

Glycosyltransferases and their products: cryptococcal variations on fungal themes

James Stacey Klutts^{1,2}, Aki Yoneda¹, Morgann C. Reilly¹, Indrani Bose¹ & Tamara L. Doering¹

¹Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, USA and ²Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA

Correspondence: Tamara L. Doering, Department of Molecular Microbiology and Washington University School of Medicine, 660 S. Euclid, St Louis, MO 63110-1093, USA. Tel.: +1 314 747 5597; fax: +1 314 362 1232; e-mail: doering@wustl.edu

Received 20 October 2005; accepted 9 November 2005.

First published online 21 March 2006.

doi:10.1111/j.1567-1364.2006.00054.x

Editor: Stuart Levtz

Keywords

glycosyltransferase; glycosylation; glycan; polysaccharide; fungi; *Cryptococcus*.

Introduction

Glycoconjugates are fundamental building blocks of organisms, contributing to the structure, integrity, and function of prokaryotic and eukaryotic cells. Individual sugars may be linked to proteins, lipids or other carbohydrates, and the resulting structures are found in every cellular compartment. Biological glycans are tremendously diverse, ranging from a single sugar moiety to the elaborate and extensive capsule polysaccharides of *Cryptococcus neoformans*. The common feature of all glycoconjugates, however, is that they are created by the co-ordinated activities of glycosyltransferase enzymes. In this article, we review current knowledge of cryptococcal glycosyltransferases, in the context of our understanding of fungal polysaccharide synthesis.

Fungi are richly endowed with polysaccharide structures. Their proteins may be extensively glycosylated, bearing hundreds of sugar residues, and their glycolipids are also quite complex. Fungal cell walls are composed primarily of carbohydrate polymers such as glucan and chitin, as detailed below. These structures are the primary mediators of cell integrity and interactions with the extracellular environment. *Cryptococcus neoformans*, the focus of this Special Issue, takes glycan

Abstract

Glycosyltransferases are specific enzymes that catalyse the transfer of monosaccharide moieties to biological substrates, including proteins, lipids and carbohydrates. These enzymes are present from prokaryotes to humans, and their glycoconjugate products are often vital for survival of the organism. Many glycosyltransferases found in fungal pathogens such as *Cryptococcus neoformans* do not exist in mammalian systems, making them attractive potential targets for selectively toxic agents. In this article, we present the features of this diverse class of enzymes, and review the fungal glycosyltransferases that are involved in synthesis of the cell wall, the cryptococcal capsule, glycoproteins and glycolipids. We specifically focus on enzymes that have been identified or studied in *C. neoformans*, and we consider future directions for research on glycosyltransferases in the context of this opportunistic pathogen.

synthesis a level further, by surrounding its cell wall with an intricate polysaccharide capsule that is crucial for virulence.

Cryptococcus neoformans is an important human pathogen, predominantly in the setting of immune compromise. Unfortunately, the antifungal compounds currently available for the treatment of cryptococcosis are associated with host toxicity and are rarely able to clear the infection completely. It is therefore critical to consider potential targets for chemotherapy, either unique to this pathogen or shared by other fungi, that may enable the development of selectively toxic compounds. In the category of unique features, *C. neoformans* offers capsule biosynthesis, a process shared only by a group of related fungal species that are not pathogenic. Based on the exquisite specificity of glycosyltransferases, at least 12 of these enzymes should be required to build the capsule components (see Fig. 1), and these are not likely to resemble host proteins. Additional synthetic processes common to *C. neoformans* and other fungal pathogens, but not to their mammalian hosts, include cell wall construction and aspects of protein glycosylation. Excitingly, studies of glycosyltransferases involved in cell wall synthesis have already yielded successful antifungal compounds (see 'β(1,3)-Glucan synthases (FKS)'). Below we review the general characteristics of glycosyltransferases, discuss the role of these enzymes in fungal glycoconjugate synthesis and consider directions for future research.

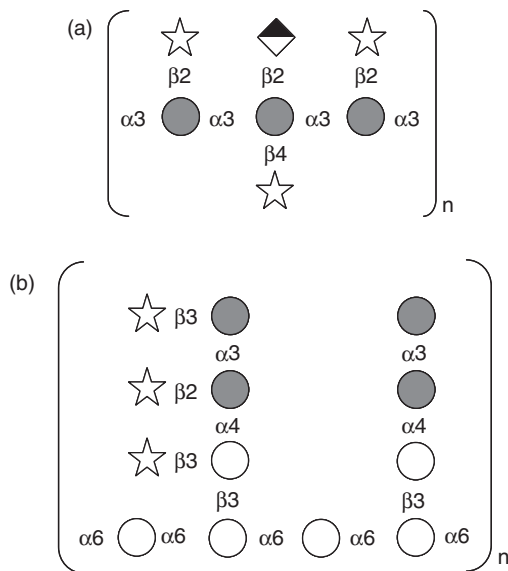


Fig. 1. Capsule polysaccharides of *Cryptococcus neoformans*. (a) Generic structure of the repeating unit of glucuronoxylomannan (GXM) (Cherniak *et al.*, 1998). The number and arrangement of the xylose residues varies in different serotypes. The mannose residues are also variably acetylated (not shown). (b) Generic structure of the repeating unit of galactoxylomannan (GalXM) (Vaishnav *et al.*, 1998). Monosaccharide moieties are denoted here and in Fig. 4 by the standardized symbols recommended by the Consortium for Functional Glycomics. Shaded circles, mannose; open circles, galactose; open stars, xylose; partially shaded diamond, glucuronic acid.

Properties and characteristics of glycosyltransferases

Glycosyltransferases catalyse the specific transfer of a monosaccharide moiety from an activated sugar donor to a distinct acceptor molecule in a particular linkage. Common sugar donors include nucleotide monophosphosugars, nucleotide diphosphosugars and dolichol-linked sugars (Fig. 2a). As will be discussed below, both saccharides as well as nonsaccharides, such as proteins and lipids, can serve as acceptor molecules. In eukaryotes, the reactions catalysed by glycosyltransferases often take place within the endoplasmic reticulum (ER) – Golgi pathway. Resident glycosyltransferases of these organelles exist as type II membrane proteins with a short N-terminal cytoplasmic domain, a membrane-spanning domain, a stem region and a globular C-terminal luminal domain.

The transfer of a glycosyl moiety from one molecule to another can occur via either an inverting or retaining mechanism. In inverting glycosyltransferases, the deprotonated hydroxyl group of the acceptor attacks the C1 anomeric carbon of the sugar donor to form a glycosidic bond, resulting in an inversion of the configuration at C1 (Qasba *et al.*, 2005). The mechanism of retaining glycosyltransferases, in which a glycosidic bond is formed between the donor and acceptor while retaining the C1 configura-

tion, is less clear. Glycosyltransferases typically require metal ion cofactors. Binding of the cofactor and/or the sugar donor molecule results in a conformational change in the protein. A number of these enzymes contain one or more flexible loop regions that re-order upon binding of the sugar donor to generate the acceptor-binding site. Once the glycosyl unit has transferred from the donor to the acceptor molecule, the saccharide product is released and the loop reverts to its native conformation, releasing any remaining moieties of the donor molecule (Qasba *et al.*, 2005).

Traditionally, glycosyltransferases were classified on the basis of their donor, acceptor and product specificity, and identified by an Enzyme Commission (EC) number (Coutinho *et al.*, 2003). With the availability of thousands of putative glycosyltransferase sequences from genome studies, a new classification scheme for these enzymes was proposed that did not require knowledge of biochemical function. Instead, these enzymes are divided into families based on similarities in amino acid sequence to one or more founding members that have been biochemically characterized (Campbell *et al.*, 1997). A database of enzymes involved in carbohydrate metabolism (CAZY, Carbohydrate Active enzymes) organized according to this classification scheme is maintained by the Glycobiology unit at AFMB-CNRS in Marseille, France, and can be found on the internet at <http://afmb.cnrs-mrs.fr/CAZY>. As of October 2005, the CAZY database contained 78 glycosyltransferase families that included 64 confirmed and putative enzymes from the *C. neoformans* var. *neoformans* JEC21 genome project.

The primary amino acid sequences of glycosyltransferases are typically very diverse, although the predicted proteins exhibit overall structural similarities. All glycosyltransferases contain distinct donor and acceptor binding domains connected by a linker region that forms the active site. On the basis of broader structural patterns, glycosyltransferases have been primarily divided into two superfamilies, GT-A and GT-B (Fig. 2b). The GT-A enzymes have two dissimilar domains: an N-terminal sugar donor-binding domain composed of several β -strands that are each flanked by α -helices to form a Rossman-like fold, and a C-terminal acceptor binding domain made up largely of mixed β -sheets (Qasba *et al.*, 2005). Members of the GT-A family frequently have a three-residue DXD, EXD or equivalent motif that is involved in the binding of a metal ion (often Mg^{2+} or Mn^{2+}), although not all GT-A enzymes require such a cofactor and some lack this motif. Enzymes of the GT-B superfamily typically have two Rossman-like folds that allow the acceptor molecule to bind at the N-terminus while the C-terminal domain is involved in binding the sugar donor (Qasba *et al.*, 2005). Although some GT-B enzymes utilize metal ion cofactors, most do not and thus GT-Bs lack a $D \times D$ motif or its equivalent, further distinguishing them from GT-A molecules. Members of the GT-B superfamily do, however,

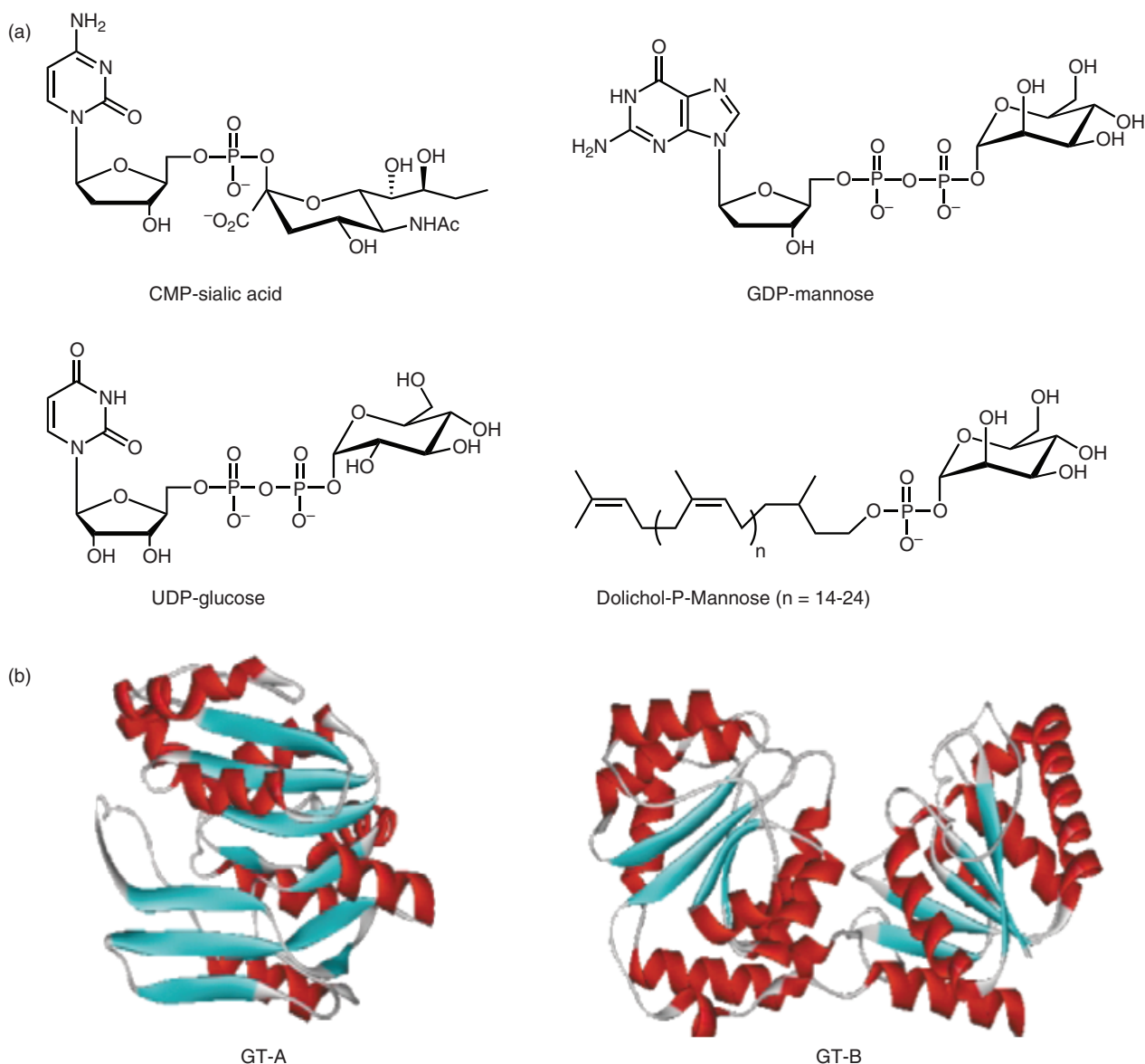


Fig. 2. Structures of monosaccharide donors and glycosyltransferases. (a) Examples of common sugar donor molecules. (b) Modeled three-dimensional structures typical of the GT-A and GT-B superfamilies. [Fig. 2b reprinted from Coutinho *et al.* (2003) with permission from Elsevier.]

exhibit a pattern of proline and glycine residues located within the donor-binding domain (Hu & Walker, 2002). In addition to the defined GT-A and GT-B superfamilies, there are a growing number of glycosyltransferases that do not fall into either of these categories on the basis of structural motifs or amino acid sequence (Kikuchi *et al.*, 2003; Liu & Mushegian, 2003; Breton *et al.*, 2005). Although a number of authors have suggested the creation of additional glycosyltransferase superfamilies to accommodate these outliers, a consensus within the field has not yet been reached.

The formation of glycoconjugates often requires several glycosyltransferases working sequentially to generate the desired linear or branched structures. These glycosyltrans-

ferases may form large protein complexes, as with the *Saccharomyces cerevisiae* M-Pol II enzyme complex discussed below (see 'N-linked glycan synthesis'). Functional redundancy of glycosyltransferases is also typical, as demonstrated by the *S. cerevisiae* Pmt and Mnt families of mannosyltransferases that act in the O-linked glycosylation of proteins (see 'O-linked glycan synthesis').

Review of fungal glycosyltransferases

Glycosyltransferases play vital roles in the biosynthesis of numerous molecules within fungi, and many glycosyltransferases are unique to these organisms, suggesting them as

Table 1. Major cell wall components of selected fungal organisms^{*,†}

	β -Glucan	α -Glucan	Mannoproteins	Chitin
<i>Cryptococcus neoformans</i>	15% [‡] mostly β -1,6 some β -1,3	35% [‡] mostly α -1,3 some α -1,4	Present	Present
<i>Saccharomyces cerevisiae</i>	50% [§] β -1,3; 10% [§] β -1,6	None	40% [§]	1–3% [§]
<i>Candida albicans</i>	40% [§] β -1,3; 20% [§] β -1,6	None	35–40% [§]	1–2% [§]
<i>Schizosaccharomyces pombe</i>	55% [§] β -1,3; 6% [§] β -1,6	28% [§] α -1,3	Likely present	0.5% [§]
<i>Aspergillus fumigatus</i> (mycelial form)	70% [‡] β -1,3; 4% [‡] β -1,6; 10% [‡] β -1,3/1,4	Present	3.5% [§]	Present
<i>Paracoccidioides brasiliensis</i>	Yeast form: 5% [‡] β -1,3 Mycelial form: Mostly β -1,3	Yeast form: 95% [‡] α -1,3 Mycelial form: Little α -1,3	Likely present	Present (yeast and mycelial)
<i>Blastomyces dermatitidis</i> (yeast form)	5% [‡]	95% [‡]	Likely present	Present
<i>Histoplasma capsulatum</i> (yeast form)	Present	Present	Likely present	Likely present

*The ratio of cell wall components is highly dependent on whether the organism is in hyphal or yeast form, so these are listed separately. Equivalent analyses have not been performed for all organisms listed.

[†]Reprinted from Bose *et al.* (2003) with permission from ASM Press. Please refer to that article for a complete list of source publications for the data in this table.

[‡]Percentage of cell wall glucan.

[§]Percentage of total cell wall mass.

attractive targets for antifungal therapy. Below we review many of the known fungal glycosyltransferases, organized by their biosynthetic role, with particular attention to what is known in *Cryptococcus*.

Glycosyltransferases involved in fungal cell wall synthesis

The cell wall is a vital structure for all fungi, lending the cell its shape and protecting the organism from the environment. This structure is composed of a number of unique interconnected polysaccharides, including chitin and a variety of glucans (see Table 1), that are not found in mammalian cells. However, as also shown in Table 1, the composition of the cell wall can differ substantially among fungal species. In addition, although the fungal cell wall is a rigid structure, it must be dynamic in order to allow for budding, growth and adaptation to environmental stress. As a result, the processes of cell wall construction and remodeling are quite complex and are poorly understood. Further evidence of cell wall importance and complexity is shown by the estimate that ~20% of all genes in *S. cerevisiae* are involved in its production (Duran & Nombela, 2004). Below we review our current knowledge of the glycosyltransferases involved in the synthesis of the cell walls of fungi.

β (1,3)-Glucan synthases (FKS)

β (1,3)-Glucan is a major structural component of the cell wall in all fungi except the zygomycetes. In *S. cerevisiae*, this moderately branched polysaccharide makes up about 50% of the dry weight of the wall, although this percentage is significantly lower in *C. neoformans* (Table 1) (Klis *et al.*,

2002; Bose *et al.*, 2003). A membrane-associated β -glucan synthase activity was described in the late 1970s that processively built a polysaccharide of 60–80 glucose residues, using UDP-glucose as substrate (Douglas, 2001). It was subsequently shown that two distinct GTP-regulated β -glucan synthase complexes could catalyse this activity. These two complexes differ only in the presence of either the Fks1 or Fks2 homologous protein subunits. In *S. cerevisiae*, these two subunits are differentially expressed, with *FKS1* being expressed during growth in the presence of high glucose and *FKS2* when glucose is limiting. Yeast strains with a disruption of either *FKS1* or *FKS2* are viable, but disruption of both genes is lethal. It has been hypothesized that the Fks1/2 proteins are the catalytic subunits of the β (1,3)-glucan synthase complex. However, purification of either Fks1p or Fks2p has proven difficult and failed to confirm that the Fks proteins have β (1,3)-glucan synthase activity (Douglas, 2001). Bussey and colleagues recently showed that disruption of *FKS1* led to a decrease in both β (1,3)- and β (1,6)-glucans in *S. cerevisiae* and suggested that the Fks proteins were involved with transporting glucans, rather than in their synthesis (Dijkgraaf *et al.*, 2002). Sequences homologous to the yeast Fks proteins have been found in many fungi. Although multiple homologs are found in *Schizosaccharomyces pombe* and other fungi, the human pathogens *C. neoformans*, *Aspergillus fumigatus* and *Candida albicans* each have only a single copy. Attempts to disrupt the Fks homolog in each of the latter organisms have not yielded viable transformants (Thompson *et al.*, 1999; Douglas, 2001), suggesting they are essential.

The echinocandins are effective antifungal compounds that have been shown to inhibit the β -glucan synthase complex in many fungi. Surprisingly, *C. neoformans* is

relatively resistant to the echinocandins, even though its β -glucan synthase can be inhibited *in vitro* by these drugs (Maligie & Selitrennikoff, 2005). This suggests that the echinocandins cannot reach their target *in vivo*, or that the organism has a way to compensate for their effect. Although the exact mechanism of resistance has not been fully elucidated, Heitman's group showed that in the presence of the echinocandin caspofungin, *C. neoformans* up-regulates expression of *FKS1* through the 'cell wall integrity MAP kinase' or Mpk1p (Kraus *et al.*, 2003). This suggests that a unique compensation mechanism could at least partially explain resistance. Finally, the *C. neoformans* β -glucan synthase appears to be unique with respect to its regulation, in that perturbation of the GTP-bound regulatory subunit, Rho1p, leads to different phenotypes than observed in other fungi (Douglas, 2001). Together with the echinocandin resistance data, this suggests unique aspects of $\beta(1,3)$ -glucan synthesis in *C. neoformans* that remain to be explored.

A second enzyme involved in $\beta(1,3)$ -glucan synthesis is not a true glycosyltransferase, but rather a transglycosidase. This enzyme (Gas1p in *S. cerevisiae*, Phr1-2p in *Ca. albicans* and Gel1p in *A. fumigatus*) catalyses the internal cleavage of $\beta(1,3)$ -glucan polymers and the transfer of the new reducing end to the nonreducing end of another $\beta(1,3)$ -glucan polymer (Mouyna *et al.*, 2000; Carotti *et al.*, 2004). It is believed that this transglycosidase is important for cell wall cross-linking. *Cryptococcus neoformans* has a homolog to this enzyme which is discussed below (see 'Glycosylphosphatidylinositol (GPI) anchor synthesis').

$\alpha(1,3)$ -Glucan synthases (AGS)

The cell wall of *C. neoformans* contains abundant $\alpha(1,3)$ -glucan. Although this polysaccharide is a constituent of the cell wall of several fungi, it is absent in *Saccharomyces* and *Candida* (Table 1). The first identified $\alpha(1,3)$ -glucan synthase, Ags1p, is a large (266-kDa) multi-domain plasma membrane protein from *S. pombe*. The speculative model for its function suggests that an intracellular synthase domain first makes $\alpha(1,3)$ -glucan. This product is then transported across the multiple membrane spanning regions of the protein and subsequently linked to the cell wall via an extracellular transglycosylase domain (Hochstenbach *et al.*, 1998). *AGS1* (also called *MOK1*) is the main member of a five-gene family in *Sch. pombe* thought to be responsible for $\alpha(1,3)$ -glucan synthesis, and two independent studies suggest it is essential in this organism (Hochstenbach *et al.*, 1998; Katayama *et al.*, 1999). *Aspergillus fumigatus* has three homologs to the *Sch. pombe* Ags1p, two of which (*AfAgs1p* and *AfAgs2p*) have been studied. Unlike the *Sch. pombe* Ags1p, neither of the *A. fumigatus* homologs appears to be essential. Disruption of one of them (*AfAgs1p*) reduced cell wall $\alpha(1,3)$ -glucan by 50%, but did not affect virulence of *A.*

fumigatus in mice (Beauvais *et al.*, 2005). In *C. neoformans*, there is only one AGS homolog. RNA interference targeting this sequence or deletion of the gene leads to a loss of cell wall $\alpha(1,3)$ -glucan, a temperature-sensitive growth defect, and a lack of virulence in mice (Reese & Doering, 2003; A. J. Reese and T. L. Doering, unpublished results). Interestingly, this mutant produces capsule polysaccharides that can be detected in the culture medium and bind to acapsular mutants, but this strain is acapsular and cannot bind capsule material from any source to its outer surface (Reese & Doering, 2003). This implicates cryptococcal $\alpha(1,3)$ -glucan in the attachment of capsule to the cell, although the exact nature of this linkage is still under investigation.

$\beta(1,6)$ -Glucan synthases

$\beta(1,6)$ -Glucan is a critical molecule in *S. cerevisiae*, as it serves as the attachment site for mannosylated cell wall proteins and thus interconnects the outer protein and inner glucan layers of the wall. Although various studies have elucidated the structure of the $\beta(1,6)$ -glucan and identified a number of genes apparently involved in its synthesis, the enzymes that build this polysaccharide remain elusive (Shahinian & Bussey, 2000). As $\beta(1,6)$ -glucan is such a vital structure for many fungi and is the most abundant β -glucan in *C. neoformans* (Table 1), this is a very attractive area for future investigation.

Chitin synthases (CHS)

Chitin is a polymer of $\beta(1,4)$ -linked *N*-acetylglucosamine (GlcNAc). This polysaccharide plays a role in a number of cellular processes, including septation, budding and apical growth, and is indispensable in many fungal species (Munro & Gow, 2001). The amount of cell wall chitin varies from 1–2% of the cell wall dry weight in *S. cerevisiae* to > 40% in some *Mucor* species (Munro & Gow, 2001). Because of its importance and diversity of function, fungi have evolved multiple synthases responsible for the construction of chitin. Although all of these chitin synthases (Chs) catalyse the same biochemical activity (i.e. addition of GlcNAc to the growing polysaccharide chain), in many fungi they perform distinct cellular functions (Munro & Gow, 2001). However, the disruption of some *CHS* genes leads to no apparent phenotype. It is currently unclear whether this lack of apparent phenotype is due to functional overlap of some of these enzymes or to the inability to identify subtle defects in cellular chitin.

The chitin synthases can be assigned to one of six classes based on amino acid sequence. Unfortunately, the functional significance of the six classes is not well understood, as the disruption of enzymes from the same class in different organisms often leads to dissimilar phenotypes (Munro &

Gow, 2001). Furthermore, there is no obvious relationship between the *in vitro* activity of chitin synthases and their importance to cell viability. It is thus difficult to draw conclusions about chitin synthetic processes in any specific fungal species by analysis of sequence or *in vitro* activities alone. In *C. neoformans*, eight chitin synthases have been identified by sequence homology. Each of these genes has been successfully disrupted, and none appears to be essential. One interesting *CHS* mutant (*chs3*) is temperature sensitive and has no chitosan in the cell wall (Banks *et al.*, 2005). Chitosan, a deacetylated form of chitin, is a more flexible and soluble polysaccharide of repeating glucosamine units. The existence of chitosan has not been fully investigated in most fungi and its biological role is not understood, but it is found in substantial concentrations in the cell wall of *C. neoformans* (Banks *et al.*, 2005). Because chitosan is produced indirectly via chitin deacetylation, Banks and colleagues suggest that Chs3p is complexed with a deacetylase and is responsible for making the chitin that eventually becomes cell wall chitosan. The cellular functions of the other cryptococcal chitin synthases are currently not known, although they might be elucidated in the future with the construction of double mutants.

Efforts to target chitin synthesis therapeutically have resulted in the discovery of two classes of compounds, the nikkomycins and polyoxins. These drugs are postulated to bind to the active site of chitin synthases, although the different synthases within *S. cerevisiae* are variably susceptible to inhibition by these drugs *in vitro* (Ruiz-Herrera & San-Blas, 2003). As *C. neoformans* is sensitive to at least one of these compounds (Nikkomycin Z) (Li & Rinaldi, 1999; Kraus *et al.*, 2003), it would suggest that elucidation of the structure of the active site and cellular function of each chitin synthase could lead to future drug design against those enzymes that are the most vital for its survival.

Glycosyltransferases involved in cryptococcal capsule polysaccharide synthesis

The unique polysaccharide capsule of *C. neoformans* is an important virulence factor for this opportunistic pathogen as strains lacking the capsule are avirulent or highly attenuated in virulence (Bose *et al.*, 2003). This suggests that capsule synthesis could be targeted therapeutically. The capsule is composed of two primary polysaccharides, glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), along with mannoproteins. GXM is a polymer of $\alpha(1,3)$ -linked mannan decorated with glucuronic acid, xylose and acetyl residues (Fig. 1) that makes up $\sim 90\%$ of the capsular mass. The arrangement of the xylose and acetyl residues in GXM differs among the four serotypes (A, D, B and C) of *C. neoformans* (Cherniak *et al.*, 1998). GalXM has

an $\alpha(1,6)$ -galactan core with $\beta(1,3)$ -galactose side chains that are further substituted with mannose and xylose (Vaishnav *et al.*, 1998) (Fig. 1). The structures of GXM and GalXM suggest that numerous glycosyltransferases are involved in their synthesis. However, no glycosyltransferase of demonstrated biochemical function has been proven to play a role in capsule polysaccharide construction to date. Kwon-Chung and colleagues have identified four 'CAP' genes (*CAP10*, 59, 60 and 64) that, when deleted, lead to viable *C. neoformans* strains that lack capsule and are avirulent in mice. However, the specific role of their protein products in capsule synthesis remains unclear (Chang & Kwon-Chung, 1994, 1998, 1999; Chang *et al.*, 1996; Bose *et al.*, 2003). Our laboratory has purified two glycosyltransferases, one mannosyltransferase and one xylosyltransferase, with activities appropriate for capsule synthesis and homologies to the CAP genes. These two enzymes are discussed briefly here.

Mannosyltransferase (Cmt1p)

An $\alpha(1,3)$ -mannosyltransferase that catalyses the transfer of mannose from GDP-mannose to an $\alpha(1,3)$ -linked mannose disaccharide was purified in our laboratory from *C. neoformans* membranes, and the corresponding gene was identified (Sommer *et al.*, 2003). This gene, *CMT1*, encodes a 46 kDa protein and is a homolog of the *CAP59* gene mentioned above. In addition, the enzyme copurified with Cas31p, a *CAP64* homolog that has been shown by Moyrand *et al.* (2004) to have a potential role in capsule acetylation and/or xylosylation. No alteration in capsule structure was detected by electron microscopy in a *cmt1* deletion strain (Sommer *et al.*, 2003), but structural analyses of the capsular polysaccharides in this mutant are still underway.

Xylosyltransferase (Cxt1p)

Our laboratory has purified and characterized a 90 kDa cryptococcal membrane protein that catalyses the transfer of xylose from UDP-xylose to an $\alpha(1,3)$ -linked mannose disaccharide. Nuclear magnetic resonance analysis of the resulting trisaccharide product revealed that xylose was added in a $\beta(1,2)$ -linkage to the reducing mannose (Klutts *et al.*, 2005). Thus, this activity is appropriate for xylose transfer into either GXM or GalXM (see Fig. 1). The corresponding gene, *CXT1* (cryptococcal xylosyltransferase 1), has been disrupted in *C. neoformans*. Interestingly, we can detect two additional xylosyltransferase activities in the *cxt1* Δ strain. We are now investigating the enzymes that catalyse these two additional activities.

Glycosyltransferases involved in protein glycosylation

There are two major types of glycosyl modifications of proteins, *N*-linked and *O*-linked. *N*-linked glycans are attached to specific asparagine residues within a protein sequence and *O*-linked glycans to either serine or threonine residues. The mechanisms and glycosyltransferases involved in the synthesis of these glycans in *S. cerevisiae* are well characterized, but there has been little study of these processes in *C. neoformans*. Protein glycosyltransferases are reviewed here, with special attention to what is known in *C. neoformans*.

N-linked glycan synthesis

Proper *N*-glycosylation of proteins may be essential for their function and, in some cases, is needed for cell survival. This pathway begins with the synthesis of a dolichol-linked oligosaccharide precursor (Dol-PPGlcNAC₂Man₉Glc₃) (Fig. 3a). This precursor is synthesized in the ER membrane by the sequential addition of monosaccharides to dolichol pyrophosphate through the function of the *ALG* family of glycosyltransferases (Burda & Aebi, 1999). The first of these is Alg7p, which catalyses the transfer of GlcNAc from UDP-GlcNAc to dolichol pyrophosphate on the cytoplasmic leaflet of the ER membrane. After the addition of a second GlcNAc by an unknown transferase, five mannose residues are transferred from GDP-mannose to the growing glycan by Alg1p, Alg2p, Alg11p and an unknown mannosyltransferase. The dolichol-linked oligosaccharide is then 'flipped' to the luminal side of the ER membrane, where Alg3p, Alg9p and

Alg12p sequentially add three more mannose residues from dolichol-*P*-mannose to the chain. In most eukaryotes, three glucose residues are then added from dolichol-*P*-glucose by Alg6p, Alg8p and Alg10p (Fig. 3a). However, similar to some trypanosomatids, *C. neoformans* lacks the Alg glycosyltransferases and thus its precursor contains no glucose (Fig. 3b; Samuelson *et al.*, 2005). In most eukaryotes, these glucose residues play an essential role for glycoprotein folding quality control. Therefore, *C. neoformans* may utilize a different strategy for this process.

In all eukaryotes, once the synthesis of the oligosaccharide chain is completed, the ER oligosaccharyl transferase (OST) complex transfers it from dolichol to an asparagine residue within a consensus sequence (NXS or NXT) of a nascent peptide. After this transfer, glycosidases remove one mannose residue and the three glucose residues of the oligosaccharide chain, and the protein is transported to the Golgi apparatus where the oligosaccharide chain may be further modified. These later Golgi modification steps of *N*-glycosylation differ considerably among various organisms, such that an assortment of side chains can be added through different linkages. In *Saccharomyces*, Golgi *N*-glycan modifications begin with the addition of a single α (1,6)-linked mannose residue by the mannosyltransferase, Och1p. The protein is then further glycosylated in one of two ways, either by the addition of several α (1,3)-linked mannose residues or by extension of the α (1,6)-mannosylation to yield a product known as mannan (Dean, 1999). Commonly found in the outer layer of fungal cell walls, mannans are not essential for *in vitro* viability, but do influence various characteristics such as cell surface hydrophobicity (Singleton *et al.*, 2005) and flocculation (Stratford, 1992). Generally, mannans have a long α (1,6)-mannose backbone with numerous mannose side chains in various linkages (Ballou *et al.*, 1994; Cutler, 2001; Munro, 2001). In *Sch. pombe*, the mannose side chains are substituted with galactose residues, thus rendering it 'galactomannan' (Ballou & Ballou, 1995). In *S. cerevisiae*, elongation of the α (1,6)-mannan backbone is mediated by mannan polymerase complex (M-Pol) I and II (Munro, 2001). *Cryptococcus neoformans* lacks most of the genes encoding M-Pol I and II subunits, suggesting a possible absence of α -1,6 mannan (see Table 2).

One of the better-studied *N*-glycosylated proteins from *C. neoformans* is a phospholipase B1 (PLB1) that is localized to the cell wall (Wright *et al.*, 2004). This protein has 17 sites with the consensus *N*-glycosylation signal; the prediction program NetNGlyc (<http://www.cbs.dtu.dk/services/NetNGlyc/>) suggests that four of these are utilized. The apparent molecular mass of glycosylated PLB1 in a serotype B strain was determined by size exclusion chromatography and differed from that observed in a serotype A strain (Wright *et al.*, 2004). This suggests possible differences in protein glycosylation between those two serotypes, although

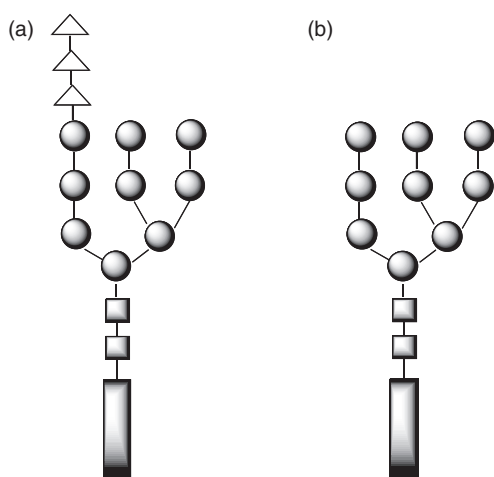


Fig. 3. Dolichol-linked *N*-glycosylation precursors. (a) Precursor in *Saccharomyces cerevisiae* and *Homo sapiens*. (b) Precursor in *Cryptococcus neoformans*. Rectangle, dolichol pyrophosphate; square, *N*-acetyl-glucosamine; circle, mannose; open triangle, glucose.

Table 2. Glycosyltransferase homologs in *Cryptococcus neoformans* involved in protein glycosylation

<i>Saccharomyces cerevisiae</i>	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i> [‡]
Protein O-glycosylation		
Pmt1p Pmt1p, Pmt5p, Pmt7p subfamily	Pmt1p, Pmt5p	144_7*/CND06150 [†]
Pmt2p Pmt2p, Pmt3p, Pmt6p subfamily	Pmt2p, Pmt6p	96_4*/CNJ01930 [†]
Pmt4p Pmt4p subfamily	Pmt4p	20_30*/CND01240 [†]
Mnt1p Mnt1p, Ktr1p, Ktr3p family	Mnt1p, Mnt2p (four other homologs with unknown function)	71_28*/CNB03210 [†]
N-linked α (1,6)-mannan synthesis		
Och1p Och1p	Och1p [‡]	14_55*/CNA07230 [†]
M-Poll Mnn9p, Van1p	Mnn9p, Van1p [‡]	None
M-PollI Mnn9p, Anp1p, Mnn10p, Mnn11p, Hoc1p	Mnn9p, Anp1p [‡] , Mnn10p [‡] , Mnn11p [‡]	Hoc1 homologs only (14_55*/CNA07230 [†])

*Twinscan predicted H99 (serotype A) protein.

[†]TIGR annotated protein, JEC21 (serotype D).

[‡]By homology only, including all *C. neoformans* sequences.

alterations in glycosylation sites or multimerization of PLB1 may also explain this result. Assessing potential serotype-specific changes in glycosylation will require detailed analysis of glycan structures. Such analysis will also be useful for predicting what glycosyltransferases are involved in cryptococcal N-glycosylation. Finally, it could shed light on the intriguing possibility that aspects of synthetic machinery may be shared between capsule synthesis and protein glycosylation.

O-linked glycan synthesis

In fungi, O-linked glycosylation is initiated by the transfer of a mannose residue from dolichol-P-mannose to a serine or threonine residue of a nascent peptide. This O-mannosylation reaction occurs in the lumen of the ER, catalysed by one of the protein mannosyltransferases (Pmts) (Strahl-Bolsinger *et al.*, 1999). Fungal Pmt family members have been categorized into three subfamilies (Pmt1, Pmt2 and Pmt4) based on sequence homology (Willer *et al.*, 2002). *Saccharomyces cerevisiae* has seven Pmt enzymes that fall into these three subgroups, whereas *Ca. albicans* has five (Cutler, 2001) (Table 2). Like *Sch. pombe* and some filamentous fungi (Prill *et al.*, 2005), *C. neoformans* appears to have only three PMT genes as identified by sequence homology (Table 2). In the Golgi, a second mannose residue is transferred from GDP-mannose in an α (1,2)-linkage by the Mnt1 family of mannosyltransferases. Although there are three redundant enzymes present to catalyse this reaction in *S. cerevisiae*, *C. neoformans* appears to have only one Mnt1 homolog (Table 2). After this step, the mannose chains can be extended in the Golgi by a variety of glycosyltransferases (Gemmill & Trimble, 1999a). In most fungi, this extension involves the addition of further mannose residues. However, in *Sch.*

pombe the addition of galactose residues has also been demonstrated (Gemmill & Trimble, 1999b).

Cryptococcal mannoproteins are cell wall-associated proteins that have been studied as immunodominant antigens for the cell-mediated immune response against this opportunistic pathogen. It is predicted that *C. neoformans* has more than 50 mannoproteins, only two of which, MP98 and MP88 (Levitz *et al.*, 2001; Huang *et al.*, 2002), have been characterized in detail. Cryptococcal mannoproteins typically contain one or more putative N-glycosylation sites and a serine/threonine-rich region that is predicted to be heavily O-glycosylated. It appears that it is the O-glycans of the cryptococcal mannoproteins that are recognized by the macrophage mannose receptor (Mansour *et al.*, 2002). This recognition of mannoproteins is inhibited by α -D-mannopyranose but not by α -D-galactopyranose (Mansour *et al.*, 2002), suggesting that these glycans end in mannose.

Glycosyltransferases involved in lipid glycosylation

Glycolipids are formed by specific glycosyltransferases. These enzymes are discussed below, grouped by each modified lipid.

Sphingolipid glycosyltransferases

Most of the knowledge on sphingolipid biosynthesis in fungi has been gathered from studies in *S. cerevisiae*. Sphingolipid biosynthesis is initiated by the condensation of serine and a fatty acyl-CoA (usually palmitoyl CoA) to form 3-ketodihydro-sphingosine. Through reduction and acylation reactions, this product is converted to ceramide, which can then be further modified to produce complex sphingolipids.

One major modification to ceramide is the addition of inositol phosphate by inositol phosphoryl-ceramide synthase (encoded by *IPC1*), to form inositol phosphoryl-ceramide (IPC). IPC may then be mannosylated in an $\alpha(1,2)$ -linkage to yield mannose-IPC (MIPC) (Patton & Lester, 1991). In *S. cerevisiae*, three genes, *SUR1* (*CSG1*, *BCL21*), *CSH1* and *CSG2*, are required for the synthesis of MIPC (Beeler *et al.*, 1997). Sur1p shows 29% and 23% similarity over a stretch of 93 amino acids to two yeast $\alpha(1,6)$ -mannosyltransferases, Och1p and Hoc1p, respectively. Csh1p is 65% homologous to Sur1p, and a *sur1 Δ csh1 Δ* strain completely lacks MIPCs, suggesting that these two proteins are involved with MIPC formation. Csg2p complexes separately with either Sur1p or Csh1p, and is also required for MIPC synthesis (Uemura *et al.*, 2003). *Cryptococcus neoformans* appears to have a single homolog to both Sur1p and Csh1p (CNH00360) and none to Csg2p.

The terminal step in *S. cerevisiae* sphingolipid synthesis is the transfer of inositol phosphate from phosphatidylinositol to MIPC to yield the major sphingolipid, mannose diinositol diphosphoryl ceramide, M(IP)₂C. The function of Ipt1p is required for this reaction. M(IP)₂C accounts for approximately 75% of the sphingolipids in *S. cerevisiae*, with the remaining 25% being divided between IPC and MIPC. An *ipt1 Δ* strain appears to grow normally (Dickson *et al.*, 1997), perhaps because *S. cerevisiae* adjusts the level of MIPC in this mutant to compensate for the absence of M(IP)₂C. The only homolog of Ipt1p in *C. neoformans* is CnIpc1p (Heidler & Radding, 2000), which functions in the synthesis of IPCs (Luberto *et al.*, 2001). Combined with the apparent lack of a Csg1p homolog, this suggests that sequence identities with proteins from other organisms may not necessarily help in the determination of function. Furthermore, these differences reveal the gaps that remain in our knowledge of the pathway of sphingolipid biosynthesis in *C. neoformans*, which may have unique features.

A second class of glycosylated sphingolipids found in fungi is the glucosylceramides. Levery *et al.*, 2000 found that in *C. neoformans* this is primarily glucosylceramide (GlcCer), made by glucosylceramide synthases (GCS) that add glucose in a $\beta(1,1)$ linkage to a ceramide acceptor. GCSs have been identified in *Ca. albicans* (van Meer & Holthuis, 2000), *Pichia pastoris* and *Magnaporthe grisea* (Leipelt *et al.*, 2001). Like their mammalian counterparts, these proteins have a putative transmembrane domain at the N-terminus and a segment of hydrophobic amino acids at the C-terminus, suggesting that they are integral membrane proteins. *Cryptococcus neoformans* appears to have a single glucosylceramide synthase gene, *GCS1* (Rittershaus & Del Poeta, 2005). The *gcs1 Δ* strain does not produce GlcCer, and is avirulent in a mouse model of infection. It is also unable to survive in conditions that mimic mammalian

lungs, i.e. alkaline pH and 5% CO₂ (Rittershaus & Del Poeta, 2005).

Sterol glucosyltransferase

Ergosterol is the most abundant sterol in fungi. This molecule and its biosynthetic precursors play roles in membrane integrity, endocytosis and vacuolar membrane fusion. Owing to the lack of ergosterol in mammalian cells, a number ofazole derivatives that inhibit its synthesis have been used as antifungals. These drugs result in the disruption of fungal membranes, leading to cytoplasmic leakage and ultimately to growth inhibition. Although the majority of cellular ergosterol is unglycosylated, in certain fungi a subset are modified into sterol glycosides. For example, a sterol glucosyltransferase gene from *P. pastoris* (*UGT51/PAZ4*) is required for the synthesis of ergosterol glucoside. A *ugt51* deletion mutant in *P. pastoris* is deficient in this product and appears to be defective in vacuole-dependent selective degradation of peroxisomes in response to glucose or ethanol (Warnecke *et al.*, 1999; Oku *et al.*, 2003). In the alkane-utilizing yeast *Yarrowia lipolytica*, a mutation in *UGT51* leads to a severe deficiency in utilization of decane (C10), but not of other *n*-alkanes (Stasyk *et al.*, 2003). Thus, the role of ergosterol glucosides appears to be diverse among fungal organisms. There is a putative sterol glucosyltransferase in *C. neoformans* (CNC04500) with 44% identity and 61% similarity to *P. pastoris* *UGT51*, but the function of this gene has yet to be ascertained.

Glycosylphosphatidylinositol (GPI) anchor synthesis

GPI anchors are glycolipids that link polypeptides to the external leaflet of the plasma membrane. (GPIs may also occur as free glycolipids or as anchors of glycans, but these have not been reported in *C. neoformans*.) This form of membrane attachment confers special properties on these proteins, in terms of their mobility, localization to membrane microdomains and potential release into the surrounding environment. In *S. cerevisiae*, GPI-anchored polypeptides also occur as biosynthetic precursors of proteins that later become anchored to cell wall $\alpha(1,6)$ -glucan (see ' $\beta(1,6)$ -Glucan synthases'). Although these proteins are initially tethered to the plasma membrane and thus surrounded by the cell wall, their subsequent anchoring to the cell wall causes them to be displayed on the cell surface and thus potentially available to interact with the environment or other cells. *Cryptococcus neoformans* generates GPI-anchored polypeptides that are both plasma membrane anchored (T. L. Doering, unpublished data) and linked to the cell wall (Djordjevic *et al.*, 2005). *In silico* analysis (De Groot *et al.*, 2005) suggests the genome encodes around 50

potentially GPI-anchored proteins (P. de Groot, F. Klis and C. Specht, pers. commun.). One of these proteins is homologous to the *A. fumigatus* Gel1p and the *S. cerevisiae* Gas1p (see Table 3), which are glucanoyltransferases important in cell wall synthesis (see ‘ $\beta(1,3)$ -Glucan synthases (FKS)’). Consistent with this finding, *C. neoformans* expresses a GPI-anchored protein that cross-reacts with polyclonal antiserum generated against yeast Gas1p (T.L. Doering, unpublished results), although its glycosyltransferase activity has not been directly tested. Another cryptococcal enzyme that is required for cryptococcal dissemination, PLB1, has recently been suggested to be GPI-anchored to the cell wall (Djordjevic *et al.*, 2005).

GPIs have a conserved linear core of three mannose residues and a nonacetylated glucosamine (Fig. 4). The reducing end of this core is linked to an inositol phospholipid, and the nonreducing end is linked via ethanolamine to the C-terminus of the anchored polypeptide. *Saccharomyces cerevisiae* typically adds additional mannose residues and two ethanolamine phosphate groups to the trimannosyl core (Fig. 4), and an acyl group to the inositol moiety. Concerted efforts in mammalian systems and *S. cerevisiae* have identified genes encoding many of the glycosyltransferases required for GPI anchor synthesis (for a recent example, see Kang *et al.*, 2005). Homologs of most of these genes are found in *C. neoformans* (Table 3), with the interesting exception of *SMP3*, which adds the fourth mannose of the anchor. None of these transferase activities has been investigated in detail in *Cryptococcus*, although mannosylated GPI intermediates have been identified *in vitro* in cryptococcal membrane preparations (T.L. Doering, unpublished results). An additional feature of GPI biosynthesis revealed by cell-free studies is that *Ca. neoformans* is much more selective than *S. cerevisiae* in terms of which acyl groups it adds to the GPI anchor inositol (Franzot & Doering, 1999).

Studies of GPI-anchored proteins in fungal pathogens have primarily focused on either specific polypeptides involved in pathogenesis, including cryptococcal PLB1 (Djordjevic *et al.*, 2005) or *Ca. albicans* adhesins (Frieman

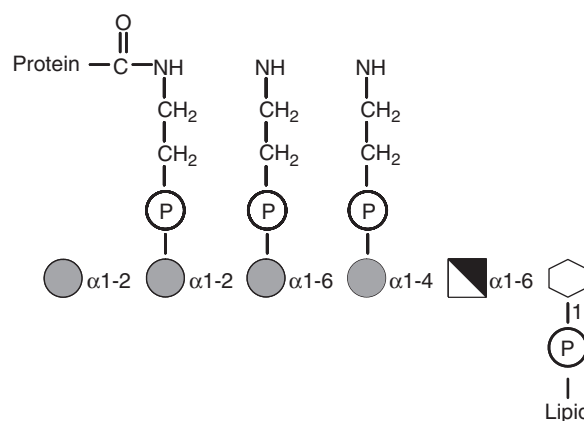


Fig. 4. Schematic diagram of a yeast glycosylphosphatidylinositol anchor linked to a protein C-terminus. Shaded circles, mannose; partly shaded box, glucosamine; open hexagon, inositol; circled P, phosphate. Inositol acylation is not shown, and anchor lipids are not detailed; for information on these components in fungi see Fankhauser *et al.* (1993), Franzot & Doering (1999), and Fontaine *et al.* (2003).

et al., 2002), or on enzymes needed for fungal-specific processes, such as Gel1p. However, there are important aspects of fungal GPI biosynthesis that are not shared with mammalian hosts, such as the timing of addition of the fourth mannose residue (Eisenhaber *et al.*, 2003), the presence of ceramide in anchor lipids (Fankhauser *et al.*, 1993; Fontaine *et al.*, 2003) and the mechanisms of cell wall linkage. These deserve additional investigation.

Future research

Studies of glycosyltransferases will increase our understanding of cryptococcal biology, and may lead to productive areas for the development of antifungal agents. Multiple strategies are available for these endeavors. For enzymes that resemble known glycosyltransferases in model systems, useful direction is provided by sequence information, enabled by recent efforts at multiple centers to sequence the cryptococcal genome (Loftus *et al.*, 2005). For example, this

Table 3. Genes encoding glycosyltransferases involved in glycosylphosphatidylinositol (GPI) biosynthesis and Gas1p

<i>Saccharomyces cerevisiae</i> gene	NCBI reference	Enzymatic activity in <i>S. cerevisiae</i> *	<i>Cryptococcus neoformans</i> protein	Percentage identity [†]	Percentage similarity [†]
<i>GPI3</i>	NP_015150	Adds <i>N</i> -acetylglucosamine to PI	CNBD4030	23	32
<i>GPI14</i>	NP_012547	Adds 1st GPI core mannose	CNBB2250	27	43
<i>GPI18</i>	NP_009558	Adds 2nd GPI core mannose	CNBC2320	21	34
<i>GPI10</i>	NP_011373	Adds 3rd GPI core mannose	CNBC2120	17	32
<i>SMP3</i>	NP_014792	Adds 4th GPI core mannose	–	–	–
<i>GAS1</i>	NP_014612	1,3-Glucanoyltransferase, putative	AAW47197	34	49

*This table does not include the multiple proteins with accessory roles in GPI biosynthetic steps, or the enzymes that add or remove nonsugar components of the GPI structure Eisenhaber *et al.* (2003). Core mannose residues are numbered starting at glucosamine (see Fig. 4).

[†]Percentage identity and similarity of the indicated *C. neoformans* sequences to the *S. cerevisiae* genes listed.

approach was recently used to investigate protein mannosyltransferases (Olson *et al.*, 2005). Cells disrupted in genes of interest that are identified by homology may then be examined with the multiple tools currently available for this pathogen, ranging from detailed imaging to animal studies. However, owing to the wealth of unusual linkages in structures like the cryptococcal capsule, the limited homology among glycosyltransferases, and the relative simplicity of the spectrum of glycosyltransferases identified even in the best-studied model yeast, many important enzymes may be impossible to identify by a bioinformatics approach. Furthermore, some interesting questions regarding the interpretation of sequence homology within *Cryptococcus* have already arisen. This is exemplified by the homology of Cap59p to the $\alpha(1,3)$ -mannosyltransferase, Cmt1p (Sommer *et al.*, 2003). The *CAP59* gene is essential for normal capsule synthesis (Chang & Kwon-Chung, 1994), but its function is not known. Does this homology to a known glycosyltransferase indicate that Cap59p is indeed a transferase, or does it have some other cellular function (Garcia-Rivera *et al.*, 2004), or both? Despite remaining questions and potential shortfalls, sequence analysis is likely to remain a mainstay of research for cryptococcal glycan synthesis.

Where homology studies are limited by the unique nature of *C. neoformans*, forward genetic approaches help to fill the gap. Techniques that are now available for these studies include insertional mutagenesis (Idnurm *et al.*, 2004; McClelland *et al.*, 2005) and RNAi library studies (I. Bose and T.L. Doering, unpublished data), in addition to more traditional techniques such as UV mutagenesis (Jacobson *et al.*, 1982; Janbon *et al.*, 2001). Studies from several groups have identified acapsular or hypocapsular mutants by colony morphology, a broad approach to identifying mutations anywhere in the biosynthetic or regulatory pathways involved in capsule synthesis. This strategy has identified proteins involved in sugar uptake and metabolism, such as UDP-glucose dehydrogenase (Griffith *et al.*, 2004; McClelland *et al.*, 2005), but has not yet led to demonstrated mutations in glycosyltransferase activity. A second screening approach, developed by the Janbon group, is to assess cells for capsule defects based on reduced reactivity with monoclonal anticapsular antibodies. This approach has identified mutants defective in upstream steps of capsule synthesis, such as UDP-glucuronic acid decarboxylase (Bar-Peled *et al.*, 2001; Moyrand *et al.*, 2002). However, as with morphology screens, mutants found in this way can have defects in many parts of the biosynthetic pathway or its regulation. To select for glycosyltransferase mutants, screening of mutagenized populations must be refined, ideally being based on assays for the activity of these enzymes. Even though this has not yet been achieved, forward genetics will continue to be an important tool for the future study of cryptococcal glycosyltransferases.

Ultimately, biochemical approaches will be needed to identify the full complement of glycosyltransferases unique to *C. neoformans*. Once structures of the glycoconjugates of interest are obtained, biochemical assays for predicted linkages can be developed. In the case of capsule, the structures have been defined (Fig. 1) and synthetic chemistry has advanced so as to allow for the generation of relevant substrates for such assays (Oscarson *et al.*, 2005). In other arenas, such as glycolipids and glycoproteins, the cryptococcal glycan structures have not yet been examined in detail and are likely to differ from other fungi (Gemmill & Trimble, 1999). In any situation where an assay can be developed, it can then be used biochemically to purify the enzyme of interest, and potentially adapted for genetic approaches or to screen inhibitors. For these reasons, further efforts to determine cryptococcal glycan structures and examine their synthesis biochemically remain a priority.

Conclusions

Our current knowledge of glycosyltransferases in *C. neoformans* is at an early stage, but the situation is changing rapidly, with advances in studies of capsule and chitin synthesis, and hints of progress in protein glycosylation as well. Identification of unique glycosyltransferases as potential drug targets is an important goal, especially as this approach has already proven effective in pathogenic fungi. Beyond therapeutic considerations, there is a tremendous amount of basic scientific knowledge to be learned from *C. neoformans*. We have known for a long time that the capsule is a unique and fascinating structure, but we have only more recently encountered unexpected features of the cell wall, and almost nothing is known about glycolipids and protein glycosylation. There will certainly be more surprises to come, as this rich system continues to teach us about fungal glycoconjugates in general as well as its own unique biology.

Acknowledgements

We thank Hong Liu and Cara Griffith for their help in collating Table 3, Stu Levitz for organizing a terrific conference, and Jenny Lodge for helpful conversations. We would also like to thank Piet de Groot, Frans Klis and Charles Specht for communicating unpublished results. J.S.K. is supported by NIH F32 GM072341. Research on cryptococcal glycans in the Doering laboratory is supported by NIH R01 GM66303 and NIH R01 GM71007.

References

- Ballou L & Ballou C (1995) *Schizosaccharomyces pombe* mutants that are defective in glycoprotein galactosylation. *Proc Natl Acad Sci USA* **92**: 2790–2794.

- Ballou CE, Ballou L & Ball G (1994) *Schizosaccharomyces pombe* glycosylation mutant with altered cell surface properties. *Proc Natl Acad Sci USA* **91**: 9327–9331.
- Banks IR, Specht CA, Donlin MJ, Gerik KJ, Levitz SM & Lodge JK (2005) A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen *Cryptococcus neoformans*. *Eukaryotic Cell* **4**: 1902–1912.
- Bar-Peled M, Griffith CL & Doering TL (2001) Functional cloning and characterization of a UDP-glucuronic acid decarboxylase: the pathogenic fungus *Cryptococcus neoformans* elucidates UDP-xylose synthesis. *Proc Natl Acad Sci USA* **98**: 12003–12008.
- Beauvais A, Maubon D, Park S, Morelle W, Tanguy M, Huerre M, Perlin DS & Latge JP (2005) Two alpha(1-3) glucan synthases with different functions in *Aspergillus fumigatus*. *Appl Environ Microbiol* **71**: 1531–1538.
- Beeler TJ, Fu D, Rivera J, Monaghan E, Gable K & Dunn TM (1997) *SURI* (*CSG1/BCL21*), a gene necessary for growth of *Saccharomyces cerevisiae* in the presence of high Ca^{2+} concentrations at 37 degrees C, is required for mannosylation of inositolphosphorylceramide. *Mol Gen Genet* **255**: 570–579.
- Bose I, Reese AJ, Ory JJ, Janbon G & Doering TL (2003) A yeast under cover: the capsule of *Cryptococcus neoformans*. *Eukaryotic Cell* **2**: 655–663.
- Breton C, Snajdrova L, Jeanneau C, Koca J & Imbert A (2006) Structures and mechanisms of glycosyltransferases superfamily. *Glycobiology* **16**: 29R–37R.
- Burda P & Aebi M (1999) The dolichol pathway of N-linked glycosylation. *Biochim Biophys Acta* **1426**: 239–257.
- Campbell JA, Davies GJ, Bulone V & Henrissat B (1997) A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities. *Biochem J* **326**: 929–939.
- Carotti C, Ragni E, Palomares O, Fontaine T, Tedeschi G, Rodriguez R, Latge JP, Vai M & Popolo L (2004) Characterization of recombinant forms of the yeast Gas1 protein and identification of residues essential for glucanosyltransferase activity and folding. *Eur J Biochem* **271**: 3635–3645.
- Chang YC & Kwon-Chung KJ (1994) Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. *Mol Cell Biol* **14**: 4912–4919.
- Chang YC & Kwon-Chung KJ (1998) Isolation of the third capsule-associated gene, *CAP60*, required for virulence in *Cryptococcus neoformans*. *Infect Immun* **66**: 2230–2236.
- Chang YC & Kwon-Chung KJ (1999) Isolation, characterization, and localization of a capsule-associated gene, *CAP10*, of *Cryptococcus neoformans*. *J Bacteriol* **181**: 5636–5643.
- Chang YC, Penoyer LA & Kwon-Chung KJ (1996) The second capsule gene of *Cryptococcus neoformans*, *CAP64*, is essential for virulence. *Infect Immun* **64**: 1977–1983.
- Cherniak R, Valafar H, Morris LC & Valafar F (1998) *Cryptococcus neoformans* chemotyping by quantitative analysis of 1H nuclear magnetic resonance spectra of glucuronoxylomannans with a computer-simulated artificial neural network. *Clin Diagn Lab Immunol* **5**: 146–159.
- Coutinho PM, Deleury E, Davies GJ & Henrissat B (2003) An evolving hierarchical family classification for glycosyltransferases. *J Mol Biol* **328**: 307–317.
- Cutler JE (2001) N-glycosylation of yeast, with emphasis on *Candida albicans*. *Med Mycol* **39**: 75–86.
- Dean N (1999) Asparagine-linked glycosylation in the yeast Golgi. *Biochim Biophys Acta* **1426**: 309–322.
- Dickson RC, Nagiec EE, Wells GB, Nagiec MM & Lester RL (1997) Synthesis of mannose-(inositol-P)2-ceramide, the major sphingolipid in *Saccharomyces cerevisiae*, requires the *IPT1* (*YDR072c*) gene. *J Biol Chem* **272**: 29620–29625.
- Dijkgraaf GJ, Abe M, Ohya Y & Bussey H (2002) Mutations in *Fks1p* affect the cell wall content of beta-1,3- and beta-1,6-glucan in *Saccharomyces cerevisiae*. *Yeast* **19**: 671–690.
- Djordjevic JT, Del Poeta M, Sorrell TC, Turner KM & Wright LC (2005) Secretion of cryptococcal phospholipase B1 (*PLB1*) is regulated by a glycosylphosphatidylinositol (*GPI*) anchor. *Biochem J* **389**: 803–812.
- Douglas CM (2001) Fungal beta(1,3)-D-glucan synthesis. *Med Mycol* **39**: 55–66.
- Duran A & Nombela C (2004) Fungal cell wall biogenesis: building a dynamic interface with the environment. *Microbiology* **150**: 3099–3103.
- Eisenhaber B, Maurer-Stroh S, Novatchkova M, Schneider G & Eisenhaber F (2003) Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. *Bioessays* **25**: 367–385.
- Fankhauser C, Homans SW, Thomas-Oates JE, McConville MJ, Desponds C, Conzelmann A & Ferguson MA (1993) Structures of glycosylphosphatidylinositol membrane anchors from *Saccharomyces cerevisiae*. *J Biol Chem* **268**: 26365–26374.
- Fontaine T, Magnin T, Melhert A, Lamont D, Latge JP & Ferguson MA (2003) Structures of the glycosylphosphatidylinositol membrane anchors from *Aspergillus fumigatus* membrane proteins. *Glycobiology* **13**: 169–177.
- Franzot SP & Doering TL (1999) Inositol acylation of glycosylphosphatidylinositols in the pathogenic fungus *Cryptococcus neoformans* and the model yeast *Saccharomyces cerevisiae*. *Biochem J* **340**: 25–32.
- Frieman MB, McCaffery JM & Cormack BP (2002) Modular domain structure in the *Candida glabrata* adhesin *Epa1p*, a beta1,6 glucan-cross-linked cell wall protein. *Mol Microbiol* **46**: 479–492.
- Garcia-Rivera J, Chang YC, Kwon-Chung KJ & Casadevall A (2004) *Cryptococcus neoformans* *CAP59* (or *Cap59p*) is involved in the extracellular trafficking of capsular glucuronoxylomannan. *Eukaryotic Cell* **3**: 385–392.
- Gemmill TR & Trimble RB (1999a) Overview of N- and O-linked oligosaccharide structures found in various yeast species. *Biochim Biophys Acta* **1426**: 227–237.
- Gemmill TR & Trimble RB (1999b) *Schizosaccharomyces pombe* produces novel Gal0-2Man1-3 O-linked oligosaccharides. *Glycobiology* **9**: 507–515.

- Griffith CL, Klutts JS, Zhang L, Lavery SB & Doering TL (2004) UDP-glucose dehydrogenase plays multiple roles in the biology of the pathogenic fungus *Cryptococcus neoformans*. *J Biol Chem* **279**: 51669–51676.
- De Groot PW, Ram AF & Klis FM (2005) Features and functions of covalently linked proteins in fungal cell walls. *Fungal Genet Biol* **42**: 657–675.
- Heidler SA & Radding JA (2000) Inositol phosphoryl transferases from human pathogenic fungi. *Biochim Biophys Acta* **1500**: 147–152.
- Hochstenbach F, Klis FM, van den Ende H, van Donselaar E, Peters PJ & Klausner RD (1998) Identification of a putative alpha-glucan synthase essential for cell wall construction and morphogenesis in fission yeast. *Proc Natl Acad Sci USA* **95**: 9161–9166.
- Hu Y & Walker S (2002) Remarkable structural similarities between diverse glycosyltransferases. *Chem Biol* **9**: 1287–1296.
- Huang C, Nong SH, Mansour MK, Specht CA & Levitz SM (2002) Purification and characterization of a second immunoreactive mannoprotein from *Cryptococcus neoformans* that stimulates T-cell responses. *Infect Immun* **70**: 5485–5493.
- Idnurm A, Reedy JL, Nussbaum JC & Heitman J (2004) *Cryptococcus neoformans* virulence gene discovery through insertional mutagenesis. *Eukaryot Cell* **3**: 420–429.
- Jacobson ES, Ayers DJ, Harrell AC & Nicholas CC (1982) Genetic and phenotypic characterization of capsule mutants of *Cryptococcus neoformans*. *J Bacteriol* **150**: 1292–1296.
- Janbon G, Himmelreich U, Moyrand F, Improvisi L & Dromer F (2001) Cas1p is a membrane protein necessary for the O-acetylation of the *Cryptococcus neoformans* capsular polysaccharide. *Mol Microbiol* **42**: 453–467.
- Kang JY, Hong Y, Ashida H, Shishioh N, Murakami Y, Morita YS, Maeda Y & Kinoshita T (2005) PIG-V involved in transferring the second mannose in glycosylphosphatidylinositol. *J Biol Chem* **280**: 9489–9497.
- Katayama S, Hirata D, Arellano M, Perez P & Toda T (1999) Fission yeast alpha-glucan synthase Mok1 requires the actin cytoskeleton to localize the sites of growth and plays an essential role in cell morphogenesis downstream of protein kinase C function. *J Cell Biol* **144**: 1173–1186.
- Kikuchi N, Kwon YD, Gotoh M & Narimatsu H (2003) Comparison of glycosyltransferase families using the profile hidden Markov model. *Biochem Biophys Res Commun* **310**: 574–579.
- Klis FM, Mol P, Hellingwerf K & Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **26**: 239–256.
- Klutts JS, Lavery SB & Doering TL (2005) A beta-1,2 xylosyltransferase from *Cryptococcus neoformans*. *6th International Conference on Cryptococcus and Cryptococcosis*. Boston, MA.
- Kraus PR, Fox DS, Cox GM & Heitman J (2003) The *Cryptococcus neoformans* MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. *Mol Microbiol* **48**: 1377–1387.
- Leipelt M, Warnecke D, Zahringer U, Ott C, Muller F, Hube B & Heinz E (2001) Glucosylceramide synthases, a gene family responsible for the biosynthesis of glucosphingolipids in animals, plants, and fungi. *J Biol Chem* **276**: 33621–33629.
- Lavery SB, Toledo MS, Doong RL, Straus AH & Takahashi HK (2000) Comparative analysis of ceramide structural modification found in fungal cerebroside by electrospray tandem mass spectrometry with low energy collision-induced dissociation of Li⁺ adduct ions. *Rapid Commun Mass Spectrom* **14**: 551–563.
- Levitz SM, Nong S, Mansour MK, Huang C & Specht CA (2001) Molecular characterization of a mannoprotein with homology to chitin deacetylases that stimulates T cell responses to *Cryptococcus neoformans*. *Proc Natl Acad Sci USA* **98**: 10422–10427.
- Li RK & Rinaldi MG (1999) *In vitro* antifungal activity of nikkomycin Z in combination with fluconazole or itraconazole. *Antimicrob Agents Chemother* **43**: 1401–1405.
- Liu J & Mushegian A (2003) Three monophyletic superfamilies account for the majority of the known glycosyltransferases. *Protein Sci* **12**: 1418–1431.
- Loftus BJ, Fung E, Roncaglia P, *et al.* (2005) The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. *Science* **307**: 1321–1324.
- Luberto C, Toffaletti DL, Wills EA, Tucker SC, Casadevall A, Perfect JR, Hannun YA & Del Poeta M (2001) Roles for inositol-phosphoryl ceramide synthase 1 (IPC1) in pathogenesis of *C. neoformans*. *Genes Dev* **15**: 201–212.
- Maligie MA & Selitrennikoff CP (2005) *Cryptococcus neoformans* resistance to echinocandins: (1,3)beta-glucan synthase activity is sensitive to echinocandins. *Antimicrob Agents Chemother* **49**: 2851–2856.
- Mansour MK, Schlesinger LS & Levitz SM (2002) Optimal T cell responses to *Cryptococcus neoformans* mannoprotein are dependent on recognition of conjugated carbohydrates by mannose receptors. *J Immunol* **168**: 2872–2879.
- McClelland CM, Chang YC & Kwon-Chung KJ (2005) High frequency transformation of *Cryptococcus neoformans* and *Cryptococcus gattii* by *Agrobacterium tumefaciens*. *Fungal Genet Biol* **42**: 904–913.
- van Meer G & Holthuis JC (2000) Sphingolipid transport in eukaryotic cells. *Biochim Biophys Acta* **1486**: 145–170.
- Mouyna I, Fontaine T, Vai M, Monod M, Fonzi WA, Diaquin M, Popolo L, Hartland RP & Latge JP (2000) Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. *J Biol Chem* **275**: 14882–14889.
- Moyrand F, Klapproth B, Himmelreich U, Dromer F & Janbon G (2002) Isolation and characterization of capsule structure mutant strains of *Cryptococcus neoformans*. *Mol Microbiol* **45**: 837–849.
- Moyrand F, Chang YC, Himmelreich U, Kwon-Chung KJ & Janbon G (2004) Cas3p belongs to a seven-member family of capsule structure designer proteins. *Eukaryot Cell* **3**: 1513–1524.

- Munro S (2001) What can yeast tell us about *N*-linked glycosylation in the Golgi apparatus? *FEBS Lett* **498**: 223–227.
- Munro CA & Gow NA (2001) Chitin synthesis in human pathogenic fungi. *Med Mycol* **39**: 41–53.
- Oku M, Warnecke D, Noda T, Muller F, Heinz E, Mukaiyama H, Kato N & Sakai Y (2003) Peroxisome degradation requires catalytically active sterol glucosyltransferase with a GRAM domain. *EMBO J* **22**: 3231–3241.
- Olson GM, Wang P, Fox DS & Buchanan KL (2005) Identification and characterization of mannosyltransferase mutants of *Cryptococcus neoformans*. *6th International Conference on Cryptococcus and Cryptococcosis*. Boston, MA.
- Oscarson S, Alpe M, Svahnberg P, Nakouzi A & Casadevall A (2005) Synthesis and immunological studies of glycoconjugates of *Cryptococcus neoformans* capsular glucuronoxylomannan oligosaccharide structures. *Vaccine* **23**: 3961–3972.
- Patton JL & Lester RL (1991) The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane. *J Bacteriol* **173**: 3101–3108.
- Prill SK, Klinkert B, Timpel C, Gale CA, Schroppel K & Ernst JF (2005) PMT family of *Candida albicans*: five protein mannosyltransferase isoforms affect growth, morphogenesis and antifungal resistance. *Mol Microbiol* **55**: 546–560.
- Qasba PK, Ramakrishnan B & Boeggeman E (2005) Substrate-induced conformational changes in glycosyltransferases. *Trends Biochem Sci* **30**: 53–62.
- Reese AJ & Doering TL (2003) Cell wall alpha-1,3-glucan is required to anchor the *Cryptococcus neoformans* capsule. *Mol Microbiol* **50**: 1401–1409.
- Rittershaus PC & Del Poeta M (2005) Sphingolipid-mediated host–pathogen interaction: the role of ceramides. *6th International Conference on Cryptococcus and Cryptococcosis*. Boston, MA.
- Ruiz-Herrera J & San-Blas G (2003) Chitin synthesis as target for antifungal drugs. *Curr Drug Targets Infect Disord* **3**: 77–91.
- Samuelson J, Banerjee S, Magnelli P, Cui J, Kelleher DJ, Gilmore R & Robbins PW (2005) The diversity of dolichol-linked precursors to Asn-linked glycans likely results from secondary loss of sets of glycosyltransferases. *Proc Natl Acad Sci USA* **102**: 1548–1553.
- Shahinian S & Bussey H (2000) Beta-1,6-Glucan synthesis in *Saccharomyces cerevisiae*. *Mol Microbiol* **35**: 477–489.
- Singleton DR, Masuoka J & Hazen KC (2005) Surface hydrophobicity changes of two *Candida albicans* serotype B *mnn4delta* mutants. *Eukaryot Cell* **4**: 639–648.
- Sommer U, Liu H & Doering TL (2003) An alpha-1,3-mannosyltransferase of *Cryptococcus neoformans*. *J Biol Chem* **278**: 47724–47730.
- Stasyk OV, Nazarko TY, Stasyk OG, Krasovska OS, Warnecke D, Nicaud JM, Cregg JM & Sibirny AA (2003) Sterol glucosyltransferases have different functional roles in *Pichia pastoris* and *Yarrowia lipolytica*. *Cell Biol Int* **27**: 947–952.
- Strahl-Bolsinger S, Gentsch M & Tanner W (1999) Protein O-mannosylation. *Biochim Biophys Acta* **1426**: 297–307.
- Stratford M (1992) Yeast flocculation: receptor definition by *mnn* mutants and concanavalin A. *Yeast* **8**: 635–645.
- Thompson JR, Douglas CM, Li W, Jue CK, Pramanik B, Yuan X, Rude TH, Toffaletti DL, Perfect JR & Kurtz M (1999) A glucan synthase *FKS1* homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. *J Bacteriol* **181**: 444–453.
- Uemura S, Kihara A, Inokuchi J & Igarashi Y (2003) Csg1p and newly identified Csh1p function in mannosylinositol phosphorylceramide synthesis by interacting with Csg2p. *J Biol Chem* **278**: 45049–45055.
- Vaishnav VV, Bacon BE, O'Neill M & Cherniak R (1998) Structural characterization of the galactoxylomannan of *Cryptococcus neoformans* Cap67. *Carbohydr Res* **306**: 315–330.
- Warnecke D, Erdmann R, Fahl A, Hube B, Muller F, Zank T, Zahringer U & Heinz E (1999) Cloning and functional expression of UGT genes encoding sterol glucosyltransferases from *Saccharomyces cerevisiae*, *Candida albicans*, *Pichia pastoris*, and *Dictyostelium discoideum*. *J Biol Chem* **274**: 13048–13059.
- Willer T, Amselgruber W, Deutzmann R & Strahl S (2002) Characterization of *POMT2*, a novel member of the PMT protein O-mannosyltransferase family specifically localized to the acrosome of mammalian spermatids. *Glycobiology* **12**: 771–783.
- Wright LC, Payne J, Santangelo RT, Simpanya MF, Chen SC, Widmer F & Sorrell TC (2004) Cryptococcal phospholipases: a novel lysophospholipase discovered in the pathogenic fungus *Cryptococcus gattii*. *Biochem J* **384**: 377–384.