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RESEARCH ARTICLE

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A computational approach to the inference of sphingolipid pathways from the genome of *Aspergillus fumigatus*

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Abstract A growing body of evidence suggests that sphingolipids are important bioactive molecules, in addition to being critical structural components of cellular membranes. These molecules have been implicated in regulating cell growth, differentiation, angiogenesis, apoptosis, and senescence. Many of the enzymes involved in sphingolipid biosynthesis are the targets of fungal toxins, thus underscoring the importance of this pathway. An international consortium has made considerable progress in sequencing the genome of *Aspergillus fumigatus*, one of the most common mold pathogens of humans; however, most genes have not yet been annotated. Here, we have identified genes involved in the sphingolipid pathway of *A. fumigatus* by comparative analysis with four other fungal species and the gene prediction program GlimmerM. Our results shows that *A. fumigatus* has most of the sphingolipid pathway genes found in other fungi, except for the *CSG2* and *IPT1* genes; the former is involved in the mannosylation of inositol phosphorylceramide (IPC) to mannose-inositol-phosphorylceramide and the latter involved in the synthesis of mannose-(inositol-P)$_2$-ceramide from mannose-inositol-phosphorylceramide.

Keywords Sphingolipid pathway · *Aspergillus fumigatus* · Gene prediction · GlimmerM

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Introduction

Aspergillus fumigatus is the most common mold pathogen of humans and causes both invasive disease in immunocompromised patients and allergic disease in patients with atopic immune systems (Denning et al. 2002). Although *A. fumigatus* only makes up a small proportion of all aerial spores, around 0.3% in the air of one particular hospital, it causes roughly 90% of invasive aspergillosis cases (Brakhage and Langfelder 2002). This suggests that *A. fumigatus* possesses certain factors that permit it to become an opportunistic human pathogen in immunocompromised patients.

To systematically identify genes and their products in this fungus, including potential targets for chemotherapy, diagnostics, and vaccine development, an international group of scientists initiated the sequencing of the *A. fumigatus* genome in 2001. The group selected the clinical isolate, Af293, as the strain to be sequenced (Denning et al. 2002).

There is urgent medical need for novel fungicidal agents that have high efficacy, lack of cross-resistance with existing agents, and low host toxicity. Compounds that target enzymes that are essential in fungi but absent in the mammalian host are attractive candidates. Such an enzyme is inositol phosphorylceramide (IPC) synthase, Aur1p, which is involved in the fungal sphingolipid biosynthetic pathway (Fig. 1). It has become increasingly evident that sphingolipids, once thought to be only structural components of cell membranes, are important molecules in cell regulation (Hannun and Obeid 1997). They have important roles in cell stress responses whereby they mediate diverse biological responses such as cell growth, apoptosis, angiogenesis, differentiation, and senescence. For example, sphingosine-1-phosphate plays a role in Ca^{2+}-mediated guard-cell closure, and a sphingosine transfer protein is involved in ceramide synthesis (Ng and Hetherington 2001). Functional studies of sphingolipids in *Saccharomyces cerevisiae* mutants have

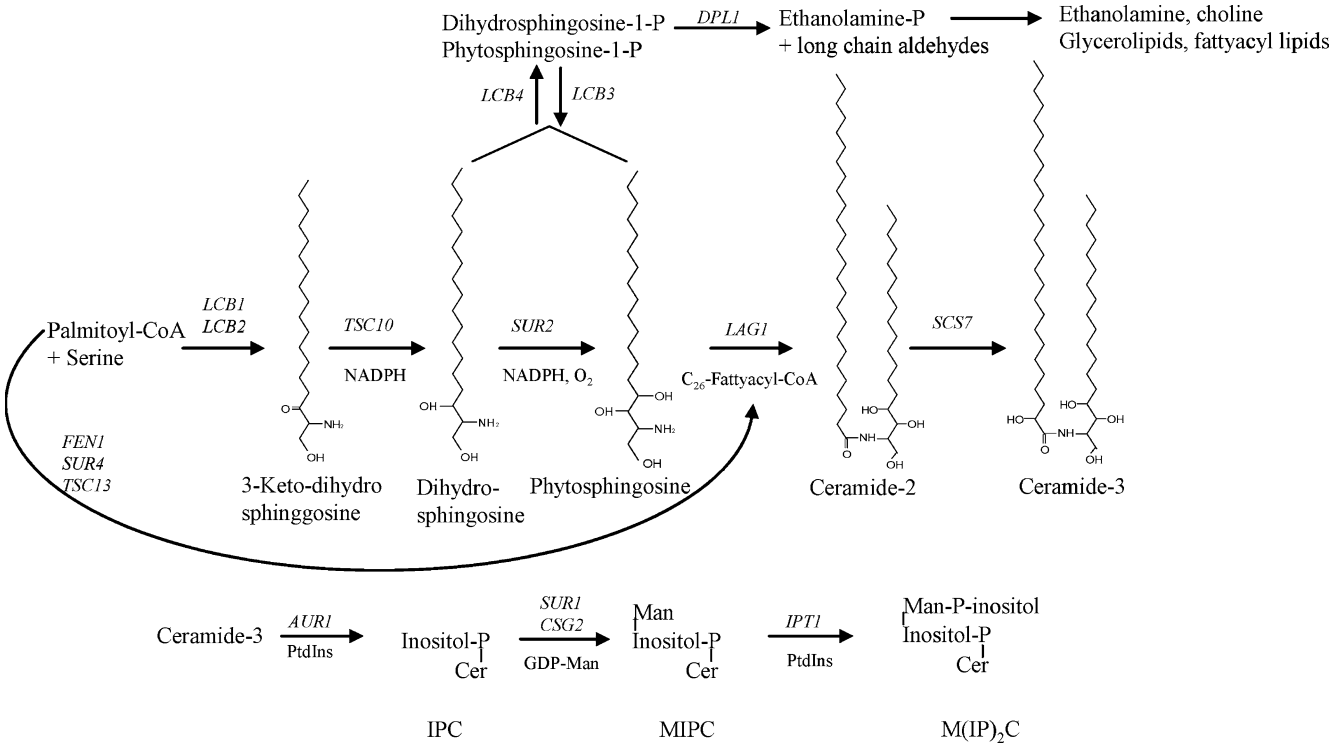

Fig. 1 Diagram of sphingolipid metabolism in *S. cerevisiae*. Gene names are shown in *italics* and the functions of their products are described in Table 2. *Dihydrosphingosine-1-P* dihydrosphingosine-1-phosphate, *Phytosphingosine-1-P* phytosphingosine-1-phosphate, *IPC* inositol phosphoceramide, *MIPC* mannose inositol phosphoceramide, $M(IP)_2C$ mannose-(inositol-P)$_2$-ceramide

provided groundwork for the identification of genes required for sphingolipid biosynthesis in other species because many of the enzymes have been conserved throughout evolution.

Sphingolipids are found in eukaryotic membranes that contain a hydrophobic segment (ceramide), which is a long-chain base (LCB) that is *N*-acylated with a very long-chain α-hydroxy fatty acid, linked to various polar head groups. The sphingolipids in mammals are sphingosine, while phytosphingosin (PHS) is the primary LCB in plants and fungi on a quantity basis. But plants and fungi contain a diversity of different LCBs in their glycosylceramides (Warnecke and Heinz 2003).

Table 1 Toxins that target sphingolipid metabolism of fungi (adapted from Obeid et al. 2002)

Toxin	Enzyme target
Myriocin	Serine palmitoyltransferase (Lcb1p)
Syringomycin E	Dihydrosphingosine hydroxylase (Sur2p)
Fumonisin B1	Ceramide synthase
Australifungin	Ceramide synthase
Aureobasidin	Inositol phosphoceramide synthase (Aur1p)

Glycosylceramides are involved in host/pathogen interactions (Koga et al. 1998), play a role fungal development (Levery et al. 2002). Many toxins target sphingolipid metabolism (Table 1).

The execution of various genetic screens in *S. cerevisiae* with the availability of its complete genomic sequence could give clues to the identification of the genes or proteins for sphingolipid biosynthesis in the opportunistic fungal pathogen *A. fumigatus* for which an unannotated genome sequence is available. In this study, we carried out a comparative genome analysis of the sphingolipid biosynthesis pathway in four fungal species including *S. cerevisiae, Schizosaccharomyces pombe*, *Candida albicans*, and *Neurospora crassa*, and attempted to identify sphingolipid biosynthesis genes in the *A. fumigatus* genome, estimated to be 30 Mb (http://www.sanger.ac.uk).

Materials and methods

Homologue search for sphingolipid biosynthesis gene(s) in *A. fumigatus*

Sequence data from the *A. fumigatus* genome were analyzed to identify *S. cerevisiae* homologues for sphingolipid biosynthesis. *S. cerevisiae* protein sequences were used as query sequences in BLAST (tBLASTn) searches (Gish and States 1993). The tenfold whole genome shotgun sequence assembly of *A. fumigatus* was obtained from The Institute for Genome Research (TIGR) website at http://www.tigr.org. The BLAST search engine runs the WU-BLAST 2.0

program (http://blast.wustl.edu). The tBLASTn program was used for the homologue search, and the statistical significance threshold for reporting matches against database sequences was 10. The e-value cutoff used to assign homologues was 1-e6. For comparative analysis of the sphingolipid biosynthetic pathway of *A. fumigatus* with that of *S. cerevisiae, S. pombe, C. albicans*, and *N. crassa*, we retrieved the genes for sphingolipid biosynthesis from several databases (geneDB, http://www.genedb.org; Candida DB, http://genolist.pasteur.fr/CandidaDB; NeurosporaBD, http://www-genome.wi.mit.edu/annotation/fungi/neurospora; SPTR, http://srs.ebi.ac.uk).

Gene prediction by GlimmerM

Regions of the *A. fumigatus* genome that possessed a high sequence similarity with *S. cerevisiae* genes for sphingolipid biosynthesis in the BLAST search were used as input for the GlimmerM program, which is specifically designed for small eukaryotic genomes with a gene density of around 20% (http://www.tigr.org) (Salzberg et al. 1999). GlimmerM contains a dynamic programming algorithm that considers all combinations of possible exons for inclusion in a gene model and chooses the best of these combinations. GlimmerM builds interpolated Markov models from a set of DNA sequences chosen for training. For coding regions, it builds three separate interpolated Markov models, one for each codon position. GlimmerM has been trained by the eukaryotic annotation team at TIGR for use with *Aspergilli* using a training set of 210 *Aspergillus* genomic sequences validated by mRNA or protein matches. This set contained 190 complete genes and 76 partial genes (13 were from *A. fumigatus)*, consisting of 39 intronless genes, 722 exons and 532 introns, and 558 acceptor sites and 555 donor sites. The genes predicted by GlimmerM were edited manually, if necessary, according to the statistical data of *A. fumigatus* genes produced by Anderson et al. (2001).

Validation of identified genes

For validation of the identified *A. fumigatus* genes, a bi-directional best-hit analysis was performed using the polypeptide sequence of the predicted *A. fumigatus* ORFs as queries for BLASTp searches of protein databases at the Swiss Institute of Bioinformatics (http://www.ch.embnet.org; http://SwissProt/TrEMBL/TREMBL_NEW) and *Saccharomyces* protein sequences in the *Saccharomyces* genome database (http://genome-www.stanford.edu/Saccharomyces). A further analysis of the predicted genes was conducted using the polypeptide sequences of the predicted *A. fumigatus* ORFs as query sequences for InterProScan (http://www.ebi.ac.uk/interpro).

Results

Comparative analysis of the biosynthetic pathway for sphingolipids in four fungal species

We surveyed various databases for the enzymes or genes from four fungal species (*S. cerevisiae, S. pombe, C. albicans*, and *N. crassa*) for comparative analysis of the sphingolipid pathway. Knowledge of *S. cerevisiae* genes necessary for sphingolipid metabolism has increased many-fold since the genome sequence was released in 1996. At least one gene is known for most steps in *S. cerevisiae* sphingolipid metabolism, which is outlined in Fig. 1. The functions of gene products are summarized in Table 2. The defining feature of sphingolipids is an LCB that is amide-linked to a fatty acid to form a ceramide. The type of LCB and fatty acid differs considerably between organisms and is not uniform even within an organism (Warnecke and Heinz 2003).

Saccharomyces cerevisiae makes two types of LCB, dihydrosphingosine (DHS) and phytosphingosine (PHS), with an additional hydroxyl on C-4 (Fig. 1). Mammals contain small amounts of both DHS and PHS, however their primary LCB is sphingosine, which is DHS with a 4, 5-double bond. *S. cerevisiae* does not synthesize sphingosine but does respond in several ways to exogenous sphingosine (Birchwood et al. 2001).

Sphingolipid synthesis begins with the condensation of palmitol-CoA and serine to yield 3-ketodihydrosphingosine (3-ketosphinganine), which is reduced to yield

Table 2 Genes for metabolism of *S. cerevisiae* sphingolipids (modified from Daum et al. 1998)

Genes	Function of gene product
AUR1	Synthesis of IPC, possible IPC synthase or a subunit of the enzyme
SUR1	Necessary for alpha-mannosylation of IPC
CSG2	Addition of mannose to IPC, function unclear
IPT1	Synthesis of $M(IP)_2C$, possible $M(IP)_2C$ synthase
FEN1	Synthesis of C24 fatty acids, possible component of fatty acid elongation system
SUR4	Conversion of C24 to C26 fatty acids, possible component of fatty acid elongation system
TSC13	Reductase of fatty acid elongation system
LCB1	Synthesis of LCBs, possible subunit of SPT
LCB2	Synthesis of LCBs, possible catalytic subunit of SPT
LCB3	Long-chain base-1-phosphate phosphatase
LCB4	Long-chain kinase
TSC10	3-Ketosphinganine reductase
SUR2	Hydroxylation of sphinganine or sphinganine-containing dihydroceramides at C4 position to yield phytoceramide
DPL1	Breakdown of LCB phosphates, possible LCB-phosphate lyase
LAG1	Acyl CoA-dependent ceramide synthase
SCS7	Hydroxylation of the fatty acid in ceramide

DHS (Fig. 1). The condensation reaction is catalyzed by serine palmitoyltransferase (SPT), a membrane-bound enzyme that is encoded by two essential genes in *S. cerevisiae, LCB1* and *LCB2* (reviewed in Obeid et al. 2002). These two genes are also found in *S. pombe, C. albicans*,and *N. crassa* (Table 3). Recently, *TSC3*, a third gene required for optimal SPT activity, was identified (Gable et al. 2000). This gene belongs to the TSC family of temperature-sensitive suppressors of calcium sensitivity.

In the second step in *S. cerevisiae* sphingolipid synthesis, 3-ketodihydrosphingosine is reduced in a reaction utilizing NADPH to produce the LCB. *TSC10* encodes 3-ketosphingosine reductase, a member of the short-chain dehydrogenase and reductase family, which is designated adh_short in the protein families database (Pfam). The adh_short domain was well conserved in the *TSC10* homologue of *S. pombe, C. albicans*, and *N. crassa* (data not shown). LCBs are phosphorylated to LCB phosphates (LCBPs) by Lcb4p kinase. *S. cerevisiae* cells contain two types of LCBPs, dihydrosphingosine-1-phosphate (DHS-P) and phytosphingosine-1-phosphate (PHS-P) (Fig. 1). These LCBPs can be degraded by the phosphatase Lcb3p to yield LCBs. LCBPs can also be degraded by *DPL1* lyase (Zhang et al. 2001). *S. cerevisiae, C. albicans*, and *N. crassa*, have the proteins necessary for LCBP metabolism (Lcb3p, Lcb4p, and Dpl1p), whereas *S. pombe* lacks *DPL1*. An *S. cerevisiae* deletion mutant of this enzyme displayed unregulated proliferation in the stationary phase, and was more resistant to killing by heat (Gottlieb et al. 1999; Zhang et al. 2001). This suggests that LCBPs regulate cell proliferation and are likely to play a role in heat stress. Zhang et al. (2001) reported that elimination of the *DPL1* lyase and *LCB3* phosphatase pathways by gene deletion in *S. cerevisiae* was lethal, indicating that these enzymes regulate LCBP levels to prevent accumulation. They also suggest that the C18 and C20 species of LCBPs are preferentially degraded by *LCB3* phosphatase, whereas the *DPL1* lyase prefers C16 DHS-P as a substrate. We propose that the breakdown of LCBPs in *S. pombe* occurs mainly through the *LCB3* phosphatase pathway because it lacks *DPL1*.

In the next step in *S. cerevisiae* sphingolipid synthesis, DHS is converted to PHS. *SUR2* is required for the hydroxylation of DHS at C-4 while *SCS7* is required for hydroxylation of the very long chain fatty acid (VLCFA) (Haak et al. 1997)

Additionally, *SUR2* deletion mutants are resistant to the fungal toxin syringomycin E (Grilley et al. 1998). The mechanism by which this occurs is unknown. However, Obeid et al. (2002) suggested that 4-hydroxylation could either directly modulate syringomycin binding by influencing sphingolipid exposure on the membrane surface, or indirectly affect syringomycin action by perturbing the lipid bilayer or by creating microdomains that facilitate ion channel formation. Both *SUR2* and *SCS7* are also found in *S. pombe, C. albicans*, and *N. crassa* (Table 3). This is consistent with the fact that the hydroxylation of C4 in the DHS is common in most fungal ceramides (Lester and Dickson 1993).

The next step in *S. cerevisiae* sphingolipid synthesis involves acylation of the LCB, PHS, to phytoceramide. This acylation involves VLCFAs, which are formed by enzymes encoded by the *FEN1, SUR4* genes (Oh et al. 1997). Fenlp elongates fatty acids by up to 24 carbons, and Sur4p is essential for the conversion of C24 fatty acids to C26 fatty acids. There are three members of the family in *S. cerevisiae, ELO1*, *FEN1*/*ELO2*, and *SUR4*/*ELO3*. The Fen1p and Sur4p proteins are required for VLCFA synthesis, with Fen1p mediating elongation of C16 or C18 to C22 or C24, but unable to direct the synthesis of C26. Sur4p displays overlapping activity

Table 3 Sphingolipid enzymes in four fungal species (in *C. albicans* and *N. crassa*, the gene identified by a best bi-directional blast hit to the yeast protein is represented by their database accession number with the prefix of CA and NCU, respectively. Databases: *C. albicans*, http://genolist.pasteur.fr/CandidaDB; *N. crassa*, http://www-genome.wi.mit.edu/annotation/fungi/neurospora)

Genes	Species			
	S. cerevisiae	*S. pombe*	*C. albicans*	*N. crassa*
AUR1	YKL004W	SPAC3H8.06	CA3353	NCU02282.2
SUR1	YPL057C	SPCC4F11.04C	CA1619	NCU07761.2
CSG2	YBR036C	SPBC405.03C	CA3315	NCU07247.2
IPT1	YDR072C	–	CA0228	–
FEN1	YCR034W	SPAC1B2.03C	CA3497	NCU06694.2
SUR4	YLR372W	SPAC806.09C	CA2166	NCU08976.2
TSC13	YDL015C	SPBC646.07C	CA4063	NCU03362.2
LCB1	YMR296C	SPBC18E5.02C	CA5101	NCU06870.2
LCB2	YDR062W	SPAC21E11.08	CA5236	NCU00447.2
LCB3	YJL134W	SPAC823.11	CA2934	NCU03504.2
LCB4	YOR171C	SPAC4A8.07C	CA4991	NCU07937.2
TSC10	YBR265W	SPCC1450.15	CA3000	NCU00302.2
SUR2	YDR297W	SPBC887.15C	CA2225	NCU06465.2
DPL1	YDR294C	–	CA4715	NCU06761.2
LAG1	YHL003C	SPBC3E7.15C	CA5446	NCU00008.2
SCS7	YMR272C	SPAC19G12.08	CA4852	NCU03492.2

with Fen1p and is also able to take C24 to C26. It is impossible, on the basis of amino acid homology, to predict the substrate specificities of the Elop proteins. However, any fatty acid with a chain length greater than C18 is generated by fatty acid elongation, and while the Elop proteins display distinct specificities for the acyl-CoAs to be elongated, the other known components of the elongating system are used to process the product of the Elop-mediated step (a 3-keto intermediate) through a reduction, dehydration and second reduction to form the final product. Kohlwein et al. (2001) indicated that *TSC13* gene encodes a protein required for elongation, possibly the enoyl reductase that catalyzes the last step in each cycle of elongation.

Although *S. pombe, C. albicans*, and *N. crassa* have the elongase complex including *FEN1, SUR4* and *TSC13* like *S. cerevisiae* (Table 3), *C. albicans* and *N. crassa* predominantly produce sphingolipids with C24 fatty acids (Wells et al. 1996). In fact, the C26 fatty acid in sphingolipids is unique to *S. cerevisiae* and is formed by a series of fatty acid synthesis and elongation steps with *ACB1, ACC1*, *FAS1, FAS2*, *FEN1, SUR4*, and *TSC13* (Sims et al. 2004). Therefore, *S. pombe, C. albicans*, and *N. crassa* might be missing one or more of these genes.

The enzyme involved in acylating these VLCFAs onto the sphingolipids, ceramide synthase or sphingolipid base *N*-acyl transferase, requires either of two redundant genes, *LAG1* or *LAC1* (Guillas et al. 2001). The *LAG1* gene was initially identified as a gene whose deletion endowed a longer lifespan phenotype on *S. cerevisiae* (D'Mello et al. 1994). Although there is little overall sequence similarity between *LAG1* proteins among various species, all these proteins possess a stretch of 52 amino acids of high sequence similarity, which has been dubbed the *LAG1* motif (Jiang et al. 1998). Comparison of the amino acid sequence of *LAG1* homologue proteins from *S. cerevisiae, S. pombe, C. albicans*, and *N. crassa* showed that the N-terminal region had low similarity, but the *LAG1* motif was detected in all four fungi following the central sequence region (data not shown).

Ceramides in *S. cerevisiae* are incorporated into complex sphingolipids that differ from mammalian sphingolipids in that the head group is comprised of an inositol phosphate instead of a choline phosphate. *S. cerevisiae* uses ceramides to make three types of complex sphingolipids: IPC, mannose-inositol-phosphorylceramide (MIPC), and mannose-(inositol-P)$_2$-ceramide (M(IP)$_2$C) (Table 4). This simplicity and the identification of many genes necessary for sphingolipid metabolism make *S. cerevisiae* an excellent organism for unraveling the roles of sphingolipids in signaling and in membrane structure and function. Ceramide is converted to IPC, the first of three so-called complex sphingolipids. Inositol phosphate is transferred from phosphatidylinositol to the C-1 OH group of ceramide (Fig. 1). This reaction is catalyzed by phosphatidylinositol:ceramide phosphoinositol transferase (IPC synthase), a membrane-bound enzyme (Becker and Lester 1980). The *AUR1* gene encodes IPC synthase or a subunit of the enzyme (Nagiec et al. 1997). IPC synthase is a promising target for the development of antifungal drugs because this enzyme is not found in mammals and its inhibition leads to fungal cell death. The homologue for *AUR1* is found in *S. pombe, C. albicans*, and *N. crassa* (Table 3), supporting the notion that the modification to C-1 of ceramide in fungi is common (Lester and Dickson 1993). IPC is mannosylated to yield MIPC, a reaction that requires the *SUR1* and *CSG2* genes (Beeler et al. 1997). The terminal step of sphingolipid synthesis in *S. cerevisiae* requires the *IPT1* gene (Dickson et al. 1997) whose product presumably catalyzes the addition of inositol phosphate to MIPC to yield M(IP)$_2$C. All the genes required for the biosynthesis of IPC, MIPC, and M(IP)$_2$C from ceramide are only found in *S. cerevisiae* and *C. albicans*. This supports the idea that *C. albicans* has the same type of IPCs as *S. cerevisiae* (Table 4). The lack of *IPT1* in *S. pombe* and *N. crassa* suggests that they have different types of IPC from those of *S. cerevisiae*.

In summary, we propose that the sphingolipid biosynthetic pathway is well conserved in the four fungal species analyzed, although there are a few exceptions (Table 3). This suggests that the other pathogenic fungus, *A. fumigatus*, might have similar sphingolipid biosynthetic pathways.

Identification of *A. fumigatus* genes for sphingolipid biosynthesis

The *S. cerevisiae* genes for sphingolipid biosynthesis were used as query sequences in a BLAST analysis to identify homologous genomic regions in *A. fumigatus*. The next step was to identify the precise protein-coding

Table 4 Fungal sphingolipids (modified from Dickson and Lester 1999)

S. cerevisiae	*C. albicans*	*N. crassa*	*A. fumiagtus*
Inositol phosphoceramides			
Cer-P-Inositol	Cer-P-Inos	Cer-(P-Inos)$_2$	Cer-P-Inos-Man-Man
Cer-P-Inos-Man	Cer-P-Inos-Man		
Cer-P-Inos-Man-P-Inos	Cer-P-Inos-Man-P-Inos		
Glycosylceramides			
Cer-Gal	Cer-Glc	Cer[(Gal)$_3$(Glc)]	Cer-Glc Cer-Gal

regions within these homologous regions. The prediction of protein-coding regions within DNA sequences in eukaryotes is not easy, possibly because of the low coding density (probably as low as 2% in humans, although the protein-coding regions within these fungal genomes have densities of at least 40%) and the presence of introns within relatively short coding regions. Various weak signals are combined, such as GC bias, splice sites, and translational start and stop sites. Different knowledge-based methods complimented by similarity searches are applied to utilize them (Guigo 1997). Commonly used methods for eukaryotic gene prediction depend on training a computer program to recognize sequences that are characteristic of known exons in genomic DNA sequences. The GlimmerM program (Salzberg et al. 1999) was trained with the *Aspergillus* training set for Glimmer provided by TIGR to enable the prediction of the positions of exons in genomic sequences and their arrangement into a predicted gene structure. We used the trained GlimmerM program to predict the positions of genes within regions of the *A. fumigatus* genome that were identified by BLAST searching with *S. cerevisiae* enzymes for sphingolipid biosynthesis.

Our results show that both *CSG2* and *IPT1* homologues of *S. cerevisiae* were not detected in the *A. fumigatus* genome (Table 5). The function of *CSG2* is not obvious, but it is required for the mannosylation of IPC to MIPC together with *SUR1*. Daum et al. (1999) suggested that these two genes are functionally redundant or that there is an unidentified pathway for making $M(IP)_2C$ because deletion of either gene reduces the level of MIPC and $M(IP)_2C$ in *S. cerevisiae*. Therefore, it is possible that the mannosylation of IPC to MIPC in *A. fumigatus* is catalyzed by *SUR1* alone, or an alternative enzyme exists that has a novel sequence compared to other fungal *CSG2* genes.

Another gene, *IPT1*, was also not identified in the *A. fumigatus* genome, nor in *S. pombe* and *N. crassa* (Table. 3, 5). $M(IP)_2C$ has been reported in *S. cerevisiae* (Steiner et al. 1969) and *C. albicans* (Wells et al. 1996) but not in *N. crassa* and *A. fumigatus*. This could be due to the lack of $M(IP)_2C$ synthase, encoded by *IPT1*. Ipt1p shows 27% amino acid identity to Aur1p, IPC synthase, over a region of 365 amino acids and both proteins use phosphatidylinositol as a substrate. Ipt1p and Aur1p are inhibited in *S. cerevisiae* by aureobasidin A, one of the strongest known fungicides, produced by *Aureobasidium pullulans* (Dickson et al. 1997). Aureobasidin A shows strong fungicidal activity against many pathogenic fungi including *Candida* spp., *Cryptococcus neoformans*, and some *Aspergillus* spp., but not *A. fumigatus* (Takesako et al. 1993). Zhong et al. (2000) suggests that the resistance in *A. fumigatus* is due to increased efflux of aureobasidin A by some transporter(s).

The fatty acid elongase complex including *FEN1, SUR4* and *TSC13* was identified in the *A. fumigatus* genome (Table 5). However, Levery et al. (2001)

Table 5 The predicted genes involved in sphingolipid metabolism in the *A. fumigatus* genome and the results of their best hit using BLASTp against the *Saccharomyces* proteome and InterProScan

Gene	Contig number/size of predicted protein	Blast results to yeast proteome: E-value/percent identity; percent similarity in amino acid overlap	InterPro accession number	Molecular function	
	AUR1	4897 (278,684–279,760)/358 aa	9.7e-70/52%; 70% in 232 aa	IPR000326	PA-phosphatase related
SUR1	4899 (192,183–193,503)/319 aa	4.0e-80/59%; 77% in 233 aa	IPR007577	Glycosyltransferase	
CSG2	–	–	–	–	
IPT1	–	–	–	–	
FEN1	4951 (248,013–249,156)/284 aa	1.4e-31/42%; 57% in 290 aa	IPR002076	Elongation of fatty acids	
SUR4	4846 (152,236–150,896)/315 aa	1E-8/24%; 40% in 315 aa	IPR002076	Elongation of fatty acids	
TSC13	4840 (47,923–48,592)/223 aa	9.0E-23/35%; 52% in 186 aa	IPR001104	Reductase	
LCB1	4941(49,885–51,593)/481 aa	2.6e-68/35%; 53% in 517 aa	IPR004839	Aminotransferase	
LCB2	4910 (29,293–31,335)/648 aa	7.4e-155/57%; 72% in 500aa	IPR004839	Aminotransferase	
LCB3	4905 (152,978–154,329)/430 aa	4.1e-19/27%; 47% in 259 aa	IPR000326	PA-phosphatase related	
LCB4	4944 (24,440–26,200)/441 aa	2.2e-61/40%; 58% in 316 aa	IPR001206	Diacylglycerol kinase	
TSC10	4837 (37,552–38,733)/338 aa	2.7e-10/31%; 42% in 196 aa	IPR002198	Short-chain dehydrogenase/reductase	
SUR2	4846 (197,717–199,056)/426 aa	3.8e-80/52%; 68% in 299 aa	IPR006087	SUR2-type hydroxylase/desaturase	
DPL1	4840 (101,259–103,208)/559 aa	1.3e-127/48%; 66% in 526 aa	IPR002129	Pyridoxal-dependent decarboxylase	
LAG1	4944(103,587–105,786)400 aa	1e-76/47%; 62% in 327 aa	IPR005547	Longevity-assurance protein	
SCS7	4942 (93,723–95,855)/562 aa	5.7e-104/52%; 67% in 385 aa	IPR006694	Fatty acid hydroxylase	

reported that *A. fumigatus* has mainly C24 fatty acids in its ceramides, as do *C. albicans* and *N. crassa*.

The glycosylceramides produced by fungi were represented in Table 4. The majority of fungi synthesize only glucosylceramide (Cer-Glc), but among *Aspergillus* species the expression of galactosylceramide (Cer-Gal) (*A. niger*), glucosylceramide (*A. oryzae; A. versicolor; A. fumigatus*), or mixture of both (*A. fumigatus*) has been reported (Toledo et al. 1999). This suggests that *A. fumigatus* might have the enzymes responsible for the synthesis of glucosylceramides, for example, sphingolipid-delta4-desaturase, sphingolipid-delata8-desaturase, glucosylceramide synthase. We could find the homologue of the *C. albicans* sphingolipid-delta4-desaturase in contig 4826 (742,151–744,788), but there is no homologue for sphingolipid-delta8-desaturase of *C. albicans*. Each homologue of *Arabidopsis thaliana* sphingolipid-delta8-desaturase and glucosylceramide synthase was found in contig 4917 (98,876–100,236) and contig 4938 (742,151–744,788), respectively.

Identification of the predicted genes of *A. fumigatus* for sphingolipid biosynthesis was validated by performing a reciprocal BLASTp search against the *Saccharomyces* proteome database. Using the predicted polypeptide sequence encoded by *A. fumigatus* genes as the query sequence, the predicted proteins for sphingolipid biosynthesis were identified (Table 5).

Each gene that was found to be involved in *A. fumigatus* sphingolipid biosynthesis was further characterized by computational functional analysis using InterProScan. All the identified genes had a significant hit for a protein family or domain and could be classified by this function or functional domain (Table 5).

Discussion

The complete sequence of the *S. cerevisiae* genome has advanced our knowledge of sphingolipid metabolism. At least one gene has now been identified for each step in yeast sphingolipid metabolism (Fig. 1). We have carried out the first trial to identify the genes necessary for sphingolipid biosynthesis in *A. fumigatus* using comparative pathway analysis and the gene-predicting program GlimmerM.

Most of the genes involved in *S. cerevisiae* sphingolipid biosynthesis are present in the *A. fumigatus* genome, except for *CSG2* and *IPT1* (Table 5). The *CSG2* is involved in the mannosylation of IPC to MIPC, and the *IPT1* is involved in the synthesis of $M(IP)_2C$ from MIPC. Levery et al. (2001) demonstrated that *A. fumigatus* and *A. blazei* contain dimannosylinositol phosphorylceramide (MMIPC) and MIPC, respectively. Thus, the mannosylation of IPC in *A. fumigatus* may be required and could occur via Sur4p or/with an unidentified enzyme having same or similar function to Csg2p.

A. fumigatus also lacks *IPT1*, inositol phosphoryl transferase, which is required for the synthesis of $M(IP)_2C$ from MIPC. *IPT1* is also absent in *S. pombe* and *N. crassa* (Table 3), which may explain why $M(IP)_2C$ has not been reported in *A. fumigatus, S. pombe*or *N. crassa*.

Even though the fatty acid elongase complex including *FEN1, SUR4* and *TSC13* has been identified in *S. cerevisiae, S. pombe, C. albicans, N. crassa*, and *A. fumigatus*, the C26 fatty acids are mainly produced in *S. cerevisiae* ceramides. This suggests that only *S. cerevisiae* has the complete set of enzymes required for the synthesis of C26 fatty acids.

Our results show that genes in *A. fumigatus* could reliably be detected using GlimmerM, which is in good agreement with our previous work (Do et al. 2004). This study was limited in computational analysis; however, it would be useful as support in the verification of genes in the sphingolipid pathway in *A. fumigatus*.

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