of functions in the near future and that the molecular mechanisms used by sphingolipids and their intermediates to mediate these functions will be elucidated.

2. Biosynthetic pathway

All organisms begin sphingolipid synthesis by condensing serine and palmitoyl-CoA to yield 3-ketodihydrosphingosine (Fig. 1). This essentially irreversible reaction is catalyzed by serine palmitoyltransferase (SPT, 3-ketodihydrosphingosine synthase; EC 2.3.1.50), for review see [21]), a pyridoxal phosphate-containing enzyme that is the target of several potent natural inhibitors (Fig. 1) including sphingofungins [22,23], lipoxamycin [24], myriocin [25], and viridofungins [26]. Two genes, *LCB1* and *LCB2*, are necessary for SPT activity and are thought to encode subunits of the enzyme [27–29]. Reduction of 3-ketodihydrosphingosine by an NADPH-requiring reaction [30] yields the long-chain base dihydrosphingosine (sphinganine). The enzyme(s) and gene(s) necessary for catalyzing this step have not been identified, and it remains one of the most poorly characterized reactions in the early part of the biosynthetic pathway.

A fatty acid, generally 26 [31], but sometimes 24 carbons long [32], is then amide-linked to dihydrosphingosine to yield dihydroceramide. This reaction, catalyzed by dihydrosphingosine (sphinganine) *N*-acyltransferase (ceramide synthase), is also poorly characterized since the enzyme has not been purified

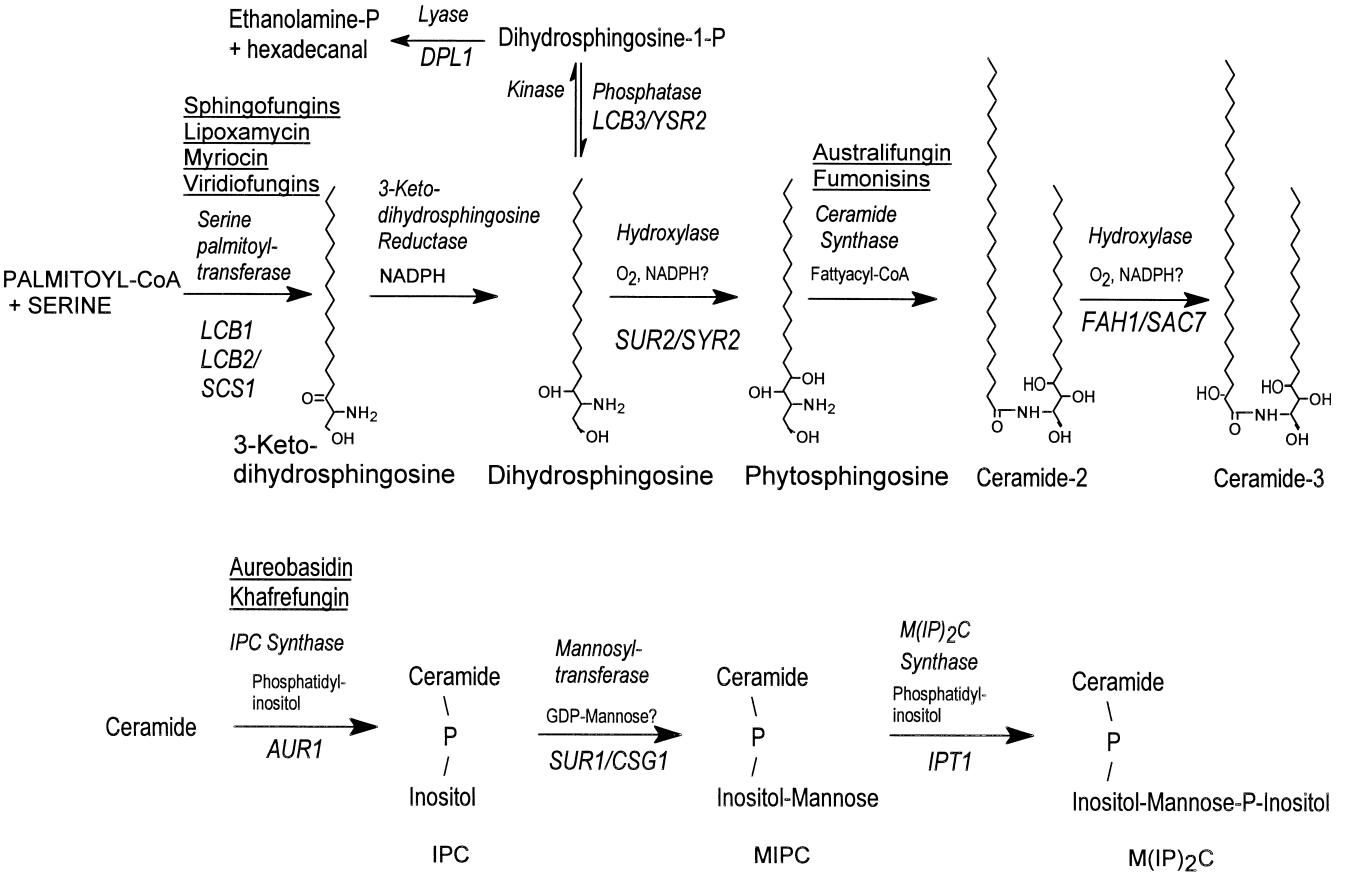

Fig. 1. Sphingolipid biosynthetic pathway in *Saccharomyces cerevisiae*. Genes are shown in capital italics and are described in more detail in Table 2. Enzyme inhibitors are underlined.

from any organism and no gene necessary for enzyme activity has been identified in *S. cerevisiae* or any other organism. Hydroxylation of C_4 in the dihydrosphingosine component of dihydroceramide yields a ceramide (sometimes referred to as phytoceramide) containing the long-chain base phytosphingosine. Phytosphingosine is the primary long-chain base found in most fungal and plant ceramides [1]. It differs from the primary long-chain base, sphingosine, found in mammalian sphingolipids because it lacks a 4,5-double bond and has instead an hydroxyl on C_4. It is unclear whether dihydrosphingosine, dihydroceramide or both are substrates for hydroxylation at C_4. A rapid accumulation of free phytosphingosine following heat shock, which precedes an increase in the level of ceramide, suggests that free dihydrosphingosine can be hydroxylated prior to incorporation into dihydroceramide, at least under these experimental conditions [4,5]. The *SUR2/SYR2* gene is necessary for hydroxylation of dihydrosphingosine at C_4 [33,34].

The Sur2 protein belongs to a family of membrane-bound, desaturase/hydroxylase enzymes distinguished by a motif containing eight histidines grouped into three clusters. The clusters are thought to be part of the catalytic site which contains oxo-diiron, Fe–O–Fe, with the O derived from O_2 [35]. The *SUR2/SYR2* gene is not essential for vegetative growth, but a deletion mutant has the interesting property of increased resistance to the antifungal compound syringomycin E [34]. It is unclear why hydroxylation of the long-chain base component of sphingolipids sensitizes cells to this drug, but understanding the mechanism of this phenomenon could provide new clues to sphingolipid functions.

Ceramide is converted to inositol phosphorylceramide (IPC) by transfer of inositol phosphate from phosphatidylinositol to the 1-OH group of ceramide

Table 1
Fungal sphingolipids: inositolphosphoceramides and glycosylceramides

Lipids	Organisms (refs.)
Inositolphosphoceramides	
Cer-P-Inos	*Saccharomyces cerevisiae* [44]; *Candida albicans* [81]; *Histoplasma capsulatum* [82]; *Phytophthora parasitica* [64]; *Cryptococcus neoformans* [83]; *Phytophthora capsici* [65]; *Aspergillus niger* [84]
Cer-$(P\text{-}Inos)_2$	*Neurospora crassa* [85]
Cer-P-Inos-Man	*S. cerevisiae* [44,76–78]; *C. albicans* [81]; *Schizosaccharomyces pombe* (Wells and Lester, unpublished data); *C. neoformans* [83]; *C. utilis* [76–78]
Cer-P-Inos-Man-Man Cer−P−Inos−Man−Man | Gal_f Cer−P−Inos−Man−Man | Gal_p	*H. capsulatum* [82,86]
Cer-P-Inos-(Man, Gal)	*A. niger* [87]
Cer-P-Inos-(Man, Gal, Glc)	*A. niger* [63]
Cer-P-Inos-(Man_2Gal_3)	*A. niger* [88]
Cer-P-Inos-Man-P-Inos	*S. cerevisiae* [89]; *C. albicans* [81]; *C. neoformans* [83]
Glycosylceramides	
Cer-Glc	*Fusicoccum amygdali* [65]; *Schizophyllum commune* [67]; *A. oryzae* [68]; *A. fumigatus*, *A. versicolor* [69]; *C. albicans* [70]; *Amanita muscaria*, *A. rubescens* [90]; *Hansenula ciferri* [91]; *Lactarius deliciosus* [92]; *Hansenula anomala* [93,94]; *Lentinus edodes* [71]; *Penicillium funiculosum* [72]; *Clitocybe geotropa*, *Clitocybe nebularis* [73]; *Sporothrix schenckii* [74]
Cer-Gal	*C. utilis*, *S. cerevisiae* [76–78]; *A. fumigatus* [69]
Cer-$[(Gal)_3(Glc)]$	*N. crassa* [85]

(Fig. 1). This modification of ceramide is also very common in other fungi (Table 1). The transfer reaction is catalyzed by phosphatidylinositol:ceramide phosphoinositol transferase (IPC synthase), a membrane-bound enzyme [36]. Available evidence indicates that *AUR1*, an essential gene, encodes IPC synthase or a subunit of the enzyme [37]. IPC synthase activity is inhibited by the antifungal agents aureobasidin A [37] and khafrefungin [38]. Both drugs are potent IPC synthase inhibitors with an IC_{50} of less than 1 nM. Since IPC synthase activity is essential and is not found in mammals, it is a promising target for antifungal drugs, of which there is a growing need because of increased use of immunosuppressive drugs in humans undergoing organ transplant or chemotherapy, or in immunocompromised humans such as those having AIDS.

IPC is mannosylated to yield mannose-inositol-P-ceramide (MIPC [1]). Deletion of either of two genes, *SUR1* [39] or *CSG2* [7], prevents synthesis of MIPC and causes IPC to accumulate. The Sur1 protein shows similarity over a stretch of 93 amino acids to two yeast α1,6-mannosyltransferases, Och1p [40] and Hoc1p [41], and it seems likely that the Sur1 protein catalyzes mannosylation of IPC. The Csg2 protein has 9–10 potential membrane-spanning domains and an EF-Ca^{2+}-binding domain, but it is unclear why this protein is necessary for mannosylation of IPC [8,42].

The terminal step in *S. cerevisiae* sphingolipid synthesis is the transfer of inositol phosphate from phosphatidylinositol to MIPC to yield the major sphingolipid $M(IP)_2C$ (Fig. 1). The similarity of this reaction to the one requiring the Aur1 protein suggested that a *S. cerevisiae* homolog (open-reading frame *YDR072c*, renamed *IPT1*, [43]) of the Aur1 protein, might be necessary for synthesis of $M(IP)_2C$. Analysis of an *ipt1* deletion mutant demonstrated the absence of $M(IP)_2C$ synthase activity and a complete absence of $M(IP)_2C$ with a compensating increase in the level of MIPC [43]. $M(IP)_2C$ normally accounts for about 75% of the sphingolipids in wild-type *S. cerevisiae* cells with the other 25% being divided between IPC and MIPC [44]. It appears that *S. cerevisiae* cells are able to sense their total sphingolipid content and adjust the level of MIPC to compensate for the absence of $M(IP)_2C$ [43,45]. The *ipt1* deletion mutant grows normally and only displays a slight

sensitivity to calcium. A genetically uncharacterized mutant strain unable to make $M(IP)_2C$ showed increased resistance to the polyene antibiotic nystatin [45]. Nystatin is thought to cause cell death by interacting with sterols and forming pores in the plasma membrane [46]. Leber et al. [45] suggest that $M(IP)_2C$, in addition to ergosterol, is necessary for nystatin action on membranes and that the two types of lipids may exist as microdomains within the plasma membrane. It needs to be determined if $M(IP)_2C$ lipids interact with ergosterol and if they do interact, is the interaction specific for $M(IP)_2C$.

The complex sphingolipids of *S. cerevisiae*, IPC, MIPC and $M(IP)_2C$, are not monomolecular species, but each is a mixture differing in the chain length and the extent of hydroxylation of both the long-chain base and the fatty acid [1]. The ceramides in cells grown at 25°C primarily contain C_{18} phytosphingosine but upon a temperature shift to 37°C, the abundance of C_{20} phytosphingosine increases [4,5]. The mechanism underlying this change is not entirely clear, but it is probably at least in part a consequence of increased synthesis of C_{18} relative to C_{16} fatty acids, as is known to occur during a heat shock [47]. As mentioned above, hydroxylation of dihydrosphingosine to yield phytosphingosine requires the *SYR2*/*SUR2* gene [33,34]. Mono- and dihydroxylation of the long-chain fatty acid requires the *SAC7* gene [33]. A nomenclature for these molecular species has been proposed [1], as has an alternative nomenclature [33]. The most abundant *S. cerevisiae* sphingolipid, designated 3, e.g., $M(IP)_2C$-3 or $M(IP)_2C$-C, contains phytosphingosine and αOH-hexacosanoic acid (C_{26}).

Little information about the domain structure of the Ipt1 and Aur1 proteins can be gained from their amino acid sequence, because neither homolog shows significant similarity to known proteins. It is disappointing that Aur1p, Sur1p and Ipt1p do not show a shared amino acid sequence that identifies a ceramide-recognition domain, since they all use ceramide or a ceramide-containing substrate. Being highly water insoluble, ceramides generally reside in membranes where they are likely recognized by one or more transmembrane helix. Extensive amino acid sequence conservation seems not to be necessary for transmembrane helices to have a similar function, and this may explain why the three yeast proteins lack any obvious 'ceramide recognition' domain.

Alignment of the Ipt1 and Aur1 proteins with putative Aur1 homologs from *Candida albicans* and *Schizosaccharomyces pombe* reveals regions with high amino acid identity and conservation of predicted transmembrane domains (Fig. 2). The region between residues 146 and about 300 of the *S. cerevisiae* Aur1 protein contains three transmembrane domains and many amino acids that are conserved in the four proteins. Transmembrane domain 3 is unusual because it contains a charged residue, His^{157} in the *S. cerevisiae* Aur1 protein, that is present in the other three proteins. This residue may be involved in substrate binding or in forming the catalytic site because a double mutation that changes it to a Tyr and changes Leu^{137} to Phe, produces a strain that is still able to make IPC but is now resistant to aureobasidin A [48]. A change of Phe^{158}, not a conserved residue, to Tyr [49], also gives resistance to aureobasidin, further indicating that this region is necessary for catalytic activity. It will be informative to determine if these variant enzymes are resistant to a newly discovered inhibitor of IPC synthase, khafrefungin [38], an aldonic acid ester linked to a C_{22} modified alkyl chain. Its structure is quite different from aureobasidin A, a cyclic, highly hydrophobic depsipeptide [50].

Transmembrane domain 4 has a region in which seven out of eight amino acid residues (FGAXPSLH), including another charged residue, His, are conserved in the four proteins. We speculate that transmembrane domains 3, 4 and 5 associate in the plane of the membrane, bind one or both substrates, and catalyze the transfer of inositol phosphate from phosphatidylinositol to the 1-OH of ceramide.

Although Aur1p lacks sequence similarity to proteins other than Ipt1p, we noted previously that it has other properties indicative of a relationship to the yeast enzymes cholinephosphotransferase (*CPT1*; EC 2.7.8.4) and ehtanolaminephosphotransferase (*EPT1*; EC 2.7.8.1). Based upon these properties and the nature of the reaction catalyzed, we suggested that these enzymes are members of a family of phospho-X-transferases [37] to which Ipt1p can now be added.

The genes known to be necessary for metabolism

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IPC1        MANPFSR------------WFLSERPPNCHVADLETSLDPHQTLLK--V    35
CAAUR1      MASSILRSKIIQKPYQLFHYYFLSEKAPGSTVSDLNFDTNIQTSLRK--L    48
SPAUR1      MSALSTLKKRLAACNRASQY------------KLETSLNPMPTFRL--L    35
IPT1        MNVIFSLASFVKNMYNASLNQRNLISLPFNFMLNFAPVFIWLSIFKRAGL    50
                                                                  
                                        1                         
IPC1        QKYKPALSDWVHYIFLGSIMLFVFITNPAP-------------WIFKIL    71
CAAUR1      KHHHWTVGEIFHYGFLVSILFFVFVVFPAS-------------FFIKLP    84
SPAUR1      RNTKWSWTH-LQYVFLAGNLIFACIVIESP-------------GFWGKFG    71
IPT1        IPIRLRPDIHSKFAFFADQFLFGDYWHELTVQLPDNTSKLFFWSFISSSA   100
                                                                  
                2                                                 
IPC1        FYCFLGTLFIIPATSQFFFNALPILTWVALYFTSSYFPDDRRPPITVKVL   121
CAAUR1      IILAFATCFLIPLTSQFFLPALPVFTWLALYFTCAKIPQEWKPAITVKVL   134
SPAUR1      IACLLAIALTVPLTRQIFFPAIVIITWAILFYSCRFIPERWRPPIWVRVL   121
IPT1        FLLVFLICIPFAIWYYIYYIKHVNYNLLEWFANIFHYPCKRKQRPIQKRF   150
                                                                  
                        α                          3    β         
IPC1        PAVETILYGDNLSDILATSTNSFL-----------DILAWLPYGLFHFGA   160
CAAUR1      PAMETILYGDNLSNVLATITTGVL-----------DILAWLPYGIIHFSF   173
SPAUR1      PTLENILYGSNLSSLLSKTTHSIL-----------DILAWVPYGVMHYSA   160
IPT1        RTIFIPFALPLFTFVILNIDHFFAYQSDANFTKTKDLLAWFSYVILHLTA   200
                                                                  
IPC1        PFVVAAILFVFGPPTVLQGYAFAFGYMNLFGVIMQNVFPAAPPWYKILYG   210
CAAUR1      PFVLAAIIFLFGPPTALRSFGFAFGYMNLLGVLIQMAFPAAPPWYKNLHG   223
SPAUR1      PFIISFILFIFAPPGTLPVWARTFGYMNLFGVLIQMAFPCSPPWYENMYG   210
IPT1        PILTAVYLYVFQPPGTLKCFSFALGLQNIAGVLTHLLVPMASPWFTHLYG   250
                                                                  
                                                    4             
IPC1        LQSA---NYDMHGSPGGLARIDKLLGINMYTTAFSNSSVIFGAFPSLHSG   257
CAAUR1      LEPA---NYSMHGSPGGLGRIDKLLGVDMYTTGFSNSSIIFGAFPSLHSG   270
SPAUR1      LEPA---TYAVRGSPGGLARIDALFGTSIYTDCFSNSPVVFGAFPSLHAG   257
IPT1        IDDTEHVNYTQEGFAAGLIRVDSHLGTHLNTKGFHMSPIVFGAVPSLHSA   300
                                                                  
                                        5                         
IPC1        CATMEALFFCYCFPKLKPLFIAYVCWLW----WSTMYLTHHYFVDLMAGS   303
CAAUR1      CCIMEVLFLCWLFPRFKFVWVTYASWLW----WSTMYLTHHYFVDLIGGA   316
SPAUR1      WAMLEALFLSHVFPRYRFCFYGYVLWLC----WCTMYLTHHYFVDLVGGM   303
IPT1        IAFQCFLFLVSRSTSLKHRFSNAGGFTMHNNDSSTFKLSEEDSEDEGDNS   350
                                                                  
IPC1        VLSYYIFQYTKYTHLPIVDTSLFCR-WSYTSIEKYDISKSDPLAADSNDI   352
CAAUR1      MLSLTVFEFTKYKYLPKNKEGLFCR-WSYTEIEKIDIQEIDPLSYNYIPV   365
SPAUR1      CLAIICFVFAQKLRLPQLQTGKILR-WEYEFV-----------------   334
IPT1        IPPTIGPNDLEMEPLGTVEPVDISNERSSSPSSSFTVSSNERSTGGGDGS   400
                                                                  
IPC1        ES-----------------------------------------V---   355
CAAUR1      NSNDNESRLYTRVYQESQVSPPQRAETPEAFEMSNFSRSRQSSKTQV---   412
SPAUR1      --------IHGHGLSEKTSNSLARTGSPYLLGRDSFTQNPNAVAFMS---   373
IPT1        IINSNGNKKPLQFVHLYDEDTNFTNKWIFKIVNDGFIPKFWAILYIILQW   450
                                                                  
IPC1        -----------------PLSNLELDFDLNMTDE----------PSVSPS   377
CAAUR1      -----------------PLSNLTNNDQVSGINEEDEEEEGD-EISSSTPS   444
SPAUR1      -----------------GLNNMELA----------NTDHEW-SVGSSSPE   395
IPT1        WATMYLDHHYRFDLFVGVLYAMTSFIIINWFVLQPKVLKKWIHIRLGDKV   500
                                                                  
IPC1        LFDG---STSVSRSSATSITSLGVKRA   401
CAAUR1      VFEDEPQGSTYAASSATSVDDLDSKRN   471
SPAUR1      PLPSPAADLIDRPASTTSSIFDASHLP   422
IPT1        DTRNEARTFGMRVFCGTKMEWFFDPLA   527
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Table 2
Genes necessary for metabolism of *S. cerevisiae* sphingolipids

Gene	Function	References
AUR1	Synthesis of IPC, possible IPC synthase or a subunit of the enzyme	[37]
DPL1	Breakdown of long-chain base phosphates, possible long-chain base-phosphate lyase	[95]
CSG2	Addition of mannose to IPC, function unclear	[7]
ELO2	Synthesis of C_{24} fatty acids, possible component of fatty acid elongation system	[32]
ELO3	Conversion of C_{24} to C_{26} fatty acids, possible component of fatty acid elongation system	[32]
IPT1	Synthesis of $M(IP)_2C$, possible $M(IP)_2C$ synthase	[43]
LBP2	Long-chain base-1-phosphate phosphatase	[6]
LCB1	Synthesis of long-chain bases, possible subunit of serine palmitoyltransferase	[96]
LCB2/SCS1	Synthesis of long-chain bases, possible catalytic subunit of serine palmitoyltransferase	[7,27]
LCB3/YSR2	Long-chain base-1-phosphate phosphatase	[6,97,98]
FAH1/SAC7	Hydroxylation of the fatty acid in ceramide	[32,99]
SUR1/CSG1	Necessary for α-mannosylation of IPC	[39]
SUR2/SYR2	Hydroxylation of sphinganine or sphinganine-containing dihydroceramides at the C_4 position to yield phytoceramide	[33,34]

of sphingolipids are summarized in Table 2. Still to be identified are the genes needed for synthesis of 3-ketodihydrosphingosine, ceramide and phosphorylation of long-chain bases. Unlike multicellular eucaryotes, which catabolize their complex sphingolipids [51], *S. cerevisiae* cells do not seem to do this, at least not during log phase growth [52]. However, because the inositol-containing sphingolipids are so abundant, 7–8% of the mass of the plasma membrane [53], breakdown of a small fraction would be difficult to detect and cannot be ruled out by current techniques. Although their function is unknown, a sphingomyelinase activity [54] and a phosphodiesterase capable of cleaving sphingolipids to yield ceramide [52] have been described in *S. cerevisiae*. It will be interesting to determine which gene(s) encodes them and what their physiological function is.

While great progress has been made in identifying genes and ascribing putative functions to them, the job of biochemically verifying these assignments remains to be done. This latter task will require purification of the proteins, something that has not been done for any of the reactions shown in Fig. 1. Purification and biochemical characterization should yield important clues about rate limiting, and thus possible regulatory, steps in the biosynthetic pathway, about catalytic mechanisms, and about preferred substrates and inhibitors, some of which may be physiologically important regulators of sphingolipid synthesis. We still have no idea how sphingolipid synthesis is integrated with the rest of cellular metabolism so that an appropriate quantity of sphingolipids are made during different growth states. Finally, although genes have been identified as being necessary for the majority of the chemical reactions shown in Fig. 1, it is very likely that one or more reactions are catalyzed by multimeric enzymes with some subunits encoded by genes that remain to be identified.

←
Fig. 2. Features of the Aur1 protein and its homologs. The Aur1 protein of *S. cerevisiae* (IPC1), and its homologs from *Candida albicans* (CAAUR1), and *Schizosaccharomyces pombe* (SPAUR1), plus the Ipt1 protein (IPT1) of *S. cerevisiae*, were aligned using the CLUSTAL algorithm [79]. Residues conserved in the four sequences are indicated by an asterisk below the alignment, and chemically similar residues are indicated by a dot. Putative transmembrane domains, indicated by a solid line over the sequences and numbered 1–5, were identified by using the TMpred algorithm [80]. Regions that could also be part of a transmembrane domain are indicated by a dashed line over the sequences. Residues 137 and 157 in the Aur1 protein of *S. cerevisiae* have an α and β symbol, respectively, over them and are discussed in the text.

3. Cellular location, abundance and sites of synthesis

IPC, MIPC and $M(IP)_2C$ are primarily located in the *S. cerevisiae* plasma membrane [53,55]. It is not known if they are present in both the inner and outer leaflet of the plasma membrane, or if there is an asymmetric distribution as there is for sphingomyelin and glycosphingolipids, which are highly concentrated in the outer leaflet of the plasma membrane of mammals ([56] and references therein). Sphingolipids are found in other locations in *S. cerevisiae* cells, especially IPC, which is found in the Golgi as well as in the vacuole (the yeast equivalent of lysosomes). Trace amounts of sphingolipids are found in mitochondria but none have been detected in lipid particles. Nuclei have not been examined [55]. It is now well established that the glycosylphosphatidylinositol-anchor of many proteins is replaced by a ceramide anchor, by an uncharacterized enzyme(s), which resides in the ER and the Golgi apparatus [16,19,20]. The details of the remodeling reactions remain to be elucidated.

Recent studies in *S. cerevisiae* have shown that C_{26} fatty acids are present in the nucleus. It is not known if they are free or incorporated into sphingolipids, glycerolipids or some novel lipid(s) species [57]. These C_{26} fatty acids have been hypothesized to play a role in stabilizing the sharp curvature in the membrane occurring at nuclear pore complexes. C_{26} fatty acids have also been found in the *sn*-2 position of the glycerol moiety of glycosylphosphatidylinositol-anchored proteins [19]. Other potential roles for long-chain fatty acids in yeasts and other organisms have been suggested [58].

Sphingolipid synthesis begins in the endoplasmic reticulum (ER) of all organisms that have been examined and proceeds up to the formation of ceramide. In *S. cerevisiae* at least some ceramide is converted to IPC before transport to the Golgi apparatus where the remaining polar head groups (Fig. 1) are added [59]. In mammals, ceramide is transported from the ER by secretory vesicles to the Golgi apparatus where it is converted to complex sphingolipids (reviewed in [60,61]). It is believed that most ceramide and IPC is transported in *S. cerevisiae* cells to the Golgi apparatus and then, following addition of mannose and inositol-P, to the plasma membrane by secretory vesicles [13,59].

4. Comparison of polar head groups found in fungal sphingolipids

The best characterized sphingolipids of fungi can be divided into two groups, the inositolphosphoceramides wherein the 1-OH of ceramide is phosphodiester linked to the 1-OH of myoinositol and derivatives, and the glycosylceramides, consisting of the 1 OH of ceramide glycosylated with a sugar or an oligosaccharide (Table 1). Complete structural characterization for many of the reported fungal sphingolipids has not yet been accomplished. Known structural details have been reviewed [1]. We present some generalizations about these sphingolipids.

In the inositolphosphoceramides, mannose has been shown to be α1,2-linked to the inositol [1]. Except for *H. capsulatum*, the structures of the more complex glycosylinositolphosphoceramides from other organisms have not been determined. We note that a glyceroglycolipid antigen of *Paracoccidioides brasiliensis* contains an inositol glycan with a structure [62] similar to the galactofuranose-containing sphingolipid from *H. capsulatum* indicated in Table 1. The predominant ceramide in the inositolphosphoceramides consists of phytosphingosine or dihydrosphingosine and a very long-chain hydroxyfatty acid, commonly 2-OH C_{26} or C_{24}. The presence of sphingosine has been reported for a mannosylinositolphosphoceramide from *Agaricus bisporus* [63] and in inositolphosphoceramides from *Phytophthora* species [64,65].

The complete structures of glucocerebrosides from several fungi are available. The glucose is β-linked to the 1-OH of a ceramide which consists of 9-methyl-C_{18} sphinga-4,8-dienine and a C_{16} or C_{18} 2-OH fatty acid [66–74]. This glucocerebroside and related ceramides have been shown to induce fruiting in *Schizophyllum*, but only when the double bond in sphingadieniene is present [75]. In organisms such as *C. albicans* there must be two quite distinct ceramide types differing in both long-chain base and fatty acid components, one destined for synthesis of inositolphosphoceramides and the other for glucocerebroside. Where the specificity lies to carry out subsequent reactions remains to be determined. Ceramide-specific glucosyl and phosphoinositol transferases or segregated reaction compartments need to be explored as possibilities.

Although not completely pure, the galactocerebroside from *A. fumigatus* [69] appears to be a β-galactosylceramide, with the ceramide composition like that of the glucocerebroside discussed above. However, the ceramide of the galactocerebroside isolated from *C. utilis* [76–78] consisted of C_{16}, C_{18} dihydrosphingosine, C_{20} sphingosine and 2-OH stearic acid. The presence of a galactocerebroside in *S. cerevisiae* has been suggested based on the presence of a compound with chromatographic properties similarity to the *C. utilis* compound [76–78]. In view of the emerging comprehensive understanding of *S. cerevisiae* sphingolipids, it seems worthwhile to verify the presence of glycosylceramides and characterize their structure.

5. Summary

Great progress has been made in identifying *S. cerevisiae* genes necessary for sphingolipid metabolism, and we feel strongly that all such genes will be identified first in this eucaryote. The challenge for the future is to purify and biochemically characterize the enzymes, most of which are membrane-bound, and establish their cellular location(s) in intact, not disrupted, cells. Compared to the glycerophospholipids and the sterols, we know very little about the function of sphingolipids and even less about how their synthesis is controlled. Identifying new functions for the polar and hydrophobic components of sphingolipids, and understanding how de novo synthesis is regulated, are two areas that could greatly benefit from insightful hypotheses and clever experimental designs.

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