

***The roles of phosphatidylserine and
phosphatidylethanolamine in Candida
albicans cell wall maintenance and
mitochondrial function***

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Abstract

Enzymes involved in phospholipid biosynthesis have been suggested as possible drug targets to treat *Candida* infections because several key enzymes, such as phosphatidylserine synthase, differ from those of the human host. We have found that phosphatidylserine synthase of *C. albicans* is required for virulence in a mouse model of systemic *Candida* infection. In addition, the *C. albicans* phosphatidylserine synthase mutant (*cho1* Δ/Δ) has no detectable phosphatidylserine and loss of phosphatidylserine causes alterations in the fungal cell wall. The *cho1* Δ/Δ mutants' cell wall defects include altered calcofluor white staining, resistance to the cell wall perturbing drug Caspofungin, and an altered physical appearance by transmission electron microscopy. We are using lipidomic analysis and transcriptomics of phosphatidylserine and phosphatidylethanolamine biosynthesis mutants to understand the role that phosphatidylserine and phosphatidylethanolamine play in controlling the cell wall in *C. albicans*. The elucidation of downstream effects of disruption of pathways involved in biosynthesis of phospholipids in *C. albicans* may validate these phospholipid biosynthesis enzymes as plausible anti-fungal drug targets. The role of phosphatidylserine and phosphatidylethanolamine in the maintenance of the fungal cell wall will also be of interest in determining how phospholipids impact signaling pathways and affect cell wall composition and architecture.

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CHAPTER ONE

Background and Introduction

Abstract

Candida albicans remains a major fungal pathogen of humans. The role of phosphatidylserine and phosphatidylethanolamine in the maintenance of the fungal cell wall will be of interest in determining how phospholipids impact signaling pathways and affect cell wall composition and architecture. It is in this light that the examination of basic metabolic processes such as cell wall generation and lipid biosynthesis may be useful in finding new therapeutic agents to treat *Candida* infections and understand the host pathogen relationship.

Introduction

Candida albicans is a leading cause of fungal infections in humans and fourth most common cause of nosocomial infections in the United States (Edmond et al., 1999). The immunocompromised, such as organ transplant and cancer patients are at particularly high risk of developing life threatening *Candida* infections. The mortality rate of these systemic fungal infections can reach as high as 40% (Pittet & Wenzel, 1995). The ability to successfully treat this fungal pathogen relies on the continued elucidation of drug targets as drug resistance remains a serious threat (Chiller et al., 2012). It is in this light that the examination of basic metabolic processes such as cell wall generation and lipid biosynthesis may be useful in finding new therapeutic agents to treat *Candida* infections.

Diseases caused by *Candida albicans*

Candida albicans normally resides in the gastrointestinal tract of healthy individuals and can establish an infection when the host defenses are compromised. Oropharyngeal candidiasis is the most common fungal infection in immunocompromised patients. This disease can present itself in the form of pseudomembranous candidiasis characterized by presence of white plaques or xerostomia in nearly 30% of HIV-infected patients (Calderone 2002; Cauda et al., 2012). Esophageal candidiasis occurs in patients undergoing treatments for chronic illnesses and frequently occurs in patients with advanced HIV infections. *C. albicans* accounts for more than 50% of vulvovaginal candidiasis episodes and 75% of women experience at least one episode of this disease in their lifetime (Cauda et al., 2012; Calderone 2002). *C. albicans* can also cause superficial infections of the skin and mucous membranes. Mortality rates associated with systemic candidiasis (bloodstream infections) is high and at least 10% of all bloodstream infections are caused by *Candida* species (Pittet, D & Wenzel, R.P. 1995).

C.albicans infections are a consequence of both the virulence properties of this organism enabling it to cause invasive infections and the immune status of the host (Singh et al., 2007). *C.albicans* is a successful commensal of the human host and is not readily eliminated since it has evolved to persist on host mucosal surfaces and utilize host resources, causing disease when host immune deficiencies occur (Cauda et al., 2012). Treatment of candidiasis is hindered by several factors, including the limited number of antifungals and, the emergence of drug resistant isolates (Morschhauser J et al., 2012).

Virulence properties of *Candida albicans*

The virulence of *Candida albicans* is mediated by a series of factors related to its ability to survive and colonize the human host (Gow et al., 2011). The ability to survive in the host is a complex issue for *C. albicans* in that it must not only acquire essential nutrients from the host, but must also evade attack and avoid clearance by the host immune system (Brown A.J. et al., 2012). Some virulence attributes of *C.albicans* include production of adhesins (which promote host recognition and colonization) secretion of proteases and phospholipases (which are digestive enzymes that aid in nutrient acquisition and invasion), and phenotypic switching associated with invasiveness of the organism (Calderone, 2002).

Many of the studies on virulence properties of *C.albicans* have been aided by gene deletion studies that result in the avirulence of the organism in mucosal and systemic animal models of candidiasis. These studies provide direct evidence that specific proteins are important in host pathogenesis. A thorough understanding of how these virulence factors contribute to disease progression, and pathogenesis is crucial so newer drugs can be designed to inhibit these factors and thus prevent colonization or persistence of the organism in the host.

Adhesins

One central problem the fungus must encounter in the host is how to adhere to the host when it comes into contact with host tissues and mucosal surfaces. Adherence to host tissues is important for disease progression, and *Candida albicans* is able to adhere to host surfaces via production of adhesins (Udea M et al., 2012). These are surface-bound polysaccharides or glycoproteins that bind to specific ligands on the host cell surface. Adhesins were mainly identified by studies in which the encoding gene was deleted, and the mutant was evaluated for its adherence to host cell monolayers *in vitro*. A number of adhesins have been identified in the organism including, but not limited to – complement receptors, fibrinogen-binding protein, integrins, lectins and proteins that bind to host proteins such as N-cadherins (Hube et al., 2011).

The importance of adhesins in *C. albicans* pathogenesis is highlighted by the contribution of adhesins to binding of plastics and biofilm formation (Filler et al., 2011). Systemic infections are usually established by contamination of indwelling catheters where (in the case of *C. albicans*), yeast cells adhere to the catheter and form a thin film which later develops into a thick layer that consists predominantly of hyphal cells. Biofilms are inherently resistant to the penetration of drugs and thus confer resistance to their inhabitants, and moreover the biofilm provides a means for the constant flow of yeast cells from the film into the bloodstream, thus further contributing to disease (Williams C et al., 2012).

Phenotypic Switching

C. albicans can exist in three different morphological forms – yeast cells, pseudohyphal cells and true hyphae. Phenotypic switching refers to the spontaneous interchange between these three different morphological states. Initially, it was believed that virulence of this organism was dependent on the hyphal form which can penetrate and invade host tissues better than the yeast form (Sherwood et al., 1992). However, the yeast cells are better at dissemination and all

three morphological forms have been identified in tissues infected with *C.albicans* (Odds, F. 1988), and these transitions are thought to promote and reflect the different stages of disease progression (Odds, F. 1994). Morphological transitions are triggered in response to growth conditions. Hyphal growth is promoted at temperatures above 35°C, pH 7, nutrient limitations, especially carbon and nitrogen starvation, presence of serum, non-fermentable carbon sources, and low oxygen concentrations (Odds, F. 1988). These conditions are presumed to affect the signals that regulate cell morphological transitions. Identification of growth-form specific genes can provide valuable insights into regulation of morphogenesis and the signaling pathways involved.

Signaling pathways

Signaling pathways that regulate morphogenetic transitions are thought to contribute to the virulence of *Candida albicans* by controlling different stages of disease establishment and progression (Brown, A.J. & Gow, N.A., 1999). In the host, *C.albicans* can survive in all three morphological forms, using environmental cues to dictate which form is more favorable in the particular host niche. Identification of the regulatory circuits that facilitate these transitions is essential to obtain a detailed understanding of morphogenesis. Many signaling pathways have been identified in the fungus, such as the Efg1 pathway (Lengeler et al., 2000), the MAP kinase pathway and a pH signaling pathway (Davis et al. 2010). Inactivation of signaling components have shown to attenuate virulence in animal models presumably by interfering with expression of virulence factors and thus influencing disease progression.

Many components of the signaling pathway have been identified using *Saccharomyces cerevisiae* as a model fungus, since there is a high degree of sequence conservation with the signaling cascades between these distantly related species (Gustin et al., 1998; Banuett, F.

1998)). Moreover, the ease of conducting genetic screens in *S.cerevisiae* has made this organism more suitable for dissecting molecular mechanisms specific to signaling.

Proteases

Secreted aspartyl proteases (Sap) are secreted proteins that can proteolytically digest exogenous host proteins for the simple purpose of acquiring nutrients (Fusek et al., 1994). The digestion of host proteins aids in host tissue colonization by digesting host cell membranes, thus facilitating host surface adhesion and penetration into tissues. In addition, secretion of proteases can also help evade host defenses by damaging cells and molecules of the host immune system. The Sap family consists of 10 genes with distinct roles in *C.albicans* virulence. Sap antigens have been detected in the sera of patients with candidiasis indicating an important role for Saps during infection (Ruchel, R. 1992; Ruchel et al., 1991). Sap genes are differentially expressed *in vitro*, for example, some SAPs are expressed only in hyphal cells (SAP 4-6)(Schaller et al., 1998), some were found both on yeast and hyphal cells. Sap1- Sap3 contributes to tissue damage (Schaller et al., 1998), Sap 1, 2 and 8 are more prevalent in vaginal infections (De bernadis et al., 1995). Some studies have suggested that early during systemic infection, SAP4-6 are important for invasion, and once the infection is established, SAP1-3 plays an important role (Staib et al.,2000). More compelling evidence for the role of Saps during infection comes from studies that demonstrate that *C.albicans* mutants lacking SAP genes are attenuated for virulence (Schaller et al, 1999). Moreover inhibition of proteinase activity using inhibitors prevented initial colonization of mucosal surfaces (Fallon et al., 1997). Thus, Saps contribute to the overall virulence of *C.albicans* by hydrolyzing host proteins, proteins of the immune system thus promoting colonization, persistence and dissemination of infection.

Phospholipases

Phospholipases are enzymes that hydrolyze ester linkages in glycerophospholipids that have been implicated in host penetration, and interaction with signal transduction pathways.

C.albicans has two phospholipases, encoded by *PLB1* and *PLB2* (Hube, B 1998; Sugiyama et al., 1999). These enzymes are categorized as virulence factors since PLB antigens have been detected in the sera of patients suffering from candidiasis and they are expressed. Mutants lacking phospholipases are significantly attenuated in virulence using animal models for systemic infections (Leidich et al., 1998; Ghannoum, M.A. 2000).

Lipases

Lipases are the least characterized of all the secreted hydrolases in *C.albicans*. Lipases are encoded by a family of 10 genes, *LIP1-LIP10* (Hube et al., 2000). Expression of these genes is usually induced in media containing lipids such as Tween 40 (Fu et al., 1997), but can be induced in a lipid independent manner too, suggesting lipases may fulfill roles other than acquisition of nutrients in *C.albicans*. Some lipases were found to be expressed during infection in mice further providing evidence that lipases may contribute to the survival and virulence of *C.albicans* in the host (Goldman et al., 2011).

Drug Targets in *Candida albicans*

Elucidating the complex interaction of *Candida albicans* with the host during infections and identifying key genes or enzymes that can assist the fungus in the colonization, persistence and invasion of tissues can lead to the development of new therapeutic strategies, through targeting these genes/ enzymes, thus extending the limited repertoire of antifungal drugs currently available. In this section, some of the currently exploited drug targets will be described with a

brief overview of potential enzymatic or biosynthetic pathways that can provide attractive targets for the development of antifungals.

Cell Wall

The cell wall is critical to the interaction of *Candida albicans* with the host, since it the outermost protective structure for the organism. The *C.albicans* cell wall is a complex, dynamic structure that undergoes significant changes in composition during growth. The different morphological forms of the organism differ in the composition of cell wall components and are also associated with differential regulation of virulence factors and proteins. The cell wall contributes not only to the cell morphology, but also protects the cell against physical, osmotic and oxidative stress and, it promotes cell-cell interactions, adherence to host surfaces, and modulates the immune response (Ruiz-Herrera et al., 2006).

The cell wall (Fig:1-1) consists of 80-90% carbohydrate in the form of glucan (which are linear and branched polymers of glucose); β 1,4 *N*-acetylglucosamine (chitin) and mannose polymers. Chitin and glucans contribute to the rigidity and morphology of the cell wall (Ruiz-Herrera et al., 2006).

Chitin is synthesized on the cytoplasmic side of the cell membrane, and then gets extruded to the cell surface where it crystallizes through extensive hydrogen bonding. Chitin synthases catalyze the polymerization of *N*-acteylglucosamine (Bulawa, C. E et al., 1993). Chitinase contributes to the maintenance and plasticity of the yeast cell wall (Cabib E et al, 1987) and disrupting the chitin synthase gene (*CTS1*) in *S. cerevisiae*, results in clumping of the cells and failure to separate following cell division (Kuranda and Robbins 1991). Thus chitin synthase are potential drug targets owing to their essential role in cell wall maintenance.

Glucans are arranged as β -(1,3)-linked residues with β -(1,6)- side chains. The enzyme that catalyzes the polymerization of glucans is glucan synthase, which links glucan synthesis to the cell cycle (Kang and Cabib 1986).

Mannoproteins comprise important constituents of the cell wall that determine the surface properties of the cell, facilitating adhesion and interaction of *C.albicans* with the host (Chaffin et al., 1998). These are proteins that are covalently attached to the mannose polymers that account for about 40% of the total cell wall polysaccharide. Mannoproteins are immunogenic, with each branch of the polymer corresponding to a specific epitope. The cell wall also consists of glycosylphosphatidylinositol (GPI) anchored mannoproteins that are covalently linked to β -1,6 glucan via a covalent bond between the GPI remnant and β -1,6 glucan (Klis et al., 2001). Some mannoproteins can be connected via disulfide bridges to other proteins in the cell wall (Boisrame et al., 2011; Casanova et al., 1989) or can be non-covalently attached to the cell wall via hydrogen bonds or hydrophobic interactions (Valentin et al., 1984).

The cell wall consists of the following layers: The inner layer- adjacent to the plasma membrane is composed of chitin and glucan polysaccharides. This is followed by a three dimensional matrix of β -1,3 glucans , to which cell wall proteins are linked via GPI anchors. Mannoproteins are found adjacent to the membrane and in the outermost layer and can be covalently linked to glucan chains. β -1,6 glucans act as an interconnector for most of the cell wall components. Other proteins called 'proteins with internal repeats' (PIR coded proteins) are covalently linked to the β -1,3-glucan scaffold.

GPI anchored proteins account for about 88% of the covalently linked cell wall proteins in *C.albicans* (Kapyten et al., 2000). GPI anchored proteins play an important role in cell wall synthesis and in modifying cross-linked polymers and thus play an integral role in governing the physiology and pathogenicity of *C.albicans* (Richard and Plaine, 2006). Proteins that are linked

to β -1,6 glucan are incorporated into the wall by a transglycosidation reaction between the glucan moiety of GPI and β -1,6 glucan (Kapyten et al., 1995; 1997). GPI can either be present on the cell surface by itself or it can be transported to the cell surface if it is covalently attached to a protein via the secretory pathway. Several genes required for GPI biosynthesis have been identified in *Saccharomyces cerevisiae*. Mutants lacking some of these biosynthetic genes have a fragile cell wall (Benachour et al., 1999), aberrant cell morphologies (Leidich et al., 1994) or have defects in GPI anchoring (Gaynor et al., 1999). *GPI* mutants also exhibit an altered composition of their cell wall (Vossen et al., 1997) and increased sensitivity to cell-wall perturbing agents (Benghezal et al., 1995; Vossen et al., 1995). Thus disruption of GPI biosynthesis can affect GPI-dependent transport of enzymes involved in cell wall assembly, and other cell wall proteins. It can also abolish GPI-dependent cross-linking of glycoproteins to the cell wall (Ram et al., 1994; Richard et al., 2002) thereby affecting cell wall organization (Fonzi 1999; Ram et al., 1998).

Since mammalian cells lack a cell wall, enzymes responsible for its structural organization or for the biosynthesis of cell wall components provide a convenient target for antifungal drugs. Few drugs have been developed that target the cell wall, these include: Nikkomycin Z and the Echinocandin class of drugs.

Nikkomycin Z inhibits chitin synthase activity and thus interferes with the chitin content in the cell wall. Echinocandins such as caspofungin and micafungin inhibit glucan synthase thereby affecting the assembly of β -glucan polymers in the cell wall. Both these classes of drugs display fungicidal activity against *C.albicans* and are effective in animal models (Calderone, 2002).

Differences in GPI biosynthesis between mammalian and yeast cells (Grimme et al., 2001; Smith et al., 1997) can also be exploited to design drugs that inhibit fungal-specific GPI biosynthetic enzymes. Some proteins that are tethered to the wall via GPI anchors are important

for adhesion to host cells and thus impact the pathogenesis and virulence of *C.albicans*. Thus, any defects in the biosynthesis of GPI anchors can have detrimental effects of the pathogenesis of *C.albicans* (Richard, M.L. and Plaine, A. 2006; Martinez-Lopez 2004; Richard et al 2002). GPI anchors also play a contributing role in the morphogenesis of *C.albicans*, and resistance to macrophages and/or oxidative stress (Richard et al., 2002). GPI proteins have also been implicated in pivotal processes such as adhesion, biosynthesis and regeneration of the cell wall and some have enzymatic functions (Klis et al., 2009; Richard, M.L. and Plaine, A 2007). Several GPI proteins have also shown to affect the virulence of *C.albicans* using a murine model of systemic candidiasis (Gelis et al., 2012; De Boer et al., 2010; Martinez-Lopez et al., 2004; Laforet et al., 2011). For example, the GPI-anchored protein Hwp1 is important for host cell attachment (Hoyer, L 2001; Staab et al., 1999). Thus considering the essential role for GPI-anchored proteins in cell wall reconstruction (Fonzi, W.A. 1999; Grimme et al., 2004), yeast-hyphal transitions, and adhesion (Castano et al., 2006; Staab et al., 1999) or virulence properties of *C.albicans* (Martinez-Lopez et al., 2004; Pardini et al., 2006, Staab et al., 1999), a better understanding of the functions of these proteins and how they influence other signaling and biosynthetic pathways and interactions with the host is needed. This could extend the number of potential targets for therapeutic treatment of *C.albicans*.

Phospholipid Biosynthesis

Phospholipids fulfill a number of vital roles in fungi. For example, in *S. cerevisiae*, they are important for cell biology and have roles in signaling and regulatory functions (Strahl and Thorner 2007), membrane trafficking (Vicinanza et al., 2008), and, anchoring membrane proteins (Roth et al., 2006; Pittet and Conzelmann 2007) as well as contributing to the membrane structure. Thus, it would be of interest to elucidate the role of phospholipids in the survival of *C.albicans* in the host and in its pathogenesis. The major phospholipids in yeast include phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC) and

phosphatidylethanolamine (PE) (Ratray et al., 1975; Paltauf et al., 1992; Guan and Wenk 2006).

In yeast, phospholipid biosynthesis (Fig:1-2) is initiated from phosphatidic acid (PA), which is partitioned to cytidyldiphosphate-diacylglycerol (CDP-DAG) or DAG by either CDP-DAG synthase (Carter and Kennedy 1966) or PA phosphatase (Han et al., 2006) respectively. CDP-DAG and DAG are then used to synthesize PE and PC by two alternative pathways (Fig:1-2): the CDP-DAG pathway and the Kennedy pathway. CDP-DAG is converted to PS by PS synthase (encoded by the *Cho1p* enzyme) in the CDP-DAG pathway. This process takes place in the endoplasmic reticulum (Atkinson et al., 1980; Letts et al., 1983; Kiyono et al., 1987; Bae-Lee and Carman 1984). The PS decarboxylases, the inner mitochondrial membrane-localized *Psd1* and the Golgi-localized *Psd2*, convert PS to PE (Clancey et al., 1993; Trotter et al., 1995; Voelker 2003). PE is then converted to PC by three sequential methylation reactions in the ER catalyzed by PE methyltransferase (encoded by *CHO2*) and phospholipid methyltransferase (*OPI3* encoded) (Kodaki and Yamashita 1987; Summers et al., 1988; McGraw and Henry 1989). Mutants that are defective in either of these pathways have choline/ethanolamine auxotrophies (Atkinson et al., 1980; Trotter and Voelker 1995; McGraw and Henry 1989).

The Kennedy pathway relies on the exogenous transport of ethanolamine and choline by a transporter encoded by *HNM1* (Nikawa et al., 1986). Cytoplasmic located ethanolamine kinase (*Eki1*) and choline kinase (*Cki1*) phosphorylate these with ATP to form ethanolamine-P and choline-P (Kim et al., 1999; Hosaka et al., 1989). These intermediates are activated by cytidylyltransferases to form CDP-ethanolamine or CDP-choline (Min-Seok et al., 1996; Tsukagoshi et al., 1987). Phosphotransferases (Hjelmstad and Bell 1988,1990,1991) then condense CDP-ethanolamine and CDP-choline with DAG to form PE and PC.

The synthesis of phospholipids in fungi is similar to that in mammalian cells, the only difference being in the synthesis of PS. In mammals, PS is synthesized from PE or PC and serine, and in fungi it is synthesized from CDP-DAG. This difference can be exploited if phospholipid biosynthesis is to be explored as a viable antifungal target. Given the ubiquitous presence of phospholipids in the cell, it is not surprising that defects in phospholipids biosynthesis can have adverse effects on the growth and proliferation of *C.albicans*. In *S.cerevisiae*, PE has been shown to play an important role in stabilizing mitochondrial protein complexes (Birner et al., 2003; Osman et al., 2009). This results in the mutant being non-viable with defects in protein and lipid scaffolding complexes (Osman et al., 2009). This in turn has dire effects on the functionality and integrity of the mitochondrial inner membrane (van Gestel et al., 2010).

Phospholipids in virulence: The *C.albicans cho1Δ/Δ* mutant is avirulent in a mouse model of systemic candidiasis (Chen et al., 2010). Loss of de novo PE (through disruption of *PSD1* and *PSD2*) also attenuates the virulence of *C.albicans*. These mutants exhibit cell wall defects as demonstrated by an increased sensitivity to caspofungin and resistance to caffeine (Chen et al., 2010). Osmostabilizers such as CaCl₂, sorbitol and NaCl rescue the growth defect of the *cho1Δ/Δ* and the *psd1Δ/Δpsd2Δ/Δ* mutants on YPD medium suggesting that loss of PE and PS may affect the cell wall (Chen et al. 2010). These findings are further supported by the observation of a thick, irregular cell wall by transmission electron microscopy in the *cho1Δ/Δ* strain (Chen et al., 2010). Furthermore, defects in PE biosynthesis also impacted the function of mitochondria, and this is not surprising since mitochondria are enriched in PE as it is the main site of de novo PE biosynthesis. Mitochondrial defects in the PS and PE deficient mutants have been demonstrated by an increase in resistance to paraquat (Chen et al., 2010). Paraquat binds to complex I in the electron transport chain leading to the generation of superoxide radicals and subsequent oxidative damage in cells with an intact mitochondrial membrane. Thus, resistance to paraquat is indicative of a defect in the respiratory chain (Cocheme et al., 2008; Blaszczyński

et al., 1985) more specifically, a defect in oxidative phosphorylation in *C.albicans* (Cheng et al., 2007). Mitochondrial respiration defects are further highlighted by the fact that these mutants take up trypan blue and eosin Y dye more readily than the wild-type strain (Chen et al., 2010; Nagai, S 1963).

Given these findings, it is clear that phospholipids play vital roles in the cell biology of *C.albicans*, but significant gaps lie in our understanding the contribution of phospholipid metabolic pathways in membrane biogenesis, signaling and cell cycle transitions. A genome-wide approach is thus required to gain a global understanding of the different regulatory networks and cell processes affected by the loss of PS and *de novo* PE.

Phospholipid structures and polymorphisms: Phospholipid species have been classified as non-lipid bilayer and lipid bilayer forming due to the cross-sectional area and shape of their respective headgroups and acyl side chains (Frolov et al. 2011; Cullis et al. 1986). Lipid bilayer forming species, such as PS and PC, are ideally suited to maintain a rigid membrane structure, whereas, PE forms a conical shape with a small headgroup and large hydrophobic tail and is thereby conducive to increasing membrane fluidity and curvature (Figure 1-3) (Cullis et al. 2002, Haque et al., 2001). A mixture of bilayer and non-bilayer forming phospholipids enables association of the membrane into a semi-permeable barrier and allows appropriate distribution for the function of integral membrane proteins (Cullis et al 1986, 2002). The dynamic nature of the lipid bilayer is evidenced by the effect of changes in tail acyl chain lengths and saturation upon cell stresses such as temperature, divalent cation concentration, and membrane disrupting detergents (Tumolo et al., 2011). Generally, the higher the saturation number and shorter the hydrocarbon length within the acyl chains, the more disordered and less rigid the membrane becomes and membrane fluidity is, therefore, increased (Cullis et al. 1986; Lindholm et al., 2004). Diversity in lipid species and function in the membrane is complicated by the presence of over 100 different lipid species in a eukaryotic membrane (Gerrit van Meer et al., 2011). The

dynamics of lipid fluidity involve the lateral displacement of lipid acyl tails and interactions of the headgroups between phospholipids and are dependent upon the phospholipid headgroup, saturation number, chain length, hydration state, protein interactions, cationic environment and interaction with other lipids such as cholesterol or ergosterol (Rajendran et al 2005; Tumolo et al., 2011).

These dynamic interactions are key to a variety of cellular processes including exocytosis and endocytosis which are thought to be at least partially mediated by the presence of cone shaped PE molecules (Farge et al., 1999). PS, the least abundant of the phospholipids, can be negatively charged due to the presence of an electronegative oxygen atom on its serine residue which affects its molecular properties such as its ability to interact with other lipid headgroups (Cullis et al. 1986; Marsh et al. 2012) at the surface of the membrane and this in turn may affect lateral diffusion of side chain packing within the membrane. At pH below 4.0, PS becomes protonated and loses its electrorepulsive properties that may result in aberrant charge distribution at the membrane surface and it is hypothesized that this facilitates improper lipid spacing and can lead to a disordered membrane consequently disrupting binding of proteins at the surface of the membrane (Cullis et al., 2002; Marsh et al 2012). PS has also been described as a co-factor for signaling pathways in fungi such as for protein kinase C (PKC) (Kamada et al., 1996) which controls cell wall integrity, as well as in calcium mediated signaling via calcineurin, which controls internal calcium levels (de Lucena et al., 2012). The diversity of lipid species, their precise functioning, and how they mediate membrane- structure function is an area of increasing interest that requires close evaluation as much remains to be known about the properties that phosphatidylserine (PS), phosphatidylethanolamine(PE) ,and phosphatidylcholine(PC) contribute to lipid interactions and how these interactions ultimately affect the cell.

Iron Sensing and Uptake

Candida albicans can colonize and grow in a variety of host environments (Calderone and Fonzi, 2001). Deep-seated tissues and organs, such as the kidneys, and mucosal surfaces like the oral and vaginal cavities, present unique environmental niches in terms of the nutrients available for uptake by microbial colonizers. Iron is an important co-factor for essential cellular processes and is present in very low amounts in the bloodstream ($\sim 10^{-24}$ M free iron) and deep tissues. The need to scavenge iron from the host has led to the evolution of distinct iron sensing and acquisition pathways in *C. albicans*. In addition, iron acquisition and utilization is tightly regulated as the organism constantly shuttles between iron -replete and -limited environments in the host, since high amounts of it can be toxic and low amounts of iron can shut down respiration and other cellular processes. In fact, in a variety of both prokaryotic and eukaryotic pathogens the ability to acquire and store iron has been shown to be an important virulence factor.

The more robust regulation of iron in *C. albicans* compared to that of *S. cerevisiae* is hypothesized to be a direct result of the environments that *C. albicans* must encounter upon infection of the host (Chen et al., 2011). Iron uptake systems have been well characterized in *S. cerevisiae* and many orthologues of the iron-import system exist in *C. albicans* (Eck et al., 1999; Ramanan and Wang, 2000; Weissman et al., 2002). Three iron-import systems have been identified in *C. albicans*. In the bloodstream, *C. albicans* can directly acquire iron from blood cells by secreting hemolysins (Manns et al., 1994; Luo et al., 2001). Rbt51/Pga10 is strongly induced in iron-depleted conditions and plays a role in heme-iron utilization (Weissman et al., 2002). Rbt5 is another GPI-anchored haem receptor (Weissman and Kornitzer, 2004) that tethers haem at the membrane, followed by uptake or internalization via endocytosis (Weissman et al., 2008). Iron can also be bound to siderophores and taken up via siderophore transporters such as Sit1 (Ardon et al., 2001; Hu et al., 2002). Finally, free iron can be taken up by iron

permeases such as Ftr1 (Knight et al., 2002) following the sequential reduction of Fe^{3+} by cell surface-bound ferric reductases (Hammacott et al., 2000; Knight et al., 2002). The GPI-anchored protein Als3 can also mediate the extraction of iron from host ferritin through a similar reductive system (Almeida et al., 2008).

Iron sensing and response is mediated by Sfu1 and Sef1/2 in *C. albicans*, in contrast to *S. cerevisiae* where ScSef1 acts directly upon ScAft2 to up-regulate iron uptake genes (Chen et al., 2011). Under iron-limited conditions, iron acquisition genes are activated through repression of Sfu1. The transcriptional repressor, Hap43 increases iron availability under these conditions by repressing genes that code for acotinase and cytochrome c both of which are involved in iron and or haeme utilization (Baek et al., 2008; Chen et al., 2011; Hsu et al., 2011; Singh et al., 2011). Sef1 is considered the central iron regulator, as it activates *HAP43* and expression and thus indirectly controls *SFU1* expression. Sef1 also activates several target genes that are regulated by Hap43 and Sfu1 such as HAP1 (Chen et al., 2011) and these genes are involved in iron response or heme biosynthesis (Chen et al., 2011).

Interestingly, it has been shown that *SEF1* is overexpressed in human plasma and is essential for bloodstream infections in a mouse model. In contrast, *SFU1* is up-regulated in the gut, and a *sfu1* Δ/Δ mutant is defective for colonization of the gut (Chen et al., 2011). This differential regulation of these co-regulated iron-response genes in different host niches highlights how *C. albicans* has evolved a tightly regulated iron regulatory circuit to promote its survival in the host. Amongst the genes regulated by Sef1 are several virulence related genes (Chen et al., 2011); including cell wall proteins, transporters and transcription factors.

Analysis of the roles of Sfu1 and Sef1 in *C. albicans* iron regulatory system has revealed that Sfu1 promotes commensalism and Sef1 promotes pathogenesis in the mammalian host (Chen et al., 2011). While Sfu1 promotes competitiveness in the gastrointestinal tract and resistance to

iron toxicity, Sef1 plays a unique role in *C.albicans* that is not conserved in other fungal species, i.e. it controls several virulence related genes. This ability to maintain expression of genes required for the survival of *C.albicans* in the host while continuously repressing toxicity factors further underscores the adaptation that this organism has undergone to promote its existence as both a commensal and pathogen in the iron-limited/ -replete host microniches. Thus any disruption of iron homeostasis in *Candida albicans* may alter the way it senses, responds to and interacts with the host.

Project Overview

In this dissertation, we demonstrate how loss of phosphatidylserine (PS) and *de novo* phosphatidylethanolamine (PE) in *Candida albicans* results in significant changes in the cell wall that can be attributed to the differential regulation of cell wall protein genes, mainly those encoding GPI-anchored proteins, and to dysfunctional mitochondria. Iron homeostasis is also affected in mutants that lack *de novo* PE but the defects are more severe in the *cho1* Δ/Δ strain which also lacks PS, as demonstrated by the up-regulation of iron regulators that function to transcriptionally repress iron-acquisition genes in conditions of low iron availability. Consequently, the mutants fail to survive in iron-limited conditions and this may also in part explain the virulence defects in a mouse model of systemic candidiasis associated with the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ strains. Furthermore, profiling the phospholipidome of these mutants has helped in the identification of key lipids that are altered upon disruption of phospholipid biosynthesis in *C.albicans*. This is the first study that links phosphatidylserine (PS) synthesis to cell wall biogenesis, mitochondrial dysfunction, and iron homeostasis in *C.albicans*.

APPENDIX AI : FIGURES

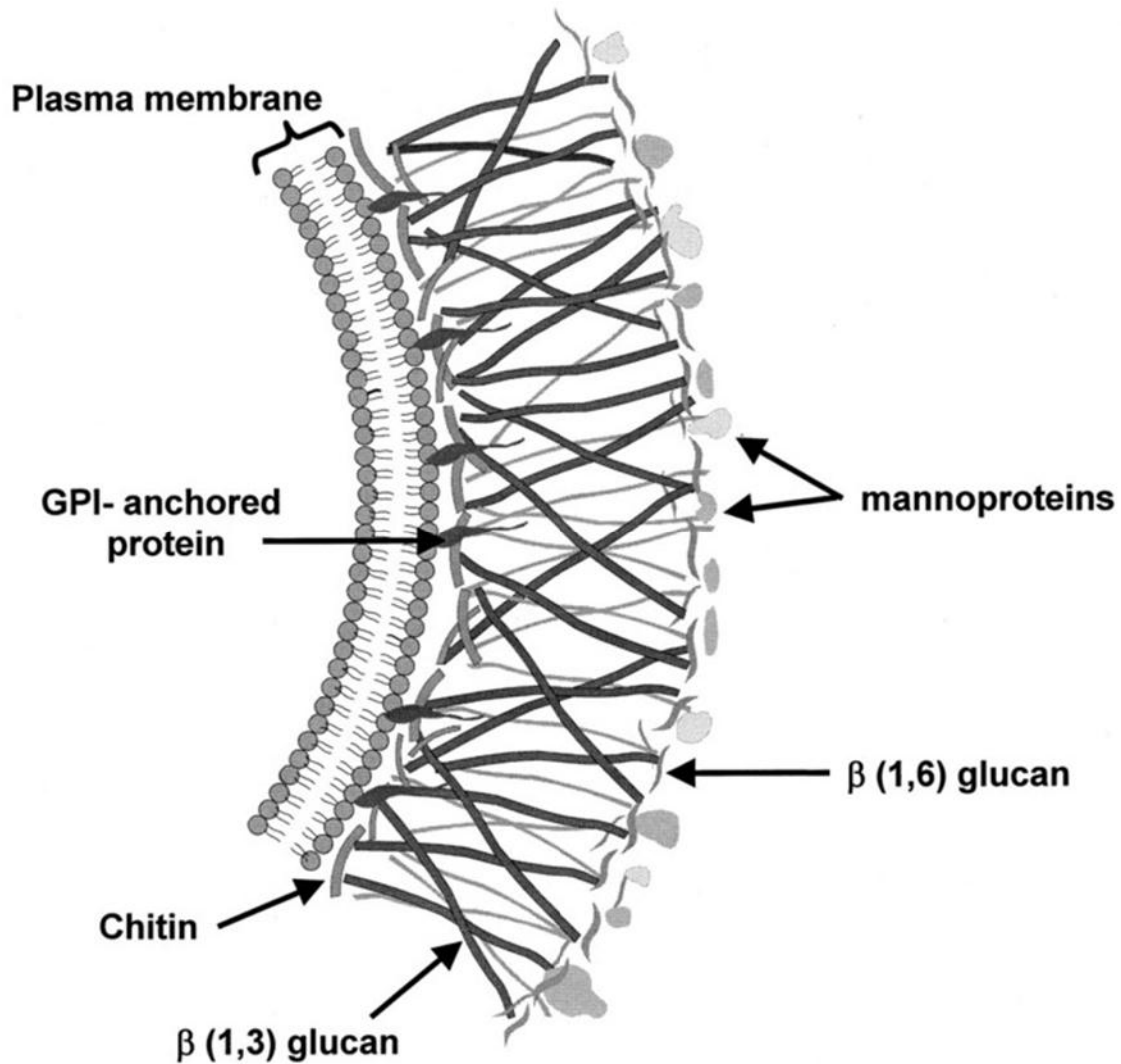


Figure 1-1: The structure of the fungal cell wall with the different polysaccharide and protein components depicted. (Image adopted from science.kennesaw.edu/fungcellwall)

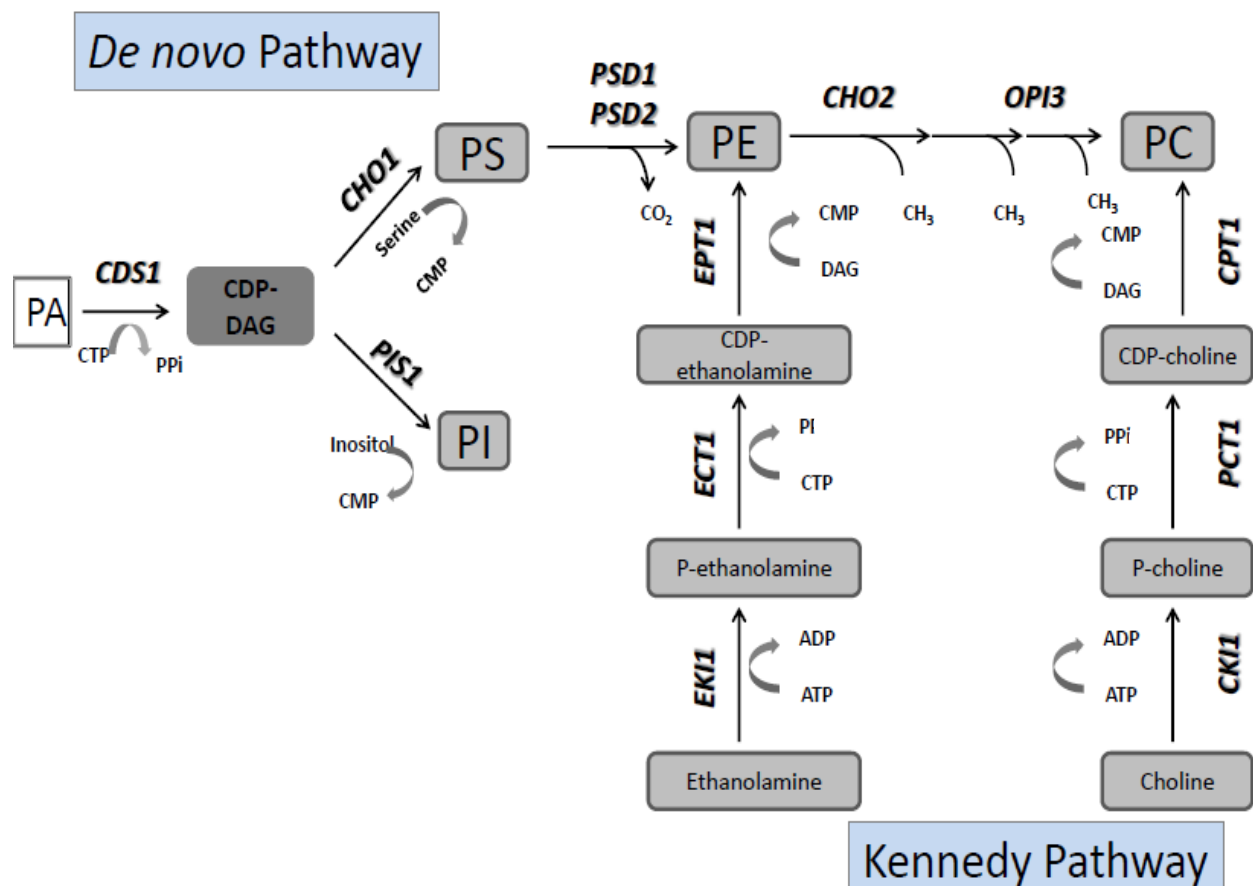


Figure 1-2: The phospholipid biosynthetic pathways in fungi (*Saccharomyces cerevisiae*). Two alternative pathways, the **de novo pathway** and the **Kennedy pathway** exist in both humans and yeast, but there are significant differences in the *de novo* synthesis of PS between yeast and humans. For a detailed explanation of the biosynthesis, refer to the text.

Model of Lipid Shape Polymorphisms

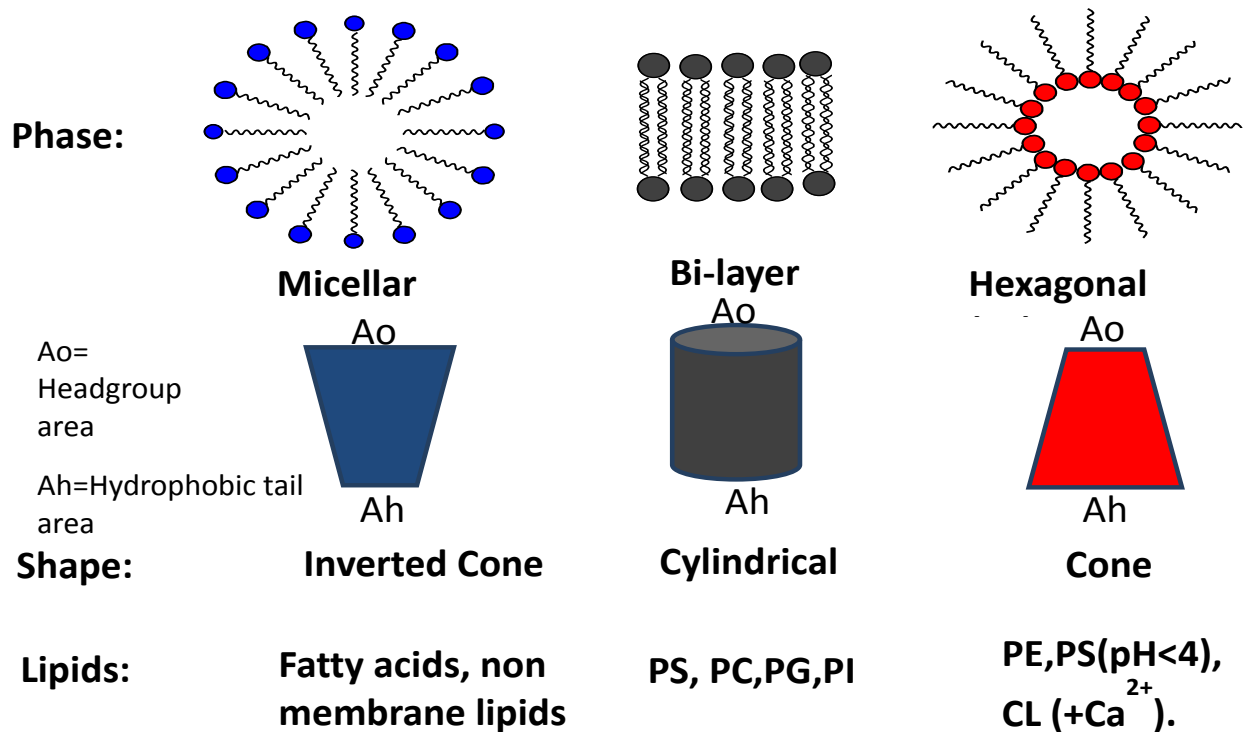


Figure 1-3: Lipid shape polymorphisms influence membrane structure and fluidity. Lipids in the eukaryotic membrane bilayer are a mixture of hexagonal and bilayer phase lipids. The relative amounts of each lipid shape can change the biophysical properties of the membrane and lead to disruption of or localization of membrane proteins and improper lipid acyl tail packing. The shape of the lipid is determined by the cross-sectional area of the headgroup(Ao) versus the cross sectional area of the hydrophobic tail(Ah). $Ao > Ah$ = inverted cone shape, $Ao = Ah$ = cylindrical shape, $Ao < Ah$ = cone shaped. PS= phosphatidylserine, PC=phosphatidylcholine, PE = Phosphatidylethanolamine, PG=phosphatidylglycerol, PI=phosphatidylinositol, CL= cardiolipin. Ionic stress and low pH can influence these properties in PS and CL as indicated (+Ca²⁺ and at pH<4).(Cullis et al., 2001; de Kruijff B, 1997).

CHAPTER TWO

***Candida albicans* *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants exhibit defects in cell wall protein expression and iron utilization**

Some of the experiments in this chapter were done in a collaborative effort and I am thankful for the help of those listed in this paragraph. I especially acknowledge John G. Gibbons and Antonis Rokas (Vanderbilt University) for contributing to the RNA sequencing data. Terry K. Smith (University of St. Andrews, Scotland) for the mass spectrometry of lipids and all his lipid advice. Sarah Davis (University of Tennessee), who helped with the mouse infection assays and immunofluorescence staining as well as Robert Wheeler (University of Maine) for the Dectin-1 binding data.

Abstract

We show that a loss of *de novo* PS and PE biosynthesis has pleiotropic effects on the *Candida albicans* cell, including cell wall defects, defects in growth under iron limited conditions, sensitivity to low pH, sensitivity to the membrane-targeting drug fluconazole and differential expression of hypoxic, cell wall, and mitochondrial related genes. We also show that changes in the phospholipidome indicate that PS 16:0/18:2 is the main lipid species trafficked through the *de novo* pathway in *Candida albicans* and that there is a subsequent increase in long acyl side chain 18:n18n PE species, presumably, from increased Kennedy pathway PE and PC synthesis. The differential effects observed between the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ strain implicate PS as having a direct role in the observed phenotypes. Therefore, we suggest a novel role for PS in *Candida albicans*, whereby, PS itself is required for cell wall maintenance and loss of PS leads to both an altered cell wall and dysfunctional mitochondria.

Introduction

The roles of phospholipids and their functions inside eukaryotic cells have been well characterized, however, there remain significant gaps in our understanding of how phospholipids affect key biological processes such as their role in the overall structure and function of organellar membranes, their contribution to protein-enriched lipid rafts and their affect on cellular metabolism (Osman et al. 2011, Dagley et al. 2011, Bishop et al. 2011). Recent advances include elucidating key protein complexes that mediate a few of these interactions, such as the discovery of the ERMES (endoplasmic reticulum-mitochondria encounter structure) (Michel A.H. & Kornmann B, 2012), which aides in tethering the mitochondria to the endoplasmic reticulum (ER) to facilitate transfer of phospholipids between these organelles. Studies of ERMES have raised questions about the structure and function of phospholipids in eukaryotic cell biology and hints that this class of lipids is underexplored (Voss et al., 2012; Osman et al., 2011). For example, the roles that different phospholipids and particular phospholipid species (*i.e.* lipids that share the same head group, but differ in fatty acid tail length or saturation) have on organelle structure and metabolism are only beginning to be understood (Osman et al. 2011, Joshi et al. 2012).

There exist two major pathways for phospholipid biosynthesis in fungi: they are the *de novo* pathway and the Kennedy pathway (Kent et al., 1995). Together these two pathways synthesize all of the cellular phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (Chen et al., 2010). The *de novo* pathway begins with the synthesis of PS from cytidyldiphosphate-diacylglycerol (CDP-DAG) and serine by Cho1p. A pool of PS is then decarboxylated to PE by the PS decarboxylases Psd1p or Psd2p to produce PE in the mitochondria or Golgi/endosome, respectively. A pool of PE is then methylated by Opi3 and Cho2p to make phosphatidylcholine (Kent et al., 1995) (Fig;1-2 p20). In contrast to *de novo* synthesis, the Kennedy pathway involves utilization of diacylglycerol (DAG) and exogenous

sources of ethanolamine or choline to synthesize PE or PC, respectively. In the Kennedy pathway PE can be directly synthesized from ethanolamine by Eki1p, Ect1p, Ept1p or PC can be synthesized from choline via Cki1p, Cct1p, and Cpt1p (Bergo et al., 2002; Kent et al., 1995) (See figure 1-2). Although the enzymes of the Kennedy pathway are conserved in mammals, some of the enzymes of the *de novo* pathway, such as Cho1p and Psd2p, do not have human homologs, and may be valuable antifungal drug targets. In mammalian cells, PS is synthesized from PE by Pss2p or directly from PC by Pss1p (Ariketh et al., 2008; Bergo et al., 2002).

A number of aspects remain unclear about how phospholipid levels and biosynthesis affect the mitochondria and how they impact other cellular structures, such as the cell wall. Phospholipid homeostasis has been linked to key cellular processes such as respiration, drug transport, and metabolism and the effects of disruption of phospholipid homeostasis on these processes warrant further study. The relationship of phospholipids to the cell wall may be through their indirect or direct effects on activation of cell wall integrity and signaling pathways such as the Rim101 pathway, HOG-1 pathway, and/or the calcineurin pathway. In *C. albicans*, cell wall stresses such as ionic stress can activate the calcineurin pathway which can alter transcription by the transcription factor CRZ1 (Li et al., 2011). In addition, activation of Rim101 can lead to changes in morphology and cell wall structure (Mitchell et al., 2000). Interestingly, the HOG1 pathway has been shown to be activated under conditions of mitochondrial dysfunction and osmotic stress (Alonso-Monge et al., 2008).

The mitochondria are key in cellular metabolism including oxidative phosphorylation and the Krebs cycle (Onishi et al., 1967; Fitton et al., 1994; Cortassa & Aon., 1994; Averet et al., 1998; Celton et al., 2012). The mitochondrion has also been suggested as the major organelle whereby PS and PE are trafficked and synthesized to be transported to the plasma membrane (Osman et al., 2011). The mitochondrial outer and inner membranes function as barriers to

establish a proton motive force for the synthesis of adenosine triphosphate (ATP) as well as to provide a favorable environment for the activity of proteins involved in the transport of electrons to the terminal oxygen electron acceptor in the mitochondrial-localized electron transport chain (Davies et al., 2012). Both membranes are essential in establishing the membrane potential that enables aerobic respiration via the Krebs cycle (Mitchell 1966; Knorre et al., 2012). Due to the presence of proteins associated with the electron transport chain, the mitochondrial inner membrane is also the most protein rich membrane of any biological membrane thus studied (Davies et al., 2012, Osman et al., 2011). Mitochondria are enriched in the lipids cardiolipin and PE and normal mitochondrial function is lost when cardiolipin or PE is removed (Joshi et al., 2012; Gohil et al., 2005; Zhong et al., 2004). In *S.cerevisiae*, the Psd1p enzyme is localized in the mitochondrial inner membrane, and functions to decarboxylate PS to PE, thereby increasing *de novo* synthesized PE within the mitochondrial inner membrane (Clancy et al., 1993). However, the contribution of PS in the mitochondria, other than as a substrate for PE synthesis, is not well understood.

In this chapter, we investigate the effects of disruption of the biosynthesis of PS and *de novo* PE on the fungal cell and provide evidence that PS itself has an effect on the mitochondria in addition to defects associated with downstream *de novo* PE synthesis. Furthermore, PS loss leads to cell wall defects in *Candida albicans*. We suggest that the defects in mitochondrial function are due to PS loss and cause specific effects on the cell wall. Finally, we demonstrate evidence that the loss of PS leads to differential phenotypes in *Saccharomyces cerevisiae* indicating that the phenotypic role of PS in cell wall maintenance is different in these two fungi.

Materials and Methods

Strains and Culture conditions

All strains used in this study are listed in Table 2-1. Unless otherwise mentioned, the strains were grown in (1%) yeast-extract (2%) peptone (2%) dextrose, (YPD) medium (Styles, 2002) at 30°C with shaking.

Phospholipid profiling

Cells were grown overnight and then inoculated at .4 O.D. / ml in 25 ml of (1%) yeast extract (2%)peptone (2%) dextrose (YPD) and grown for 6 hours at 30°C with shaking. The cells were then pelleted at 13,000xG for 1 min and washed with 1ml H₂O. Next, the cells were suspended in 1 ml of 100 % methanol. 5 mg of glass beads were added and the sample was bead-beated five times using a mini-Bead beater-1 Biospec beater on ice with 30 second incubation on ice in between bead beating. Next, 3 ml of chloroform was added to the cell suspension and the solution was mixed by vortexing for 30 seconds. Cells were then transferred to a separatory funnel and washed with 900 µl of 0.9% NaCl solution. The bottom organic layer (1 ml) was then collected using a separatory funnel and dried under N₂ gas for 1 hour. Prior to organic extraction and separation, internal standards of 10 µg of PE 28:0, PC 28:0 and PS 30:0 were added for quantification. The lipid pellets were then analyzed by electrospray ionization tandem mass spectroscopy (ESI-MS/MS) and the relative amounts of each class of phospholipids were quantified relative to the standard.

RNA sequencing

Overnight cultures were added to 25 ml of YPD at .4 O.D./ml and allowed to grow with shaking at 30°C for 5 hours. 10 O.D.(A₆₀₀)equivalents per strain were collected by centrifugation. Total RNA was extracted using the phenol method (Dhomedy et al., 1991). Extracted RNA was treated with DNase following the manufactures protocol (Ambion® (TurboDnase)). It was then assayed for purity by gel electrophoresis. DNase treated total RNA was then cleaned with an RNeasy column (Qiagen) according to the manufacturer's instructions. mRNA libraries were constructed and sequenced at the Vanderbilt Genome Technology Core following Illumina® specifications on an Illumina HiSeq 2000. 50bp read sets from each of the four samples were independently mapped to the *Candida albicans* SC5314 reference transcriptome using BLAST. Over 20 million reads were mapped for each sample (*cho1Δ/Δ*: 34,537,503; *psd1Δ/Δ*: 21,041,937; *psd1Δ/Δpsd2Δ/Δ*: 21,271,203; and *cho1Δ/Δ::CHO1*: 25,147,891). In all samples, 95-96% of mapped reads uniquely mapped to a single transcript. The nucleotide content of mapped reads represented an average 128x coverage of the *C. albicans* SC5314 reference transcriptome (*cho1Δ/Δ*: 193x; *psd1Δ/Δ*: 117x; *psd1Δ/Δpsd2Δ/Δ*: 119x; and *cho1Δ/Δ::CHO1*: 140x). Reads were trimmed from 101 bp to 50 bp by removing the first 11 bp and last 40 bp of each read to maximize overall read quality. Gene expression levels were quantified as previously described (Gibbons et al. 2012) by mapping reads against the *Candida albicans* SC5314 Assembly 19 reference transcriptome using the SeqMap software, version 1.08 (Jiang and Wong 2008 – doi:10.1093/bioinformatics/btn429) allowing 2 mismatches per read. For each gene, gene expression levels were quantified in terms of reads per kilobase per million mapped reads (RPKM) (Mortazavi et al. 2008) using the rSeq software, version 0.0.5 (Jiang and Wong 2009 –doi:10.1093/bioinformatics/btp113). False discovery Rate (FDR) was used to determine statistical differences between the mutants and the wild type strain. A p-value was set as 1E-10 as statistically significant (Conesa et al., 2011, Mortazavi et al., 2008). The data was then

normalized to five housekeeping genes ACT1, PMA1, RIP, RPP2B and LSC2 (Nelis et al., 2006). RNA sequencing results were verified by immunofluorescent staining for *ALS* and *HWP1* genes as described below.

Sensitivity to cell wall and membrane perturbing drugs

For the spot dilution tests, overnight cultures of *C.albicans* strains were washed twice in 1 ml phosphate buffered saline (PBS), and resuspended at 1 O.D./ml in 1 ml of H₂O. The cells were then serially five-fold diluted and spotted onto minimal media (Yeast Nitrogen Base(YNB) + 2 % glucose+ histidine +lucine+1mM ethanolamine) containing either calcoflour white (20µg/ml) or congo red (10µg/ml) or Fluconazole (2µg/ml). All plates were imaged after incubation at 37°C for 48h.

Fluorescence Microscopy

The *C.albicans* strains were grown overnight in YPD at 37°C for 24 hours and then collected by centrifugation at 13,000x g for 1 min, and washed thrice in phosphate buffered saline (PBS), Next, the cells were diluted to .4 OD/ml in 25 ml of YPD and grown for 5 hours at 30°C. Cells were collected washed with 1 ml H₂O and then stained with monoclonal antibodies against Als1p, Als3p and Hwp1p obtained from Lois Hoyer (University of Illinois, Urbana-Champaigna). Samples were further stained with a goat anti-mouse secondary antibody conjugated to Cy3. Microscopic images were then taken under bright field or TRITC filter to observe fluorescent staining.

Growth in low pH conditions

For growth in low pH media, cells were grown overnight in YPD then inoculated at .1 O.D./ml into 5 ml of minimal media (Yeast Nitrogen Base(YNB)+glucose+uracil+lucine+histidine+1 mM Ethanolamine) plus phosphate citrate buffer to pH 4.0 and pH 2.7 and allowed to grow for 48 hours at 37°C with shaking. The pH was measured before and after the assay using a pH meter to assure correct pH.

Iron-limitation assay

The cells were inoculated from overnight culture into minimal media with addition of 1 mM of the Iron chelator bathophenanthroline disulfonate (BPS), or in minimal media containing 1 mM BPS plus the addition of 100 μ M or 300 μ M Iron (II) Sulfate. The cells were inoculated into 5 ml of minimal media and allowed to grow at 37°C for 48 hours with shaking. Growth was then measured by optical density at 600 nm. For solid media, cells were inoculated in spot dilutions from a starting concentration of 1 O.D./ml minimal media (Yeast Nitrogen Base + 2% glucose+uracil+lucine+histidine+1 mM Ethanolamine +3 % agar) plates with or without 1mM BPS, or 1mMBPS + 100 μ M Iron (II) Sulfate. Images were taken after 48 hours of growth at 37°C.

Chitin Staining

Strains were grown overnight in YPD at 37°C with shaking. 1ml at 5 O.D./ml of cells were collected and washed with 1 ml of water. Cells were then stained by addition of 1 ml of 1mg/ml calcofluor white and rocked at room temperature for 5 min. The cells were centrifuged and washed three additional times and then resuspended in 500 μ l water and 5 μ l was spotted onto a slide for microscopy. The cells were visualized at 200X magnification under bright field and fluorescence (UV-DAPI filter) microscope. Results are expressed as percentage of cells that

exhibited increased fluorescence out of the total cells counted. The statistics are based on the average of three different fields of view. Approximately ~400 cells were counted for each strain.

Transmission Electron Microscopy

Cells growing at log phase in 25 ml of YPD were harvested and fixed for electron microscopy using a modification of a protocol by Wright (Wright, 2000). 1ml of 10 O.D/ ml of cells were collected and resuspended in a fixative consisting of 3% glutaraldehyde and 3% paraformaldehyde in 0.1 M phosphate buffer. Cells were centrifuged, then re-suspended in fixative and incubated overnight at 4°C. Cells were then washed in 0.1 M phosphate buffer for 10 mins. Cells were washed in 1% KMnO₄ and re-suspended in fresh KMnO₄ for an additional 90 min. Cells were then en-bloc stained in 1% aqueous uranyl acetate for 60 min and then dehydrated in a graded ethanol series at 15 min intervals. This was repeated twice. Cells were treated with Spurr resin/ethanol over a 24 h period. Spurr resin was replenished and samples were heated in a 68°C oven for 24 h. Thin sections were cut with a Reichert OMU3 ultramicrotome, mounted on copper grids, stained with lead citrate, and examined with a HitachiH800 transmission electron microscope operating at 75 KeV.

Statistical Analysis

Prism 4.0 software (Graph Pad) was used to determine the significance of differences in growth under low pH and iron-limitation conditions. Statistically significant differences were determined using the two-tailed unpaired *t* test. Statistical significance was set at a *P* value of <0.05.

Results

Lipid profiling of phospholipid biosynthesis mutants

In order to better understand the specific changes associated with disruption of PS and/or *de novo* PE biosynthesis, global lipid profiles of *cho1Δ/Δ* and *psd1Δ/Δ psd2Δ/Δ* mutants were

compared to wild-type. For the lipidomic analysis, *C. albicans* strains were grown in YPD medium for 6 hours at 30°C with shaking and total lipids were extracted as described in the materials and methods. Extracted lipids were subjected to Electrospray Ionization Tandem Mass spectrometry (ESI-MS/MS). The total lipids were quantified and normalized to internal standards PE (14:0/14:0), PC 14:0/14:0 and PS (14:0/14:0) (These lipid standard species are not found in *Candida albicans*).

PS species in wild-type were all of the 16:n/18:n variety (16 and 18 indicate the number of carbons in the sn-1 and sn-2 fatty acid tails, respectively, and “:n” indicates the number of unsaturated bonds in that particular tail). The most abundant species of PS species in the wild-type were 16:0/18:2 or 16:1/18:2 (Table 2-2); all of these species were completely absent in the *cho1Δ/Δ* mutant, as expected. In contrast, there is a build-up of PS in the *psd1Δ/Δ psd2Δ/Δ* mutant (Table 2-2).

In contrast to PS, PE in wild-type is represented by species that include 16:n/18:n (34%), 18:n/18:n (41%), and 18:n/20:n (25%) (Table 2-2). The most abundant wild-type 16:n/18:n PE species is 16:0/18:2, which makes up 20% of total PE. In the *cho1Δ/Δ* and *psd1Δ/Δ psd2Δ/Δ* mutants, the substantial drop in PE that was noted previously by TLC, appears to be almost exclusively due to a decrease in 16:n/18:n PE, with the most substantial decrease occurring in 16:0/18:2 PE. Conversely, the other species of PE do not decrease, and instead become a larger percentage of the total PE. These other species are likely generated by the Kennedy pathway from exogenous ethanolamine in YPD medium.

PC is also represented by 16:n/18:n (59%), 18:n/18:n (36%), and 18:n/20:n (5%), with a small percentage of 14:n/16:n in the wild-type (Table 2-2). In *cho1Δ/Δ* and *psd1Δ/Δ psd2Δ/Δ* there is a substantial loss of the 16:n/18:n species, but a strong increase in 18:n/18:n species, that may be due to compensation from the Kennedy pathway.

Thus, in *C. albicans*, the majority of species contributed to PS, PE, and PC by the *de novo* pathway appear to be 16:n/18:n, and the Kennedy pathway contributes species with longer fatty acid tails.

Transcriptional profiling of PS- and *de novo* PE- deficient *Candida albicans* mutants by RNA sequencing

The lipidomics data suggested that loss of the *de novo* pathway specifically blocks synthesis of 16:n/18:2 PS, PE, and PC. The *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants both have a similar drop in 16:n/18:2 PE and PC, but only the *cho1* Δ/Δ mutant has a complete loss of PS as well. Both mutants have mitochondrial and cell wall defects and in both cases, the defects seem to be greatest in the *cho1* Δ/Δ mutant. In order to determine what effects the losses of these species of PE and/or PS have on the mitochondria, cell wall or other organelles, transcriptional profiles of the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants were compared to wild-type using RNA sequencing (RNA seq). Genes in either strain that showed ≥ 3 -fold up or down regulation compared to wild-type, after normalization, were selected for further analysis.

There are 114 genes that are transcriptionally up-regulated by ≥ 3 -fold in the *cho1* Δ/Δ mutant compared to the WT (SC5314), and 129 genes are up-regulated by ≥ 3 -fold in the *psd1* Δ/Δ *psd2* Δ/Δ double mutant v/s the WT strain (Appendix Tables A-3 and A-5). Of the 114 genes up-regulated in the *cho1* Δ/Δ mutant, 47 (41%) were also up-regulated by ≥ 3 -fold in the *psd1* Δ/Δ *psd2* Δ/Δ mutant compared to wild-type. The total number of genes up-regulated in each mutant were sorted according to gene ontology (GO) term corresponding to subcellular localization (component) using the GO SLIM Mapper function in the *Candida* Genome Database (CGD). This analysis revealed that in both mutants, 16-24% of the genes that were up-regulated compared to wild-type related to the mitochondria (Fig 2-1).

As both of these mutants have cell wall defects, we more carefully examined cell wall genes that were up-regulated in the mutants compared to wild-type (Tables 2-3 to 2-5). Interestingly, both mutants up-regulated a number of cell wall genes that are involved in hyphal growth, even though they are being grown in YPD at 30°C, a condition that is normally not conducive to forming hyphae. These genes included *ALS1-3*, *PGA6*, and *PGA10* (Table 2-5). Some other cell wall genes that are usually associated with hyphal formation like *HWP1* and *RBR1* were up-regulated very highly in the *cho1ΔΔ* mutant (Table 2-3), but were not up-regulated in the *psd1ΔΔ psd2ΔΔ* strain.

In order to further explore the expression changes in the cell wall genes, we focused first on the *ALS* genes, which represent a highly conserved family of glycosylphosphatidylinositol (GPI)-anchored cell wall adhesin genes. This was explored using a set of monoclonal antibodies specific to the Als1, Als3, and Als4 proteins and Hwp1p (Coleman et al., 2012;

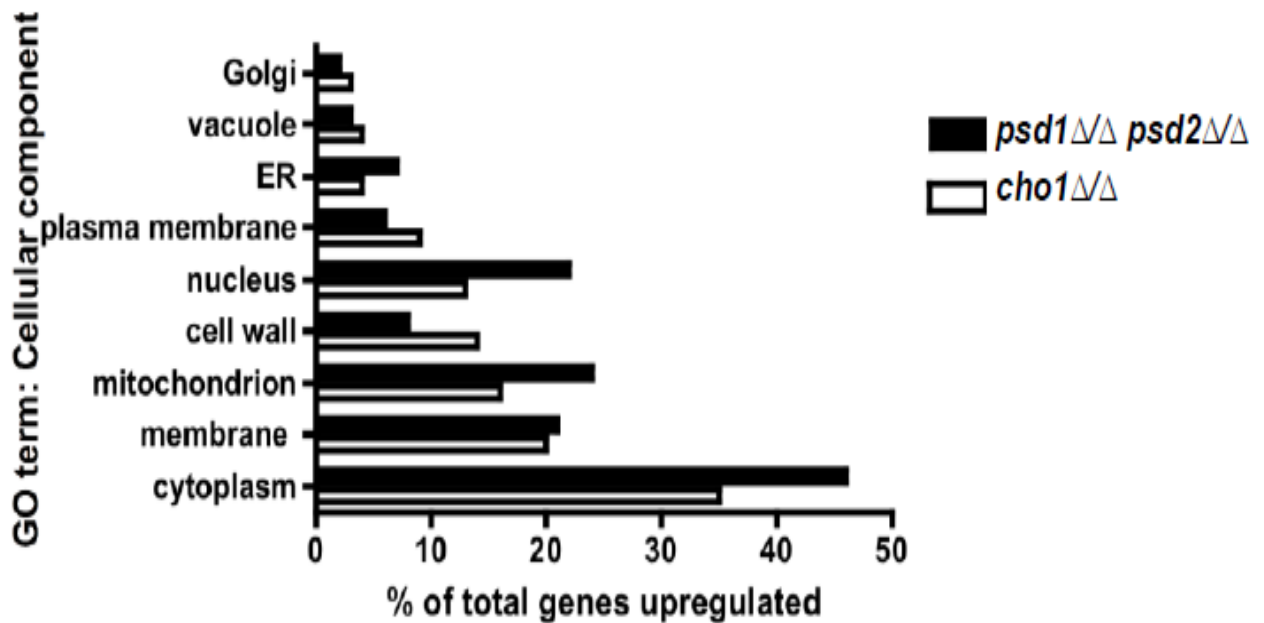


Figure 2-1: Gene ontology (GO) comparison of all genes UP regulated in the *cho1*Δ/Δ and *psd1*Δ/Δ*psd2*Δ/Δ mutants. Genes were input into the Candida Genome Database (CGD) SLIM mapping tool and mapped for their cellular components. Gene ontology is expressed as both component and process and the percentages are displayed comparing the *cho1*Δ/Δ strain and the *psd1*Δ/Δ*psd2*Δ/Δ strain .

Coleman et al., 2009), which is another GPI-anchored cell wall protein that is not highly conserved with *ALS1-4*.

Fluorescence microscopy of wild-type, *cho1Δ/Δ*, and *psd1Δ/Δ psd2Δ/Δ* cultures grown in YPD at 30°C for 5 hours (under identical conditions as the RNAseq experiment) revealed that in wild-type, *psd1Δ/Δ psd2Δ/Δ* and *cho1Δ/Δ* strains the Als1p and Als4p were expressed (Fig 2-2, data not shown), but Als1p was slightly higher in *cho1Δ/Δ* and *psd1Δ/Δ psd2Δ/Δ* than wild-type. In contrast, Als3p was not expressed very strongly in any strain except *cho1Δ/Δ*, and this was primarily associated with a higher number of hyphal-like cells found in the culture (Fig 2-2).

These data, especially regarding Als3p, do not agree with the RNAseq data which indicates that *ALS3* is up-regulated in both *cho1Δ/Δ* and *psd1Δ/Δ* (Table 2-5). However, this may be because *ALS1* was more highly expressed and *ALS1* and *ALS3* are practically identical over much of their sequence (data not shown). Thus, the BLAST searches matching the RNA sequencing library to the database may have generated artifacts due to this close homology.

In order to clear this up, another hyphal cell gene, *HWP1* was tested, since it shows much higher expression in *cho1Δ/Δ* than in the *psd1Δ/Δ psd2Δ/Δ* strain (Table 2-3), but has little homology with the *ALS* gene family. It was found that Hwp1p was highly expressed in *cho1Δ/Δ* hyphal cells, but not in the other strains, including *psd1Δ/Δ psd2Δ/Δ* (Fig 2-2). The wild-type and *psd1Δ/Δ psd2Δ/Δ* cultures grew mainly as yeast cells. Thus, the *cho1Δ/Δ* mutant appears to have more hyphae in YPD at 30°C.

In addition to the up-regulation of cell wall genes that are involved in hyphal formation, the *cho1Δ/Δ* mutant up-regulated several genes involved in cell wall repair including *PGA31*, *PGA13*, *IFF11*, and *ECM331*. *ECM331* was up-regulated in *psd1Δ/Δ psd2Δ/Δ* as well, although it was more highly expressed in the *cho1Δ/Δ* mutant. Both mutants exhibit cell wall defects, although the defects are greater in the *cho1Δ/Δ* mutant. These include an altered cell wall

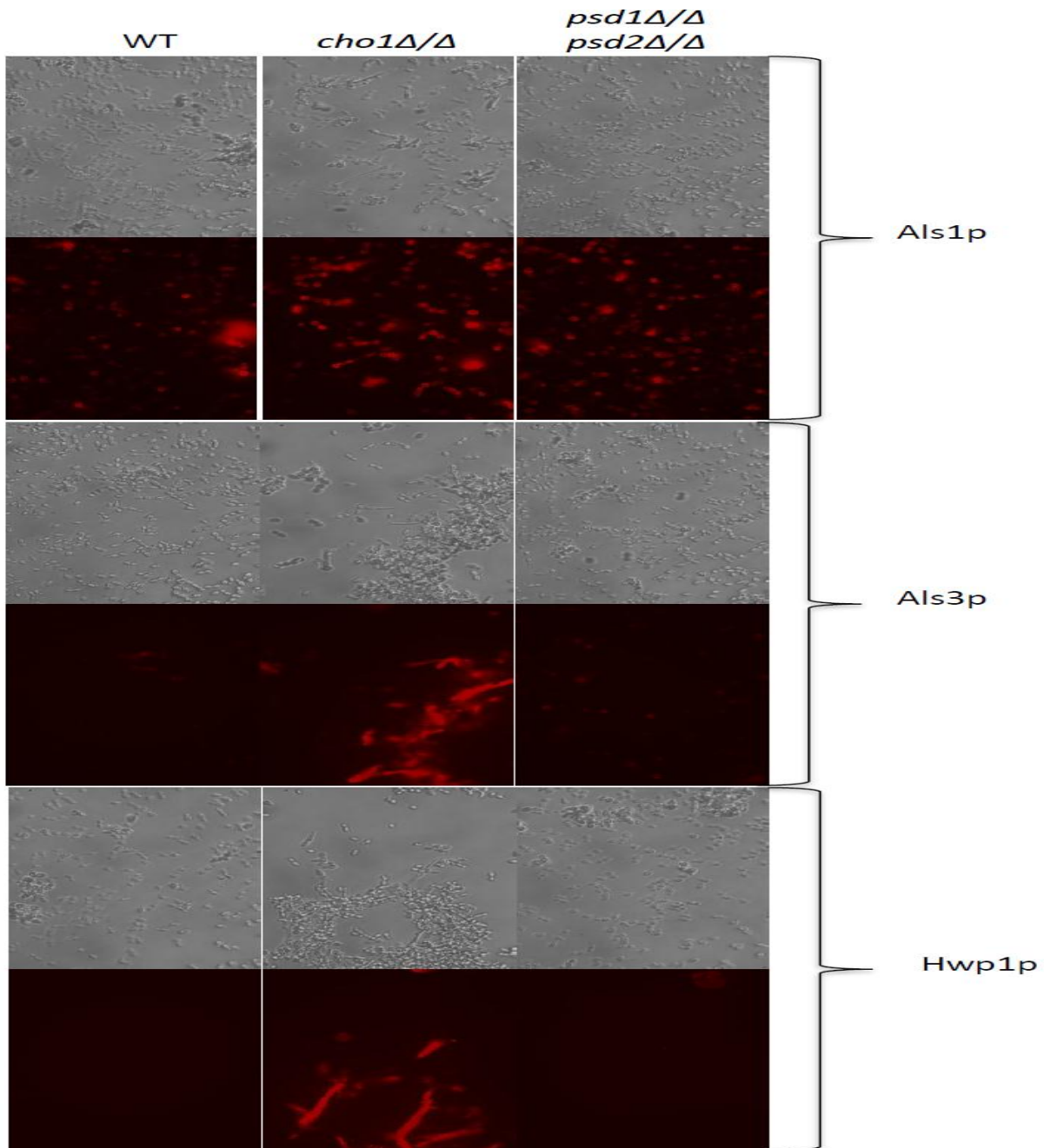


Figure 2-2: Immunostaining of *C.albicans* strains to determine expression of Als1, Als3 and Hwp1. Candida cells were grown in YPD medium at 30 °C, washed in saline, counted and transferred into pre-warmed RPMI medium at a cell density of 5×10^6 cells ml^{-1} . Fixed cells were immunosaturated with anti-Als1 or –Als3 and –Hwp1. Cells were observed by fluorescence microscopy. The *cho1Δ/Δ* mutants showed increased expression of Als3 and Hwp1 proteins (middle and last panels) and both the *cho1Δ/Δ* mutant and *psd1Δ/Δ psd2Δ/Δ* mutant showed increased expression of Als1 (first panel). (Sarah Davis contributed to this figure).

structure, based on transmission electron microscopy (TEM) and increased staining for chitin (Chen, et al 2010).

It was hypothesized that the alterations in the wall in these mutants would result in increased sensitivity to cell wall stress-inducing agents. This was previously tested and found not to be the case using YPD media. However, when it was re-examined on minimal media, it was discovered that the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ mutants display increased sensitivity to both calcofluor white and congo red compared to the wild-type strain (Fig: 2-3). The *cho1* Δ/Δ mutant shows greater cell wall defects which is consistent with its other cell wall defects (Chen et al., 2010).$

Down-regulated genes in *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ are enriched for iron uptake

In addition to the genes up-regulated by the loss of PS and *de novo* PE, there were genes that were down regulated compared to wild-type as well. There were 29 genes that were down-regulated in the *cho1* Δ/Δ mutant compared to wild-type by ≥ 3 -fold (Appendix Table A-4). There were 103 down-regulated genes (≥ 3 -fold) in the *psd1* Δ/Δ *psd2* Δ/Δ mutant compared to wild-type (Appendix Table A-6). The genes are summarized based on their categorizations according to cellular component GO terms (Fig: 2-4).

Among the genes down-regulated in *cho1* Δ/Δ compared to wild-type, there were five (~17%) that had to do with iron uptake or utilization including *CFL2*, *FRE10*, *FTR1*, *orf19.6873.1*, and *CHA1* (Table 2-6). Iron related genes make up only 2.4% of the genes in the whole genome, so the down-regulated genes in *cho1* Δ/Δ are substantially enriched for iron uptake genes. Of these 5 genes, 2 are down-regulated in *psd1* Δ/Δ *psd2* Δ/Δ compared to wild-type as well, *CFL2* and *FRE10* (Table2-6).

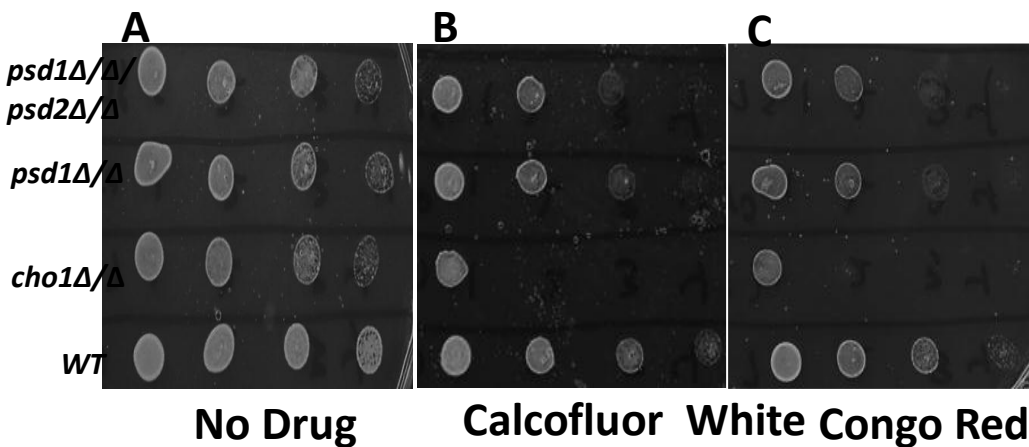


Figure 2-3 :Sensitivity to the cell wall perturbing drugs Calcofluor White and Congo Red. The *C. albicans* *cho1Δ/Δ*, *psd1Δ/Δ*, and *psd1Δ/Δpsd2Δ/Δ* mutants are sensitive to the cell wall perturbing drugs Calcofluor White and Congo Red but the *cho1Δ/Δ* mutant's sensitivity is greater as its growth was highly diminished in the presence of either Calcofluor White or Congo Red (Panel B and C). Serial dilutions of 5 μ l of 1 O.D./ml of overnight cultured cells were spot inoculated on solid minimal media (Yeast Nitrogen Base +dextrose, +uracil+histidine+1 mM Ethanolamine) either with (A) no drug (B) 20 μ g/ml Calcofluor white, a chitin synthesis inhibitor or (C) 40 μ g/ml Congo Red, a β -1-3 glucan synthesis inhibitor. The plates were incubated at 37°C and images were taken at 24 hours.

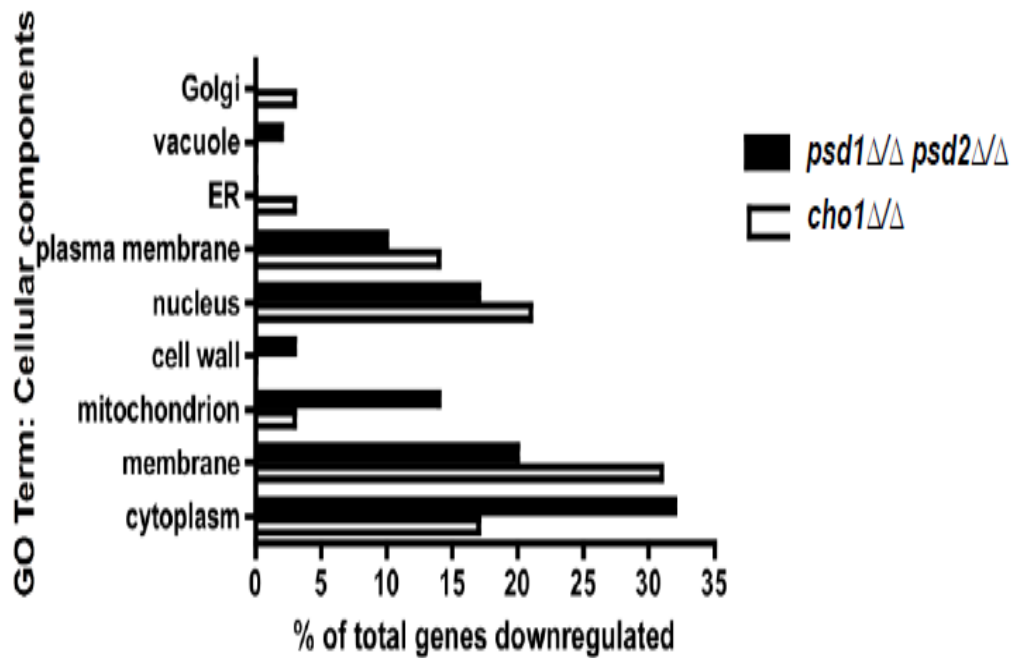


Figure 2-4: Gene ontology (GO) comparison of all genes DOWN regulated in the *cho1*Δ/Δ and *psd1*Δ/Δ*psd2*Δ/Δ mutants. Genes were input into the Candida Genome Database (CGD) SLIM mapping tool and mapped for their cellular component. Gene ontology is expressed as both component and the percentages are displayed comparing the *cho1*Δ/Δ mutant (white) and the *psd1*Δ/Δ*psd2*Δ/Δ mutant (black).

The down-regulation of iron utilization genes suggested that the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants might have defects in the ability to grow in iron limiting conditions. Therefore, overnight growth of the strains was compared in minimal media containing or lacking the iron chelator bathophenanthroline disulfonate (BPS), and minimal media + BPS with iron sulfate added back (100 μ M or 300 μ M). This experiment revealed that growth of the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants was significantly attenuated compared to wild-type in media where iron was limiting (100 μ M or 300 μ M, Fig: 2-5; data not shown). Thus, iron limitation does inhibit the growth of both the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants.

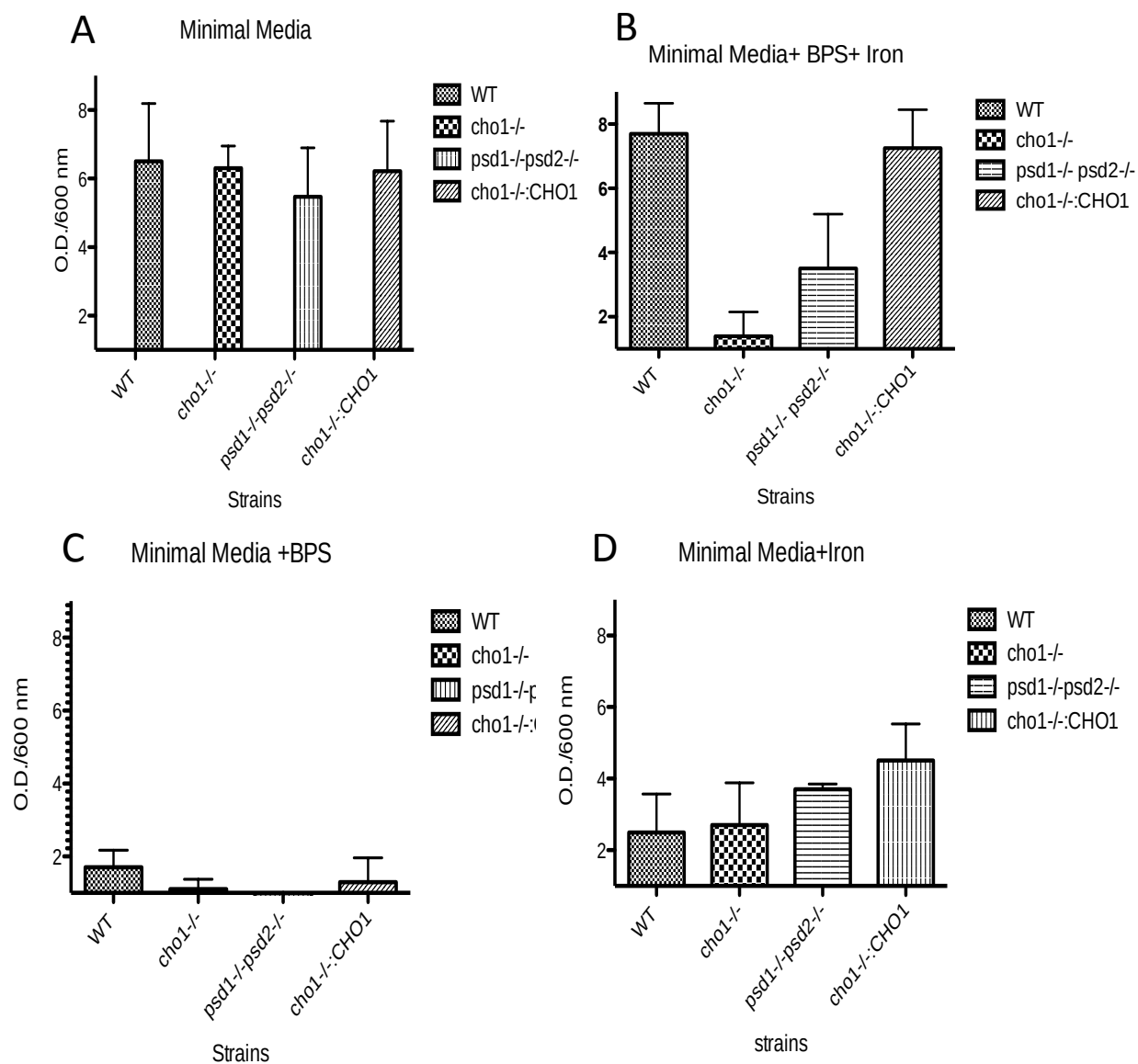


Figure 2-5: Growth response to iron limitation in liquid media. Overnight cultures were diluted to 0.1 OD/ml in 5 ml of minimal media (Yeast Nitrogen Base + dextrose + histidine + leucine + 1mM ethanolamine) (A) with no chelator or (C) + 1mM of the iron chelator, BPS or (B) with 1mM of the iron chelator BPS and 100 μ M Iron(II) sulfate. Toxicity to iron was assayed in minimal media with the addition of 100 μ M of Iron (II) Sulfate to the medium (Panel D). Cells were grown with shaking at 37°C and optical density was measured after 48 hours. (* indicates a p-value < .05 ** indicates a p-value < .001)

The *cho1* Δ/Δ mutant showed greater attenuation compared to *psd1* Δ/Δ *psd2* Δ/Δ , and this might be because it had more iron utilization genes down-regulated (Table 2-7). A comparison of the 50 genes that are more down-regulated in the *cho1* Δ/Δ mutant than the *psd1* Δ/Δ *psd2* Δ/Δ mutant by ≥ 2 fold, revealed that 20% of them are involved in iron utilization (Table 2-7, Appendix Table A-4). This would help explain why *cho1* Δ/Δ is more sensitive to iron depletion. These results are limited to liquid aerated culture and were not observed in the same media on agar plates (Fig: 2-6). On plates containing BPS with iron added back, the growth between the strains was similar (Fig: 2-6).

In contrast to down-regulating genes involved in iron uptake and utilization, both the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ strains up-regulated genes known to be involved in response to hypoxia (low oxygen) (Table 2-9). A number of these genes are known to be induced under hypoxic conditions (Synnott et al., 2010) in *Candida albicans*. We observed a more robust (higher fold) up-regulation of some of these genes in the *cho1* Δ/Δ strain compared to the *psd1* Δ/Δ *psd2* Δ/Δ strain. This data suggests that while both strains exhibit a transcriptional response similar to a response to hypoxia, the *cho1* Δ/Δ mutant response is indicative of a greater hypoxic response compared to the *psd1* Δ/Δ *psd2* Δ/Δ strain.

In addition, genes involved in oxidative stress response are up-regulated in the *cho1* Δ/Δ mutant compared to the *psd1* Δ/Δ *psd2* Δ/Δ strain (Table 2-8). These genes include the alternative oxidase (*AOX2*), which may help shunt electrons away from the classical electron transport chain to avoid oxidative damage. In addition, genes that neutralize reactive oxygen and nitrogen species were also up-regulated like *SOD5* and *YHB1*. Several alcohol dehydrogenases including *IFE2* and *ADH5* were up-regulated which may help to metabolize

increased alcohol production if fermentation is increased to compensate for decreased respiration (data not shown).

Reactive oxygen species (ROS) have been shown to be generated in the mitochondrial inner membrane under hypoxic conditions (Murphy et al., 2009; Cap et al. 2012). Consistent with the *cho1* Δ/Δ mutants' observed mitochondrial defects (Chen et al., 2010), ROS may be produced in the mitochondria under hypoxic conditions in the PS lacking *cho1* Δ/Δ strain resulting in more defective mitochondria than the *de novo* PE lacking *psd1* Δ/Δ *psd2* Δ/Δ strain.

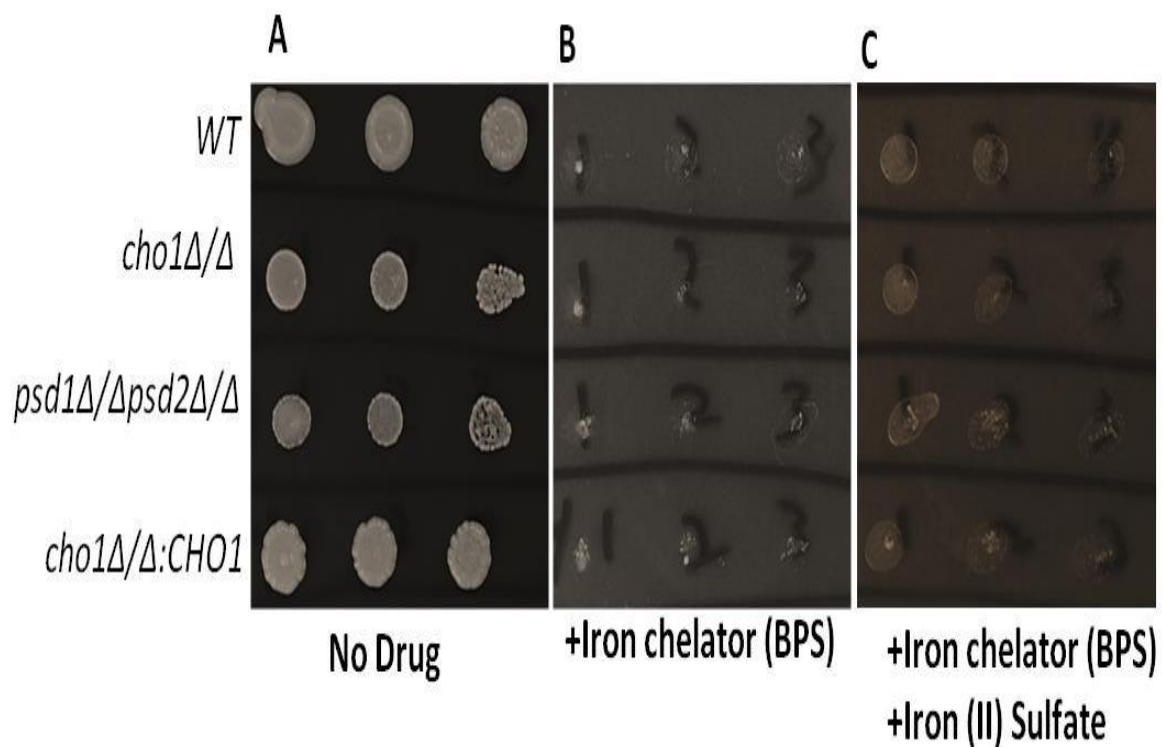


Figure 2-6: Growth on solid minimal media under iron limited conditions. The *cho1Δ/Δ*, and *psd1Δ/Δpsd2Δ/Δ*, and *cho1Δ/Δ:CHO1* strains exhibit no differences in growth under iron limited conditions on solid minimal media compared to the WT (SC5314) strain (Panel C). Serial dilutions of 5 μ l of 1 O.D./ml of overnight cultured cells were spot inoculated on solid media (Yeast Nitrogen Base + 1mM ethanolamine + uracil + histidine + Leucine + glucose) on minimal media plates (A) without chelator or iron or (B) + 1 mM of the iron chelator, Bathophenanthrolinedisulfonic acid (BPS) or (C) + 1mM BPS + 100 μ M Iron (II) Sulfate. The plates were incubated at 37°C and images were taken at 48 hours.

***Saccharomyces cerevisiae cho1*Δ mutant has less severe cell wall defects compared to the *Candida albicans cho1*Δ/Δ mutant**

S. cerevisiae has served as a template for studying phospholipid biosynthetic pathways in *C. albicans* (Kent et al 1995). To compare the cell wall phenotypes between the *C. albicans cho1*Δ/Δ mutant and *S. cerevisiae cho1*Δ mutant, the susceptibility of the *S. cerevisiae cho1*Δ mutant to cell wall perturbing drugs was tested. It was observed that like the *C. albicans cho1*Δ/Δ mutant, the *S. cerevisiae cho1*Δ mutant is sensitive to congo red (Fig: 2-7), however, unlike the pathogen, it is not sensitive to calcofluor white.

The *cho1*Δ/Δ mutant in *C. albicans* has higher chitin content in the cell wall. The loss of PS in *S. cerevisiae* does lead to increased chitin content as evidenced by calcofluor white (CFW) staining (Fig: 2-8). However, the increase in chitin is about two- fold in the *S.cerevisiae cho1*Δ mutant, whereas in *C. albicans* the *cho1*Δ/Δ mutant has a ten-fold higher chitin content compared to the wild-type strain (Chen et al., 2010). Therefore, the effect of loss of PS on the cell wall is less severe in *S. cerevisiae* compared to *C. albicans*.

Phenotypic differences in response to a loss of PS between *C. albicans* and *S. cerevisiae* are further highlighted by the fact that the ultrastructure of the cell wall differs between the two mutant strains of different species. Transmission electron microscopy (TEM) was performed to examine the ultra-structure of the cell wall and quantify its thickness. As we previously reported, the *C.albicans cho1*Δ/Δ mutant displays a thick, irregular cell wall compared to the wild-type strain (Chen et al, 2010). However, unlike in *C. albicans*, the *S. cerevisiae cho1*Δ mutant does not display a disordered or thick cell wall. The cell wall appears to be identical to the wild type strain in both thickness and observable structure (Fig: 2-9).

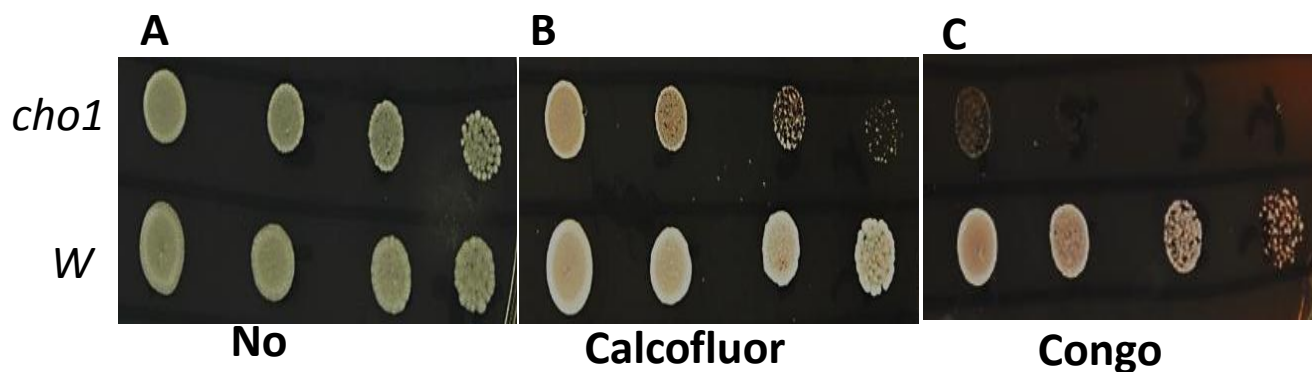


Figure 2-7: Sensitivity of the *Saccharomyces cerevisiae cho1*Δ mutant to the cell wall perturbing drugs Calcofluor White and Congo Red. Serial dilutions of 5 μl of 1 O.D./ml of overnight cultured cells were spot-inoculated on minimal media containing (A) no Drug or (B) 20 μg/ml Calcofluor White, a chitin synthesis inhibitor or (C) 40 μg/ml Congo Red, a β-1-3 glucan synthesis inhibitor. Images were taken after 48 hours of growth at 30°C. The *cho1*Δ mutant is hypersensitive to the cell wall perturbing agent Congo Red compared to WT (Panel C) but is not hypersensitive to Calcofluor white (Panel B). The plates were incubated at 30°C and images were taken at 24 hours.

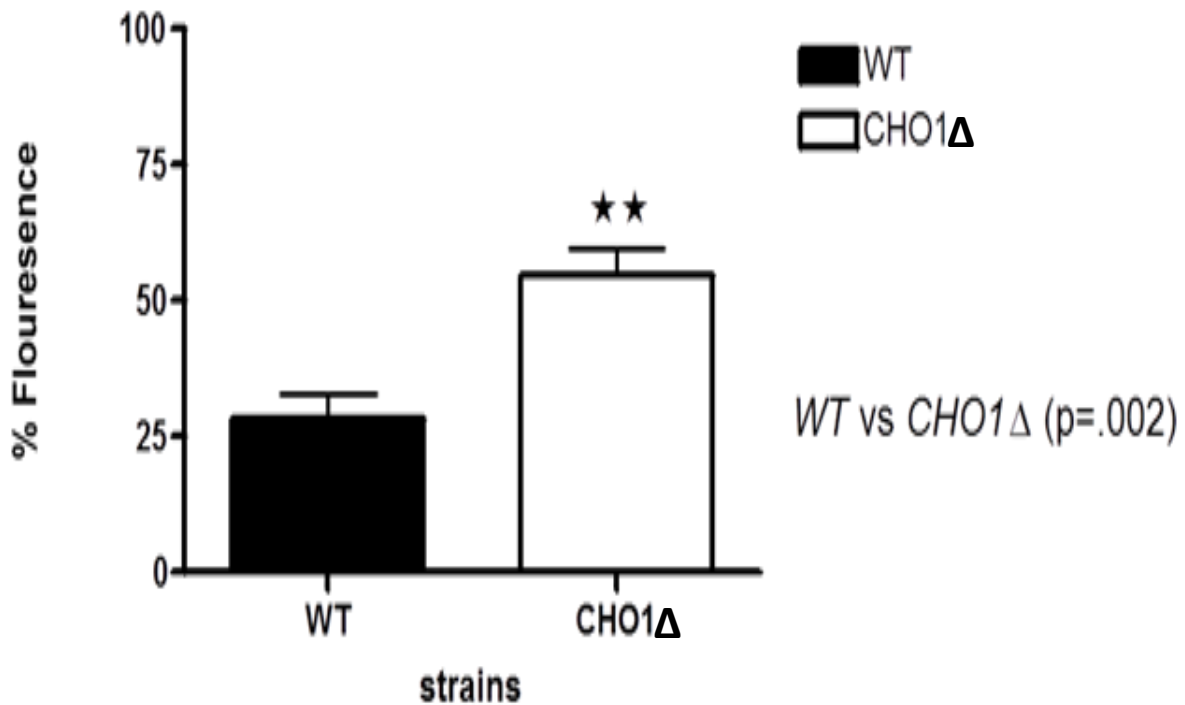


Figure 2-8: Chitin content of the *Saccharomyces cerevisiae* *cho1*Δ strain. The *Saccharomyces cerevisiae* *cho1*Δ mutant has a higher chitin content compared to the wild type strain. However, unlike the ten-fold increase in chitin observed in *C. albicans* (Chen et al., 2010), the increase is only two-fold in *S. cerevisiae*. Cells were grown overnight in 2% Yeast extract 2% Peptone 2% Dextrose (YPD) at 30°C and 1ml of 1 O.D./ml of cells were stained with calcofluor white (1mg/ml) for 5 mins, washed with 1 ml H₂O, and visualized under a light microscope equipped with a fluorescent DAPI filter. Percentage fluorescence at low exposure is expressed as a percentage of total cells. ~400 cells were examined per strain. (** *p* value < 0.001)

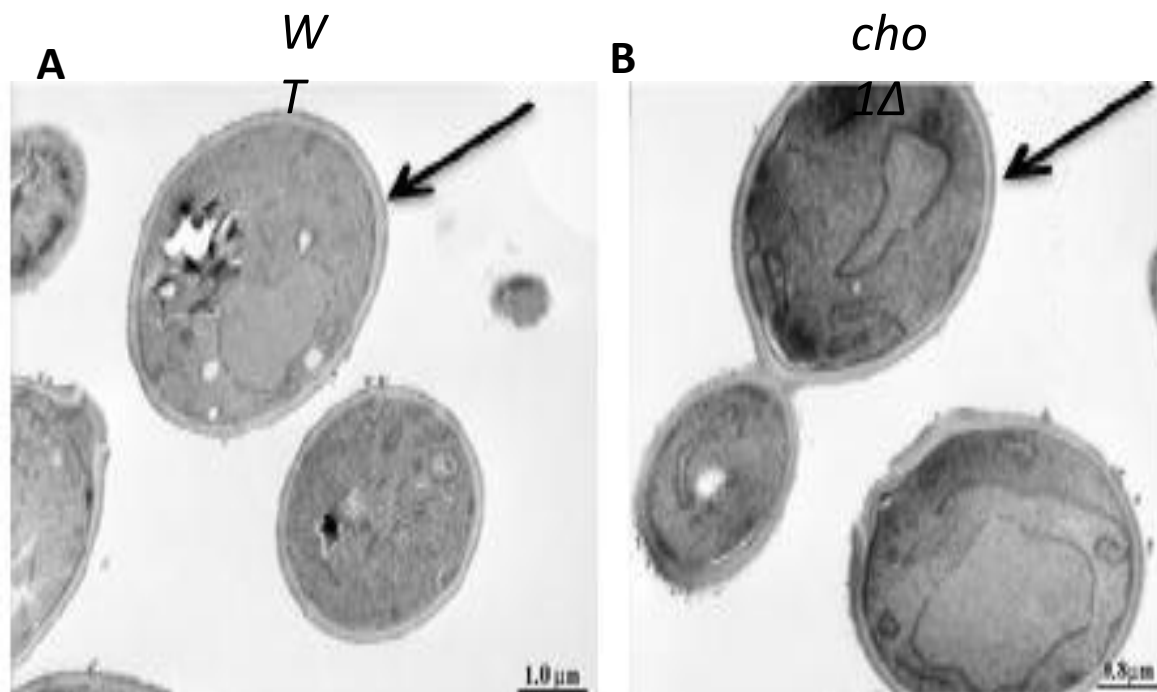


Figure 2-9: Transmission Electron Microscopy of the *Saccharomyces cerevisiae* *cho1*Δ strain. TEM images reveal that the *cho1*Δ strain (Panel B) shows no observable differences in cell wall ultrastructure compared to the WT strain (Panel A). Arrows indicate the outer surface of the cell wall. Note that the cell wall thickness and order is not changed, unlike that observed in the *Candida albicans* *cho1*Δ/Δ strain (Chen et al., 2010). Overnight cultures were growing at log phase were harvested using a modification of a protocol by Wright (2000). Cells were resuspended in a fixative consisting of 3% glutaraldehyde and 3% paraformaldehyde in 0.1 M phosphate buffer, washed in 1% KMnO₄ and then en-bloc stained in 1% aqueous uranyl acetate for 1 hour. Cells were infiltrated with gradually increasing ratios of Spurr resin/ethanol over a 24 h period. The final 4 h of incubation were in 100% Spurr resin. Image is representative of the ~150 cells examined by transmission electron microscopy. Scale Bar= 1 μm (panel A) and 0.8 μm (Panel B).

Discussion

C. albicans is an important fungal pathogen and accounts for a majority of the cases of invasive candidiasis. Phospholipid biosynthesis enzymes, in particular the PS synthase Cho1p, have recently been shown to be required for virulence in a mouse model of systemic candidiasis (Chen et al., 2010). The mechanism underlying the avirulence of the *cho1* Δ/Δ mutant remains unknown. However, we have found that this mutant, along with the *psd1* Δ/Δ *psd2* Δ/Δ mutant, has a defective cell wall as demonstrated by increased chitin content and disordered ultrastructure using transmission electron microscopy (Chen et al., 2010). An enormous amount of scientific effort has been spent in understanding the fungal cell wall as a plausible and effective antifungal drug target and to elucidate the biogenesis of this durable and complex structure (Blankenship et al., 2010; Plaine et al., 2008; Mora-Montes et al., 2012). In fact, the cell wall has often been referred to an organelle because of its dynamic structure as well as its essential role in survival (Chaffin et al., 1998; Nobel et al., 2000; Bussey et al., 2004). In *C. albicans*, the cell wall plays a crucial role in maintaining cell structure and regulates morphogenesis (Gelís et al., 2012; Cabib et al., 1997; Martinez-Lopez et al., 2004; Kelly et al., 2004). It is crucial to environmental sensing and dictates interactions with the host by facilitating adhesion, evading immune recognition (McKenzie et al., 2010; Lewis et al., 2012; Mora-Montes et al., 2012), and activating signaling cascades allowing the fungus to respond to its environment (Birkaya et al., 2009; Blankenship et al., 2010; LaFayette et al., 2010; Rauceo et al., 2008). Therefore, a better understanding of how phospholipids influence the cell wall and its maintenance is necessary for a more complete view of how phospholipids mediate cellular processes in *Candida albicans*.

The *cho1* Δ/Δ mutant has stronger effects on cell wall genes than the *psd1* Δ/Δ *psd2* Δ/Δ mutant

Despite their strong effect on the cell wall, there is currently a lack of information on why losses of certain phospholipids, in particular PS and de novo PE, affect the cell wall in *C. albicans*. To better understand the roles of PS and PE biosynthesis in maintaining the integrity of the cell wall, we examined whether specific lipid species were affected and what transcriptional changes occur upon loss of these species of PS and/or PE. RNA sequencing was used to measure changes in transcripts associated with blocking PS and *de novo* PE synthesis (*cho1* Δ/Δ) or *de novo* PE synthesis alone (*psd1* Δ/Δ *psd2* Δ/Δ). Three fold up- and down-regulated genes were then annotated by Gene Ontology (GO) using GO Slim Mapper tool on the *Candida* Genome Database (CGD) website <http://www.candidagenome.org/> based on gene component (Figure 2-1). Both mutants were compared to the wild type strain, and we found that the *cho1* Δ/Δ mutant up-regulated a greater percentage of genes related to cell wall maintenance than the *psd1* Δ/Δ *psd2* Δ/Δ mutant (Figure 2-1).

While several cell wall proteins (GlycosylPhosphatidylinositol (GPI)-anchored) were up-regulated in both the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants (Tables 2-3 and 2-4), the up-regulation of *ALS1* in both was confirmed using monoclonal antibodies, but Als3p was up-regulated only in the *cho1* Δ/Δ mutant (Fig:2-2). This may have been due to the fact that many *ALS* genes are very similar in sequence. In fact, BLAST searches using *ALS1* reveal large regions with very strong homology to *ALS3*, *ALS2*, *ALS5*, *ALS 4*, and *ALS9*, (in that order), where *ALS3* and *ALS2* are the strongest. Since RNAseq works by BLASTing the results of the sequenced library against the database, and *ALS1* is strongly expressed in *psd1* Δ/Δ *psd2* Δ/Δ , this may have caused an artifact that suggested increased expression of *ALS3*, as it is the closest *ALS* gene in homology.

In contrast, expression of the *HWP1* gene was reflected very accurately by the RNAseq data (Table 2-3), as the *cho1Δ/Δ* mutant clearly overexpressed it, but the *psd1Δ/Δ psd2Δ/Δ* had wild-type levels, based on immunofluorescence (Fig 2-2). This is supported by the fact that *HWP1* has little homology to the ALS genes.

Immunofluorescence staining revealed that the *cho1Δ/Δ* mutant, despite having problems filamenting at 37°C in response to some of the canonical stimuli such as neutral pH or nitrogen starvation (Chen et al, 2010), has a population of hyperfilamentous cells at 30°C (Fig 2-2). This is clearly a consequence of the loss of PS since the *psd1Δ/Δ psd2Δ/Δ* mutant, with a similar loss of *de novo* PE, does not share this phenotype. The pathways affected by the loss of PS that lead to this phenotype, however, remain to be discovered.

There are a number of cell wall genes (GPI-anchor) up-regulated in the *cho1Δ/Δ* mutant, but not in the *psd1Δ/Δ psd2Δ/Δ* strain (Tables 2-3 and 2-4), and several are involved in cell wall repair including *IFF11*, *PGA13*, and *PGA31* (Richard et al., 2007). GPI-anchored proteins regulate cell wall integrity and cell-cell interactions in *C.albicans* (Richard et al., 2007; Plaine et al., 2008). Two recently published studies reported that phospholipid levels play an important role in cell wall biogenesis and that mitochondrial defects may lead to cell wall defects in *C. albicans* (Qu et al., 2012, Dagley et al., 2010). Down-regulation of cell wall genes may help explain why the *cho1Δ/Δ* mutant is more sensitive to cell wall insults like calcofluor white and Congo red than the *psd1Δ/Δ psd2Δ/Δ* mutant (Fig: 2-3), and has a more disordered cell wall and greater levels of chitin (Chen et al., 2010).

Disruption of phospholipids biosynthesis results in defects in iron utilization

We examined genes that were down-regulated in the mutants compared to the wild-type, we found that there was enrichment for genes that are involved in iron uptake or utilization (Tables

2-6 and 2-7). This result led to the hypothesis that our phospholipid biosynthesis mutants would be defective for growth in iron-limiting conditions. Therefore, we assayed growth of the mutants under iron-limiting conditions by addition of the iron chelator, bathophenanthroline disulfonate (BPS), to deplete the media of iron, and amendment of the media with a low concentration (100 μ M or 300 μ M) of iron sulfate. Both mutants exhibit defective growth in response to iron limitation compared to the wild type strain (Fig: 2-5). However, the *cho1* Δ/Δ mutant showed a more severe defect in response to iron limitation than the *psd1* $\Delta/\Delta*psd2* Δ/Δ strain, as addition of 100 μ M iron did not rescue growth of *cho1* Δ/Δ to the extent it did for the *psd1* $\Delta/\Delta*psd2* Δ/Δ mutant (Fig: 2-5). Surprisingly, the phenotypic difference in iron response was limited to liquid culture, as we saw no observable difference on solid media (Fig: 2-6).$$

The *cho1* Δ/Δ mutant actually exhibits a greater decrease in iron responsive genes than the *psd1* Δ/Δ *psd2* Δ/Δ strain (Table 2-7), which probably explains the greater growth defect in iron limiting media for the *cho1* Δ/Δ mutant than the *psd1* Δ/Δ *psd2* Δ/Δ mutant. In addition, the *psd1* $\Delta/\Delta*psd2* Δ/Δ strain exhibited better growth under iron limited conditions compared to the *cho1* Δ/Δ strain. In correlation to this, we observed that the *psd1* $\Delta/\Delta*psd2* Δ/Δ mutant up-regulates a subset of genes involved in iron uptake response that were not up-regulated in the *cho1* Δ/Δ strain (Table 2-10). These genes include *SEF1*, *SEF2*, and *HAP2*, *CCC2* and orf19.2863.1. *SEF1* is a transcriptional activator of iron-uptake genes that include *SEF2*, *CCC2*, and the iron-utilization gene *HAP2* (Chen et al., 2011). This may be an attempt to abrogate effects of iron limitation due to a loss of PS. The host bloodstream is an iron-limited environment, and it may be that the poor growth in iron-limiting conditions prevents the mutants from being able to grow in these conditions.$$

The *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains exhibit a hypoxic response

We examined genes known to be differentially regulated under hypoxic conditions in *Candida albicans*. We found that both the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ showed an up-regulation of genes associated with a hypoxic response compared to the wild type strain (Table 2-9). Specifically, both the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains up-regulated *ECM331*, a GPI anchored protein gene induced under low oxygen conditions, however the *cho1*ΔΔ strain up-regulates this gene nine-fold whereas the *psd1*ΔΔ*psd2*ΔΔ strain only induces a five-fold up-regulation. In addition, *WH11*, a phenotypic switching gene, strongly induced under hypoxia is also more highly up-regulated in the *cho1*ΔΔ (6-fold) versus the *psd1*ΔΔ*psd2*ΔΔ strain (2-fold). In addition, we did find that both *ERG11* and *ERG3*, both involved in the biosynthesis of ergosterol, were up-regulated in both the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains. It is also known that these genes are induced under hypoxic conditions (Synnott et al., 2010). Taken together, this data suggests that both the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains exhibit a hypoxic response but that the *cho1*ΔΔ strains response is stronger and is indicative of a more hypoxic environment or a response that mimics this hypoxic state.

In contrast to the results above with *ERG11* and *ERG3* upregulation, it was observed that the sensitivity of both the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strain to the antifungal drug fluconazole were increased (Fig: 2-10). Fluconazole inhibits the function of *ERG11* (lanesterol -14-demethylase), and therefore blocks ergosterol biosynthesis. The sensitivity to fluconazole in the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains in spite of their upregulation of *ERG11* may be due to an overall defect in ergosterol biosynthesis, which can lead to increased sensitivity to fluconazole (Flowers et al. 2012).

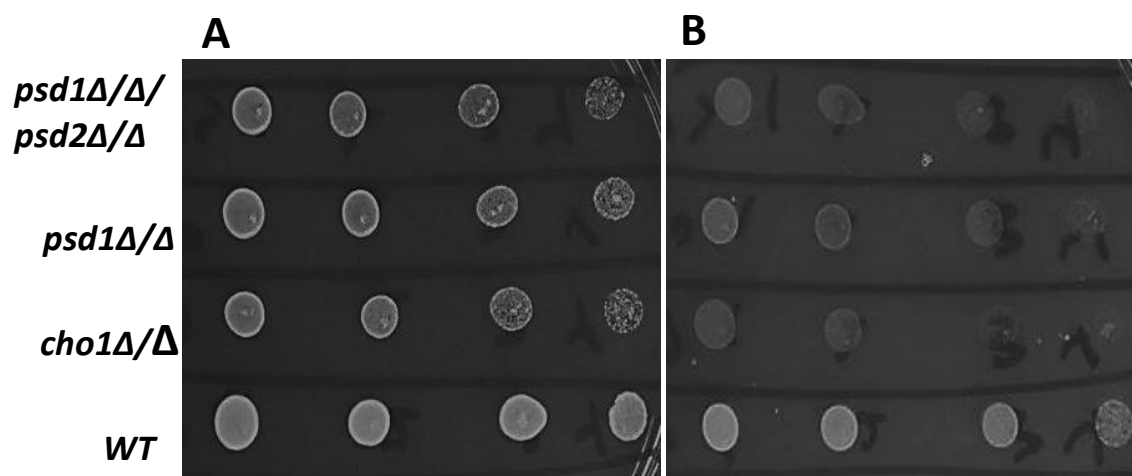


Figure 2-10: Sensitivity to the antifungal drug Fluconazole. The *cho1Δ/Δ*, *psd1Δ/Δ*, and *psd1Δ/Δpsd2Δ/Δ* strains are hypersensitive to the antifungal drug fluconazole, which targets lanosterol-4-demethylase, an enzyme involved in the biosynthesis of the fungal sterol; ergosterol. Serial dilutions of 5 μl of 1 O.D./ml of overnight cultured cells were spot inoculated on solid media containing either (A) no Drug or (B) 5 μg/ml drug of Fluconazole. Images were taken after 48 hours of growth at 37°C.

Role of specific lipids in affecting the cell wall, iron-limitation, and other phenotypes

Lipidomics analysis revealed that in addition to the loss of PS, specific species of PE, 16:n/18:n species were depleted. This is consistent with the fact that these species are most likely derived from PS, which itself is composed of 16:n/18:n species. We suspect that the defects seen in the *psd1* Δ/Δ *psd2* Δ/Δ mutant are due to specific loss of this species of PE, and not just general decreases in PE, as the Kennedy pathway can make more PE to compensate for loss of the *de novo* pathway, but its species are generally larger (18:n/18:n and 18:n/20:n). However, this remains to be tested experimentally.

Since PS is converted to PE in the mitochondria and endosome/Golgi by Psd1p and Psd2p, respectively, the observed phenotypes may be partly due to mitochondrial defects because of specific loss of 16:n/18:n PE or even endosomal sorting defects. Defects in mitochondrial function affect the cell wall in *C. albicans* (Dagley et al., 2011), and endosomal defects affect signaling pathways that regulate the cell wall, like the Rim101p pathway. Again, this remains to be explored.

Finally, the *cho1* Δ/Δ mutant has added defects, as it is missing *de novo* PE (16:n/18:n) and PS. PS has been shown to be enriched in the endosomal and plasma membranes, and could affect a number of signaling proteins that affect the cell wall such as the Rim101p pathway, which depends on the endosomal components for proper processing (Davis et al, 2010). Pkc1p has not been implicated and our lab has demonstrated that it is likely not significantly involved in these events (data not shown: Sarah Davis personal communication). Finally, PS is required for calcineurin to associate with membranes (Kennedy et al., 1997), *in vitro*, so this cell wall pathway could be affected as well. We also noted an increase in phosphatidylglycerol (PG) and phosphatidylinositol (PI) upon blocking *de novo* PE biosynthesis, suggesting a possible shuttling of CDP-DAG into PG and PI biosynthesis to prevent a build-up

of CDP-DAG in the cell (Blankenship et al. 2005). We observed differences in sensitivities to low pH that may be due to the cell wall defects (Figure 2-11).

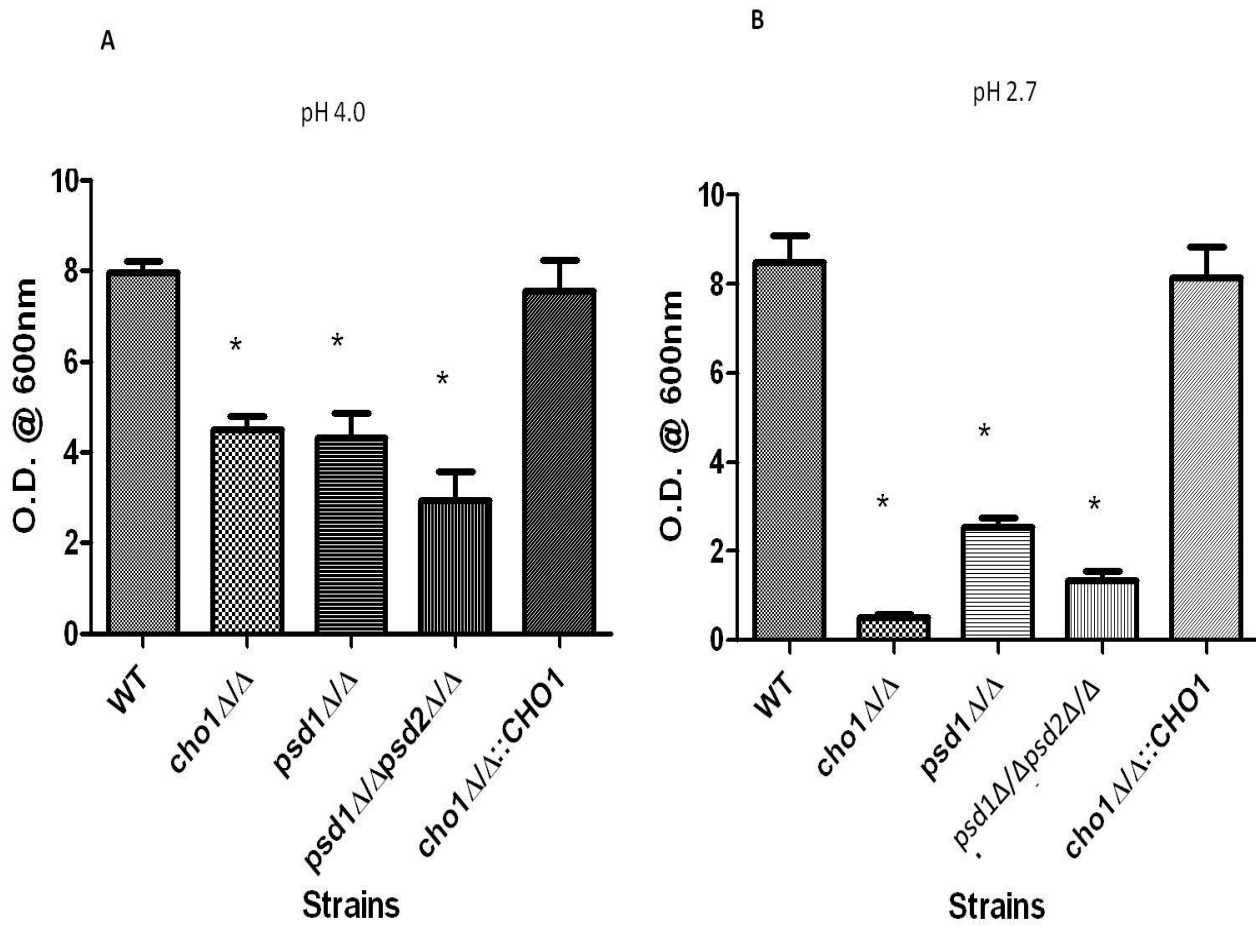


Figure 2-11: Sensitivity of *C.albicans* strains to low pH. Overnight cultures of *C.albicans* were inoculated into 5 ml of minimal media at the indicated pH (Panel A) pH 4.0 and (Panel B) pH 2.7 . The pH was adjusted by phosphate citrate buffer. The strains were grown at 37°C with shaking and the optical density was measured after 48 hours of growth.

Proposed model for the phenotypic effects observed upon a loss of *de novo* PS and PE in *Candida albicans*

We propose that the loss of phosphatidylserine (PS) in the fungal membrane leads to pleiotropic effects in *C. albicans*. These include, a defective cell wall as determined by sensitivity to the cell wall perturbing drugs, calcofluor white and congo red (Fig: 2-3) , differential expression of cell wall or glycosylphosphatidylinositol (GPI) anchored proteins (Tables 2-3 and 2-4), a defective response to iron limited conditions (Fig:2-5) and sensitivity to low pH (pH<4) (Fig:2-11). We hypothesize that the differences observed between the *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* mutants may be due to dysfunctional mitochondria as the *cho1Δ/Δ* strain shows a lowered expression of mitochondrial related genes as well as an up-regulation of genes involved in the neutralization of reactive oxygen species (ROS) generated within the mitochondria compared to the *psd1Δ/Δpsd2Δ/Δ* strain. These differences may be caused by changes in membrane fluidity due to a disruption of *de novo* aminophospholipid biosynthesis in the *cho1Δ/Δ* mutant or by a direct chaperone activity of PS, but the exact mechanism remains to be explored. We examined the phospholipidome of the mutants to determine what species of lipids were changed upon disrupting the *de novo synthesis* pathway. We discovered that the major lipid species absent in the *cho1Δ/Δ* mutant was PS 16:n/18:2 (Table 2-2) and noted a subsequent decrease in similar species of downstream *de novo* synthesized PE and PC. We also observed an increase in longer acyl chained 18:n/18:n PE species suggesting that the Kennedy pathway up-regulates synthesis of this PE species upon disruption of the *de novo* pathway. It is possible that loss of the anionic PS and a shift toward longer acyl chained Kennedy pathway derived PE and PC may result in a more rigid membrane structure and this could lead to improper protein localization inside the mitochondrial membrane, however, more experiments are necessary to directly test this hypothesis. We show that the *cho1Δ/Δ* strain has much more severe cell wall and mitochondria defects compared to the *psd1Δ/Δpsd2Δ/Δ* and

wild type strains. Our data suggest that mitochondrial dysfunction and generation of reactive oxygen species, in part, may account for the observed phenotypes in the *cho1Δ/Δ* strain and we demonstrate a novel role for PS in mitochondrial function. Firstly, transcriptional data is consistent with a hypoxic stress response in both the *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* strains (Table 2-9). Also, in support of this idea, the PS deficient *cho1Δ/Δ* strain up-regulates genes involved in neutralization of reactive oxygen species (ROS) (Table 2-8). ROS are generated within defective mitochondria and can lead to cell damage and/or death (Murphy et al., 2009). We observed an up-regulation of genes involved in breaking down the reactive oxygen species such as nitric oxide (NO) and hydrogen peroxide (H₂O₂) in the *cho1Δ/Δ* mutant but did not see these genes up-regulated in the *psd1Δ/Δpsd2Δ/Δ* strain. Previously published data demonstrated the *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* strains have defects in mitochondrial function but the full implications and differences between the strains remained unknown (Chen et al., 2010). Our transcriptional data indicates that *cho1Δ/Δ* has much more severe mitochondrial defects than *psd1Δ/Δpsd2Δ/Δ* which implicates PS in a previously unknown role in mitochondrial function. It is known that nitric oxide is generated when iron bound by a porphyrin (heme) fails to receive electrons from cytochrome c (Nicholls et al., 1998; Yi et al., 2012), and instead reacts to produce damaging nitric oxide in the mitochondrial inner membrane. It will be of interest to investigate if PS itself, through its anionic headgroup mediates this interaction either directly or indirectly, and how a loss of PS could affect electron transport and generation of ROS. We also demonstrate that, in support of this model, the *cho1Δ/Δ* strain represses iron-uptake genes and unlike the *psd1Δ/Δpsd2Δ/Δ* mutant does not up-regulate genes involved in iron uptake such as the canonical iron response regulator *SEF1*. This may be due to a shift toward glycolysis and a shut-down of aerobic respiration that mitigates production of oxygen radicals in the mitochondria in an effort to protect itself from generating damaging intracellular free radicals. The effect of this damage on cell signaling such as activation of ion or osmotic stress pathways like Hog1 and Rim101 remains to be explored. We did not see any transcriptional changes in Rim101 or Hog1,

but that does not necessarily mean that these pathways are not being activated as transcriptional activation is only a small part of mediating activation of signaling pathways as proteolytic cleavage and phosphorylation are more common ways to initiate a quick response to cell damage. It is possible that a combination of the cellular energy and metabolic state, the generation of reactive oxygen species, and defects in signaling pathways, all related to PS and/or PE deficiencies, lead to defects in cell wall protein gene expression and thereby lead to defects in the structure of the wall.

Losses of PS and PE have different consequences for *S. cerevisiae* and *C. albicans*

Phospholipid biosynthesis pathways are practically identical between *S. cerevisiae* and *C. albicans* (Chen et al., 2010). However, we wanted to determine if the phenotypes that result from altered aminophospholipids between the *S. cerevisiae cho1Δ* mutant and that of *C. albicans cho1Δ/Δ* are conserved. It was demonstrated that the *S. cerevisiae cho1Δ* mutant does not have a disordered and thick cell wall (Fig: 2-9). The chitin content, while increased, was only two-fold (Fig:2-8) compared to a ten-fold increase in the *C. albicans cho1Δ/Δ* mutant (Chen et al., 2010). Therefore, there exist differences in the phenotypic response to loss of PS between *S. cerevisiae* and *C. albicans*. It may be that the ability of *S. cerevisiae* to tolerate mitochondrial dysfunction compared to *C. albicans* (which unlike *S. cerevisiae* cannot survive a loss of the mitochondrial genome) may contribute to these cell wall phenotypes as cell wall and mitochondrial function have been linked by the cell signaling pathway Hog-1. This may indicate a fundamental difference between these two fungi, whereby in *C. albicans* cell wall maintenance and mitochondrial function may be more strongly linked compared to *S. cerevisiae*. However, the mechanism behind this difference remains to be tested. It may also be related to differences in conserved, but distinct, cell wall signaling pathways that exist in both microbes, such as calcineurin and/or Rim101p.

Conclusions

We show that a loss of *de novo* PS and PE biosynthesis has pleiotropic effects on the *Candida albicans* cell, including cell wall defects, defects in growth under iron limited conditions, sensitivity to low pH, sensitivity to the membrane-targeting drug fluconazole and differential expression of hypoxic, cell wall, and mitochondrial related genes. We also show that changes in the phospholipidome indicate that PS 16:0/18:2 is the main lipid species trafficked through the *de novo* pathway in *Candida albicans* and that there is a subsequent increase in long acyl side chain 18:n18n PE species, presumably, from increased Kennedy pathway PE and PC synthesis. The differential effects observed between the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ strain implicate PS as having a direct role in the observed phenotypes. Therefore, we suggest a novel role for PS in *Candida albicans*, whereby, PS itself is required for cell wall maintenance and loss of PS leads to both an altered cell wall and dysfunctional mitochondria. This is evidenced by the increased defects observed upon loss of PS compared to the defects observed upon a loss of PE alone. We also demonstrate that *C. albicans* and *S. cerevisiae* have different PS-deficient cell wall phenotypes and this highlights a major difference between the response of these fungi to changes in phospholipid composition. Therefore *S. cerevisiae* cannot be used as a model to fully extrapolate phospholipid-related phenotypes or responses in *C. albicans*. It will be of interest to further explore how these interactions are mediated and, in particular, to elucidate a direct mechanism for PS in cell wall maintenance and mitochondrial function.

APPENDIX B: TABLES

Table 2-1: *Candida albicans* (Ca) and *S. cerevisiae* (Sc) strains used in this study

Strain	Genotype	Reference
SC5314	Ca. wild-type	Gillum et al. 1984
YLC 337	<i>Ca.cho1Δ/Δ</i>	Y.Chen et al. 2010
YLC 344	<i>Ca.cho1Δ/Δ::CHO1-SAT1</i>	Y.Chen et al. 2010
YLC 345	<i>Ca.cho1Δ/Δ::CHO1-SAT1</i>	Y.Chen et al. 2010
YLC 280	<i>Ca.psd1Δ/Δ</i>	Y.Chen et al. 2010
YLC 294	<i>Ca.psd1Δ/Δ::PSD1-SAT1</i>	Y.Chen et al. 2010
YLC 375	<i>Ca.psd1Δ/Δpsd2Δ/Δ</i>	Y.Chen et al. 2010
TRY 153	<i>Sc.ura3Δ,his3Δ</i>	This study
TRY 155	<i>Sc.ura3Δ,his3Δ,cho1::KanMX6</i>	This study

Table 2-2: Differences in phospholipids species of the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains compared to the wild type strain. Values are relative amounts(ug) of each phospholipid species that were normalized to PE (phosphatidylethanolamine), PS (phosphatidylserine), and PC (phosphatidylcholine) standards in their own classes. The standards used were PE 28:0, PS30:0, and PC 28:0.

		Strains		
Species	m/z	Lipid	WT	<i>psd1</i> ΔΔ <i>psd2</i> ΔΔ
PE				
	710.4	16:1/18:3	8	6
	712.4	16:1/18:2	35	8
	714.4	16:0/18:2	134	18
	716.4	16:0/18:1	37	5
	736.4	18:2/18:3	64	51
	738.4	18:2/18:2	75	57
	740.4	18:2/18:1	70	63
	742.4	18:0/18:2	29	79
	744.5	18:0/18:1	17	18
	746.5	18:0/18:0	15	17
	762.5	18:2/20:4	17	11
	764.5	18:1/20:4	41	38
	766.4	18:0/20:4	47	57
	768.4	18:1/20:2	23	41
	770.4	18:0/20:2	16	14
	772.4	18:0/20:1	15	14
	Total amount		643	496
PS				
			WT	<i>psd1</i> ΔΔ <i>psd2</i> ΔΔ
	760.7	16:0/18:1	5	12
	756.7	16:1/18:2	51	127
	758.7	16:0/18:2	23	46
	Total amount		79	185
PC				
			WT	<i>psd1</i> ΔΔ <i>psd2</i> ΔΔ
	730.4	16:0/16:2	10	10
	732.5	16:0/16:1	12	12
	734.4	16:0/16:0	18	18
	752.4	14:0/20:5	9	9
	754.4	16:2/18:2	62	15
	756.5	16:1/18:2	107	22

Table 2-2 continued.				
m/z	lipid	WT	<i>psd1</i>Δ/<i>psd2</i>Δ/Δ	<i>cho1</i>Δ/Δ
760.5	16:0/18:1	137	34	33
762.6	16:0/18:0	112	24	21
778.4	16:2/20:4	78	112	121
780.4	16:1/20:4	106	147	157
782.5	18:2/18:2	66	108	132
784.5	18:1/18:2	49	84	91
786.5	18:1/18:1	35	44	52
788.5	18:0/18:1	24	29	26
808.5	18:1/20:4	6	13	10
810.5	18:0/20:4	8	24	24
812.5	18:1/20:2	12	33	13
814.5	18:0/20:2	14	26	14
816.5	18:0/20:1	7	10	8
Total amount		1025	811	814

Table 2-2. n.d.= not detected. m/z= charge to mass ratio of species.

Lipid component is described as number of carbon atoms: saturation number/ number of carbon atoms: saturation number.

Table 2-3: Cell Wall related genes up-regulated in the *cho1* Δ/Δ mutant compared to wild type

Gene	Fold up-regulated	Function	p- value
<i>PGA31</i>	84	Cell wall protein	1.0E-300
<i>PGA23</i>	32	GPI-anchored protein transcription is negatively regulated by Rim101p	1.0E-300
<i>RBR1</i>	31	GPI-anchored cell wall protein required for filamentous growth at acidic pH	4.6E-70
<i>PGA58</i>	13	Putative GPI-anchored protein	1.9E-158
<i>PGA13</i>	6	cell wall regeneration and core caspofungin response	1.0E-300
<i>IFF11</i>	5	Secreted protein required for normal cell wall structure and for virulence	7.1E-46
<i>AMS1</i>	4	Adhesin-like cell wall protein	7.0E-180
<i>HWP1</i>	3	N-linked mannoprotein of cell wall and membrane	1.0E-300
<i>SAP9</i>	3	Secreted aspartyl protease; roles in adhesion	1.0E-300
<i>orf19.2076</i>	3	N-linked mannoprotein of cell wall and membrane	4.2E-54
<i>PGA44</i>	3	GPI-anchored protein	6.5E-12
<i>SAP10</i>	2	Secreted aspartyl protease	1.6E-12
<i>IFF6</i>	2	Putative GPI-anchored adhesin-like protein	2.6E-20
<i>orf19.12206</i>	2	Cell wall adhesin	5.4E-50

Table 2-4: Cell wall-related genes up-regulated in the *psd1* Δ/Δ *psd2* Δ/Δ mutant compared to wild type.

Gene	<i>psd1</i>Δ/Δ <i>psd2</i>Δ/Δ > WT (fold up-regulated)	Function	p value
<i>RBT5</i>	4	GPI-anchored cell wall protein involved in hemoglobin utilization	7.08E-55
<i>PGA54</i>	3	Putative GPI-anchored protein	3.47E-149
<i>PGA18</i>	3	GPI-anchored protein	7.99E-51
<i>PGA27</i>	3	GPI-anchored protein	3.40E-15
<i>PGA55</i>	3	GPI-anchored protein; adhesin-like protein; filament induced	7.66E-23
<i>PLB5</i>	2	GPI-linked phospholipase B	6.87E-27
<i>PGA1</i>	2	induced during cell wall regeneration; required for normal adhesion	1.76E-19
<i>CHT2</i>	2	GPI-linked chitinase required for normal filamentous growth	1E-300
<i>HYR4</i>	2	GPI-anchored adhesin-like protein	1E-16
<i>orf19.2677</i>	2	Putative GPI transamidase component	2.9E-48
<i>PGA25</i>	2	GPI-anchored adhesin-like protein	4.5E-24

Table 2-5: Cell wall genes up-regulated in both *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ mutants compared to the wild-type

Gene	<i>cho1</i>ΔΔ	<i>psd1</i>ΔΔ<i>psd2</i>ΔΔ	Function	p value <i>psd1</i>ΔΔ<i>psd2</i>ΔΔ Δ vs wt	p value <i>cho1</i>ΔΔ
<i>PGA10</i>	9	4	Plasma membrane protein of heme-iron utilization	2.4E-17	7.8E-98
<i>ECM331</i>	9	5	GPI-anchored protein	1.2E-62	1.5E-200
<i>ALS2</i>	8	4	Cell wall adhesion	1.1E-65	1.0E-300
<i>ALS3</i>	5	5	Cell wall adhesin	3.1E-177	1.0E-300
<i>HYR1</i>	4	3	Hyphal-induced GPI-anchored cell wall protein; macrophage-induced	2.6E-30	1.4E-50
<i>ALS4</i>	4	3	Cell Wall adhesin	1.3E-77	1.0E-300
<i>GPI1</i>	4	4	Protein GPI synthesis	1.1E-48	3.5E-48
<i>ALS1</i>	3	5	Adhesin	1.0E-300	1.0E-300
<i>PGA6</i>	3	6	cell wall adhesion	1.0E-300	1.0E-300
<i>HYR3</i>	3	2	adhesin-like protein	7.1E-99	1.1E-174
<i>IFF5</i>	2	2	GPI-anchored protein; adhesin-like protein	9.6E-23	1.35E-35
<i>ALS9</i>	2	3	Cell wall adhesin	7.3E-32	2.7E-34
<i>PGA45</i>	2	2	GPI-anchored protein	4.5E-194	9.4E-211

Table 2-6: Iron response genes down-regulated ≥ 3 fold in the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants compared to wild-type

Gene name	WT > <i>cho1</i> Δ/Δ (fold downregulated)	WT> <i>psd1</i> Δ/Δ <i>psd2</i> Δ/Δ (fold downregulated)	Cellular function	P-value
<i>CFL2</i>	-11.3	-10	Putative oxidoreductase, iron utilization	1E-300, 1E-300
<i>FRE10</i>	-5.0	-4	Ferric reductase	1E-300, 1E-294
<i>FTR1</i>	-4.3	no change	High affinity iron permease	4.2E-171
<i>CHA1</i>	-3.4	no change	Regulated by Rim101p and iron	3.0E-67
<i>orf19.2067</i>	no change	-3	Putative protein with a predicted role in mitochondrial iron metabolism	7.4E-284

Table 2-7: Iron response genes down-regulated ≥ 2 fold in the *cho1* Δ/Δ mutant compared to *psd1* Δ/Δ *psd2* Δ/Δ mutant

Gene name	<i>cho1</i> Δ/Δ <i>psd1</i> Δ/Δ <i>psd2</i> Δ/Δ	Cellular function	p-value
<i>RBT5</i>	-5	Cell wall-Hemoglobin utilization	2.2E-88
<i>orf19.4459</i>	-3	Heme binding	2.2E-90
<i>HAP3</i>	-3	Iron regulation gene	8.0E-18
<i>orf19.6976</i>	-3	Heme carrier protein	3.1E-49
<i>CCC2</i>	-2	Iron assimilation	2.3E-61
<i>PGA32</i>	-2	Cell wall-regulated by iron	4.8E-16
<i>FTR1</i>	-2	High affinity iron permease	7.0E-47

Table 2-8: The *cho1* Δ/Δ strain up-regulates genes associated with neutralization of reactive oxygen species (ROS).

Gene	Fold up-regulated in <i>cho1</i>Δ/Δ vs <i>psd1</i>Δ/Δ<i>psd2</i>Δ/Δ	Function	P-value <i>cho1</i>Δ/Δ vs <i>psd1</i>Δ/Δ<i>psd2</i>Δ/Δ
orf19.10993	112	H ₂ O ₂ stress	1.0E-300
AOX2	63	Alternative oxidase	1.0E-300
SOD5	15	superoxidedismutase; oxidative stress	1.0E-300
YHB1	7	Nitric oxide dioxygenase	1.0E-300
orf19.711	7	Uncharacterized ORF induced by nitric oxide	1.0E-34
PCK1	5	Phosphoenolpyruvate carboxykinase;H ₂ O ₂ stress	1.0E-300

Table 2-9: Hypoxic genes up-regulated in the *cho1*ΔΔ and *psd1*ΔΔ*psd2*ΔΔ strains compared to WT.

Gene	<i>cho1</i> ΔΔ	<i>psd1</i> ΔΔ <i>psd2</i> ΔΔ	Function	p value <i>cho1</i> ΔΔ vs WT	p-value <i>psd1</i> ΔΔ <i>psd2</i> ΔΔ vs WT
<i>ECM331</i>	9	5	GPI-anchor protein; hypoxia induced	1.5E- 200	1.2E-62
<i>WH11</i>	6	2	cytoplasmic protein; hypoxia induced	6.0E-146	2.1E-10
<i>orf19.1691</i>	3	3	uncharacterized	1.0E-300	1.6E-200
<i>ERG3</i>	3	3	C-5 sterol desaturase;	1.0E-300	3.3E-168
<i>ERG11</i>	3	3	Lanosterol 14-alpha- demethylase	1.0E-300	1.0E-300
<i>SLD1</i>	3	3	Sphingolipid delta-8 desaturase	1.7E-148	5.5E-82
<i>RBE1</i>	no change	2	Cell wall protein	6.1E-31	3.4E-64
<i>orf19.6117</i>	2	no change	uncharacterized; hypoxia induced	1.3E-97	1.9E-72
<i>CSH1</i>	3	2	Aldo-keto reductase family member	3.1E-245	2.5E-78
<i>PNG2</i>	6	3	Putative peptide:N- glycanase	1.0E-300	1.8E-160
<i>GDH3</i>	8	11	NADP-glutamate dehydrogenase	1.0E-300	1.0E-300

Table 2-10: Iron uptake genes up-regulated in *psd1* Δ *psd2* Δ mutant compared to the wild type

Gene	<i>psd1</i> Δ / <i>psd2</i> Δ (fold up-regulated)	Function	p-value
orf19.1195	5	Cellular iron ion homeostasis	1.0E-300
RNR3	5	Ribonucleotide reductase	1.7E-82
PGA10	4	Protein of heme-iron utilization	2.4E-17
ALS2	4	Adhesin	1.1E-65
SFU1	4	Transcriptional regulator of iron	8.0E-149
SEF2	3	Putative zinc cluster protein	8.0E-95
FTR2	3	High-affinity iron permease	1.0E-300
SEF1	3	Regulates iron uptake	1.5E-64
orf19.2863.1	3	Cellular iron ion homeostasis	2.1E-74
FTH2	2	Putative iron transporter	4.5E-52
HAP2	2	CCAAT-binding factor	2.0E-21
MNT4	2	Mannosyltransferase	2.5E-11
CCC2	2	Copper-transporting ATPase	2.3E-24
MNN2	2	Alpha-1,2-mannosyltransferase	5.1E-75

CHAPTER THREE

Conclusions and future directions

Abstract

In this study, we examine specific changes to the phospholipidome that may help explain some of the observed phenotypic differences for the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ strains.$

Phospholipid profiling revealed that PS 16:018:2 is the major PS species absent in the *cho1* Δ/Δ strain and this is likely the major lipid species trafficked through the *de novo* pathway in *Candida albicans*. We also noted a shift toward longer acyl chained PE species in the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ strains suggesting that these species are synthesized via the Kennedy pathway in *C. albicans*. Defects in mitochondrial function are a consequence of phosphatidylethanolamine (PE) deficiency, and loss of PS causes a loss of *de novo* synthesized PE (Kent et al., 1995; Chen et al., 2010). However, a comparison between mutants lacking just *de novo* PE (*psd1* $\Delta/\Delta *psd2* Δ/Δ) and both PS and *de novo* PE (*cho1* Δ/Δ) reveals that mitochondrial and cell wall defects are more severe in the *cho1* Δ/Δ mutant, suggesting a direct role for PS in both of these functions.$$

Introduction

The aim of our study is to elucidate the functions of *de novo* phospholipid biosynthesis in *Candida albicans*. We found that disruption of *de novo* phospholipid biosynthesis leads to both a defective cell wall and dysfunctional mitochondria. Our data suggests that loss of phosphatidylserine (PS) itself specifically affects mitochondrial function and leads to the down-regulation of iron uptake genes and up-regulation of hypoxia induced genes as well as genes involved in cell wall repair, and generation of a transcriptional response to neutralize intracellular reactive oxygen species (ROS) in the fungal pathogen *Candida albicans*. Defects in mitochondrial function are a well-known consequence of phosphatidylethanolamine (PE) deficiency, and loss of PS causes a loss of *de novo* synthesized PE (Kent et al., 1995; Chen et al., 2010). However, a comparison between mutants lacking just *de novo* PE (*psd1* Δ/Δ *psd2* Δ/Δ) and both PS and *de novo* PE (*cho1* Δ/Δ) reveals that mitochondrial and cell wall defects are more severe in the *cho1* Δ/Δ mutant, suggesting a direct role for PS in both of these functions.

The defects in the cell wall in the *cho1* Δ/Δ mutant may be partially or completely attributable to mitochondrial defects as recent studies link cell wall defects to mitochondrial dysfunction (Dagley et al. 2011, Qu et al., 2012). The role of PS in mediating mitochondrial function has not been well described. We propose a model wherein PS loss leads to dysfunctional mitochondria, which in turn regulates the pathogen's response to hypoxia and iron limitation. In response to a loss of PS, we propose that *cho1* Δ/Δ activates compensatory pathways involved in cell wall integrity and neutralization of damaging oxygen species. We also report that PS plays divergent roles in *Saccharomyces cerevisiae* as we do not see similar cell wall defects in the *S. cerevisiae cho1* Δ strain. This demonstrates a fundamental difference in phospholipid roles between *Candida albicans* and *Saccharomyces cerevisiae* and suggests that

this model organism should be used only with caution in elucidating the roles of these phospholipids in *Candida albicans*.

Summary of Results

In this study, we examine specific changes to the phospholipidome that may help explain some of the observed phenotypic differences for the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ strains.$

Phospholipid profiling revealed that PS 16:018:2 is the major PS species absent in the *cho1* Δ/Δ strain and this is likely the major lipid species trafficked through the *de novo* pathway in *Candida albicans*. We also noted a shift toward longer acyl chained PE species in the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ strains suggesting that these species are synthesized via the Kennedy pathway in *C. albicans*.$

Linking cell wall defects and mitochondrial dysfunction in the *C. albicans cho1* Δ/Δ strain

A comparison of the transcriptional profiles of the *cho1* Δ/Δ strain to the *psd1* $\Delta/\Delta*psd2* Δ/Δ strain indicates that the *cho1* Δ/Δ strain has more defective mitochondria as it up-regulates genes involved in neutralization of mitochondrial-generated reactive oxygen species (ROS). Recent advances in the study of mitochondrial and ER-localized protein complexes provide a direct link between mitochondrial dysfunction and cell wall defects in *Candida albicans* (Qu et al., 2012, Alonso-Monge et al., 2009). For example, the SAM (Sorting and Assembly Machine) complex localized within the mitochondria has been shown to be important for maintaining mitochondrial function and its deletion (*sam37* Δ/Δ) leads to cell wall defects in *Candida albicans* (Qu et al., 2012). In addition, this complex has been hypothesized to be involved in the transfer of phospholipids to and from the mitochondria as it shares Mdm10, a key component of the ER-Mitochondrial Encounter Structure (ERMES) which aides in tethering mitochondria to the ER to facilitate phospholipid transfer (Dagley et al., 2011 Osman et al., 2012). The *sam37* Δ/Δ mutant, like the *cho1* Δ/Δ mutant, exhibits increased chitin content and sensitivity to the cell wall$

perturbing drugs calcofluor white and congo red (Qu et al., 2012). Interestingly, Qu et al. demonstrate that the *sam37* Δ/Δ mutant showed a loss of mitochondrial DNA (mtDNA) indicating that the mitochondrial genome is partially lost upon disruption of this SAM37 complex. This data correlates with the defects in the cell wall observed in the *sam37* Δ/Δ mutant, including the observation that, like the *cho1* Δ/Δ strain, *sam37* Δ/Δ has a thickened cell wall by transmission electron microscopy, suggesting that a deletion of SAM37 leads to cell wall defects and an inability of *Candida albicans* to maintain the mitochondrial genome by an as yet unidentified signaling pathway (Qu et al., 2012). It therefore may be possible that loss of PS affects this complex, directly or indirectly and that some of the shared phenotypes may be due to a conserved mechanism linking mitochondrial dysfunction to cell wall defects.

We speculate that the phospholipid environment of the mitochondria, in particular the loss of PS, may lead to a loss of either the mitochondrial genome or transcription of many of its genes. We also identified nuclear gene products localized to the mitochondria that were down-regulated in the *cho1* Δ/Δ versus *psd1* $\Delta/\Delta*psd2* Δ/Δ strain including *MCD1*, *SEN2*, *orf19.2090* and *orf19.4273*. *MCD1* acts to regulate mitochondrial membrane potential upon oxidative stress in *S. cerevisiae* (Yang et al., 2008) but has not been studied in *Candida* species, *SEN2* is a mitochondrial localized endonuclease and *orf19.2090*, *orf19.4273* are found in the mitochondria but are of unknown functions (Candida genome database). In addition, we have identified several genes such as *COX2*, *COX3A*, *COX3B*, *NAD1*, *NAD3*, and *NAD6*, which are all encoded by the mitochondrial genome and directly involved in the electron transport chain, that are down-regulated in the *cho1* Δ/Δ mutant compared to the *psd1* Δ/Δ *psd2* Δ/Δ mutant suggesting a difference in mitochondrial genome stability. However, these genes need to be independently verified by other means such as qPCR as in our study they were not significantly different despite their two fold down-regulation. Taken together, this data suggests an inhibition of the electron transport chain, which may result in respiratory deficiency and the production of$

reactive oxygen species (ROS). In addition, the observed loss of mitochondrial function and the hypothesized electron transport dysfunction may explain the link between cell wall defects as these genes are down-regulated in both the *cho1* Δ/Δ and *sam37* Δ/Δ strains (Qu et al., 2012).

It may be that mitochondrial dysfunction in both the *cho1* Δ/Δ and *sam37* Δ/Δ mutants may lead to activation of cellular mechanisms normally expressed in response to changing metabolic and oxygen conditions in *Candida albicans*, which can be induced upon mitochondrial-generated ROS (Synnott et al., 2010). It is known that hypoxic conditions can cause changes in expression of iron uptake genes and lower sterol levels in the *Candida albicans* membrane (Dhamgaye et al. 2010; Sosinska et al. 2008; Mungai et al., 2011). It may also be that loss of PS causes defects in respiration that lead to the observed increase in ROS-related genes which may also be tied to the down-regulation of iron transport genes (the products of which are integral to the electron transport chain via heme interactions with cytochrome c) and an increase in hypoxia induced genes. It is also possible that the Hog1 signaling pathway may be activated upon loss of PS due to a more fluid membrane as it is known to be activated by changes in membrane fluidity (Hickman et al., 2011). Therefore, *C. albicans* may have evolved a mechanism where mitochondrial dysfunction leads to production of ROS which initiates a response that alters cell wall structure, regulates iron uptake, neutralizes damaging free radicals, and thus allows the fungus to thrive in diverse environmental niches of varying carbon sources and environmental stresses. In addition, it is known that respiratory deficiency in *S. cerevisiae* leads to increased mRNA stability of the *cho1* Δ transcript which also supports the idea that PS has some function in mitochondrial maintenance (Carmen et al., 2007). Our work examines some of these connections and presents a model where PS is critical for normal cell wall structure and mitochondrial function *C. albicans*.

A proposed role for phosphatidylserine in hypoxic response and iron uptake

We found that both the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ strains are sensitive to the anti-fungal drug fluconazole, although *cho1* Δ/Δ was much more sensitive than *psd1* Δ/Δ *psd2* Δ/Δ .

Fluconazole targets the Erg11 (lanosterol-14 α -demethylase) enzyme that is involved in the biosynthesis of the major fungal sterol, ergosterol. It is known that *ERG11* is up-regulated under hypoxic conditions (low oxygen, ~1% O₂) and, sterols are important markers of hypoxic response (as molecular oxygen is required for ergosterol biosynthesis) in several fungi including *Candida albicans*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Aspergillus fumigatus* (Hughes et al., 2006; Blatzer et al., 2011; Synnott et al., 2010; Hickman et al., 2011; Blosser et al., 2012). The decreased expression of mitochondria related genes and the production of ROS in *C. albicans* may mimic hypoxia. In *Candida albicans* and *Saccharomyces cerevisiae*, heme biosynthesis is also central in mediating the response to hypoxia (Lai et al., 2008; McDonagh et al., 2011; Sosinska et al. 2008). In *Candida albicans*, the transcription factor *UPC2* directly activates hypoxic response upon lowered sterol levels (Synnott et al., 2010). In parallel, the heme binding transcription factor *HAP1* acts to reduce aerobic respiration and activates expression of hypoxic genes in low oxygen environments (Synnott et al., 2010; Sing et al. 2011). In *S. cerevisiae*, under normoxic conditions *HAP1* binds directly to heme and activates the transcriptional repressor *ROX1*, but upon loss of molecular oxygen, heme biosynthesis ceases, as oxygen is required for the biosynthesis of heme, and *HAP1* can no longer bind to heme, thereby causing the loss of the repressor and activation of hypoxia induced genes (Synnott et al., 2010). The ortholog to *ROX1* in *C. albicans*, *RFG1*, does not mediate hypoxic response but rather activates cell wall enzymes required for filamentation (Synnott et al., 2010). This may, in part, explain how *cho1* Δ/Δ exhibits changes in cell wall structure, increased filamentation (Fig 2-2) (Chen et al. 2010), and the repression of iron uptake genes. In *C. albicans*, an increase in ROS by decreased expression of the

alternative oxidase (AOX1) has been shown to induce hypersensitivity to fluconazole (Yan et al., 2009). Thus, increased ROS due to a loss of PS may be responsible for the observed phenotypes in the *cho1Δ/Δ* mutant. This is consistent with our proposed model of mitochondrial dysfunction leading to these phenotypes as decreased oxygen levels have been linked to generation of ROS (Mungai et al., 2011) and a reduction in heme biosynthesis.

In *psd1Δ/Δpsd2Δ/Δ* increased ROS may be mitigated by activation of the iron uptake transcriptional activator *SEF1* and this may explain why growth under iron limited conditions, while defective, is not as deficient as that observed in the *cho1Δ/Δ* strain. *SEF1* acts to up-regulate heme biosynthesis genes and iron utilization genes (Chen et al., 2011). This may aide in stabilizing the slight defects in the mitochondria observed in the *psd1Δ/Δpsd2Δ/Δ* strain by increasing heme biosynthesis and thereby reducing generation of ROS.

In addition to less of a defect with regards to iron assimilation genes, the *psd1Δ/Δpsd2Δ/Δ* strain grew better than the *cho1Δ/Δ* strain under iron limited conditions. This indicates a role for PS in iron response in *Candida albicans*. It could be that loss of PS inherently alters iron uptake and this causes defects in iron related processes such as heme biosynthesis and uptake. Alternatively, the observed hypoxic-like response causes decreased expression of iron uptake genes as oxygen and iron sensing are tightly linked (Sosinska et al., 2008, Dhamgaye et al., 2012, Chen et al., 2011). This suggests that *cho1Δ/Δ* may shift away from aerobic respiration possibly to mitigate damaging effects of ROS due to dysfunctional mitochondria. The observed up-regulation of AOX2 (Alternative Oxidase 2) in *cho1Δ/Δ* compared to the *psd1Δ/Δpsd2Δ/Δ* supports this idea, as AOX2 is an electron acceptor which has recently been shown to be involved in the neutralization of reactive oxygen species generated from leaky electron transport chains (Cvetkovska et al., 2012).

We found that the *cho1* Δ/Δ mutant up-regulated genes involved in hypoxic response and neutralization of H₂O₂ (hydrogen peroxide) and NO (Nitric oxide) compared to the wild type strain. These reactive oxygen species can be generated by dysfunctional mitochondrial electron transport chains, NO is produced upon failure of cytochrome c to transfer electrons to heme during aerobic respiration (Bourens et al., 2012). It is also known that reactive oxygen species (ROS) can inhibit mitochondrial function and biogenesis in *S. cerevisiae* but little is known of their effect in *C. albicans* (Chevtzoff et al., 2010).

Taken together our data indicate a novel function for phosphatidylserine (PS) in maintaining mitochondrial function and cell wall structure. We propose a model whereby loss of PS leads to a hypoxic type response that shuts down iron uptake and heme biosynthesis, and generation of reactive oxygen species that activate cell wall integrity stress pathways and leads to differential cell wall protein expression. This could be due to changes in membrane fluidity caused by the loss of PS but more experiments are necessary to confirm the role of membrane fluidity in these processes. It may also be that PS acts directly as a molecular chaperone either through interaction with Sam37p or with components of the electron transport chain such as cytochromes. As PE has been shown to act as a molecular chaperone in *E. coli*, through interaction with the membrane protein lactose permease (Xie et al., 2006), a chaperone function has not yet been described for PE or PS in yeast. The pathways most likely to be activated upon loss of PS are the Rim101 pathway, shown to be linked to hypoxia and ion stress, or the Hog1 pathway which has been linked both to mitochondrial defects and changes in membrane fluidity in *S. cerevisiae* (Hickman et al., 2011; Ikeda et al., 2008).

The defects observed in *psd1* Δ/Δ *psd2* Δ/Δ are not as severe as in the *cho1* Δ/Δ and they can possibly be explained by the observed shift in acyl length of the phosphatidylethanolamine (PE) species leading to less severe changes in mitochondrial function when compared to *cho1* Δ/Δ mutant, as it still has PS present in the membrane. It may also be that greater changes in

membrane fluidity due to a loss of phosphatidylserine (PS) cause these differential wall and mitochondrial phenotypes in *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* strains. In *S. cerevisiae*, hypoxic response has been directly linked to changes in membrane fluidity via the transcription factors *MGA2* and *SPT23* (Jiang et al., 2001, Kelley et al., 2009) but this remains to be tested in *Candida* species.

Our study also demonstrates that *C. albicans* and *S. cerevisiae* do not conserve cell wall phenotypes associated with disruption of *de novo* phospholipid biosynthesis, suggesting they have evolved different mechanisms to respond to stresses associated with disruption of phospholipid biosynthesis. In support of this, it has been shown that the hypoxic response and iron sensing pathways are not identical between *S. cerevisiae* and *C. albicans*. This may be due to the fact that *C. albicans* is not viable upon loss of its mitochondrial genome (a petite negative yeast) whereas *S. cerevisiae* (a petite positive yeast) is better able to overcome mitochondrial genome loss and the problems associated with loss of PS. In *S. cerevisiae* *psd1Δ psd2Δ* and *cho1Δ* strains require PE for growth on non-fermentable carbon sources and this growth is correlated to an increase in mitochondrial PE content (Birner et al. 2001). PE depletion also causes increased respiratory deficiency suggesting that PE itself may play a more direct role in *S. cerevisiae* mitochondrial dysfunction and cell wall phenotypes. This is in contrast to *C. albicans* where a loss of PE alone does not explain the observed cell wall and mitochondrial defects suggesting that PS has an independent function in this petite negative yeast.

Future Directions

To test the hypothesis that the *cho1Δ/Δ* strain produces more reactive oxygen species (ROS) compared to the *psd1Δ/Δpsd2Δ/Δ* and wild type (SC5314) strains, the dye Lucigenin (Invitrogen™) that specifically binds to reactive oxygen species and produces a quantifiable colorimetric signal can be used to measure differences in ROS between *cho1Δ/Δ*, *psd1Δ/Δpsd2Δ/Δ*, and wild type (SC5314) strains. Also, it will be of interest to delete *AOX2* in

the *cho1Δ/Δ* strain, since it may be involved in preventing electron leakage from the electron transport chain and thereby compensate for generation of ROS in the *cho1Δ/Δ* strain. A *aox2Δ/Δ cho1Δ/Δ* double mutant could be used to test if this can further increase damage caused by ROS generation compared to wild-type, *cho1Δ/Δ*, and *aox2Δ/Δ* single mutants. Alternatively, the AOX inhibitor, salicylhydroxamic acid (SHAM) can be added to the media to test if blocking Aox1p and Aox2p function enhances the mitochondrial and cell wall defects observed in the *cho1Δ/Δ* strain. Specific dyes that bind to nitric oxide, hydrogen peroxide and O₂-species, such as MitoSOX red (Mukhopadhyay et al., 2007, Cvetkovska et al., 2012), or DAF-FM (which measures NO production) can also be utilized in our mutant strains. These experiments will provide a method to directly test our hypothesis that the *cho1Δ/Δ* strain produces reactive oxygen species (ROS) due to dysfunctional mitochondria.

Transcriptional profiling revealed a possible down-regulation of mitochondrial-encoded genes such as *COX1* and *COX2*. This can be directly tested by qPCR of DNA. It is speculated that down-regulation of mitochondrial genes reflects a partial loss of mitochondrial genome in *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* mutants, and this can be assayed by staining the cells with 4',6-diamidino-2-phenylindole (DAPI) to visualize for punctate spots that are indicative of a loss of the mitochondrial genome. Alternatively, mitochondrial morphology changes can be examined using Mitotracker® Red (a dye that stains mitochondria) and transmission electron microscopy (TEM) for more detailed analysis.

Since there are many similarities between the *sam37Δ/Δ* and the *cho1Δ/Δ* mutants, it might also be relevant to delete the SAM37 complex component, Mdm10, to test if this complex is responsible for any of the phenotypes seen in the *cho1Δ/Δ* strain. Since mislocalization of Mdm10 causes mitochondrial and cell wall defects (Qu et al., 2012), examining the localization of a C-terminally GFP-tagged Mdm10p or Sam37p may be useful to determine if this key protein is mislocalized due to loss of PS in the *cho1Δ/Δ* mutant. This can be accomplished by

fluorescent microscopy. Conversely, it will be of interest to also measure the phospholipid composition of the mitochondria in both the *sam37Δ/Δ* and *cho1Δ/Δ* strains to directly measure differences in phospholipids and how this may directly affect mitochondrial function by coupling it with morphological studies and oxygen consumption assays to measure electron transport chain activity.

The cell wall phenotypes will also be tested under low and high oxygen conditions (using an anaerobic chamber to control oxygen levels) to assay if hypoxic conditions exacerbate these cell wall defects in the *cho1Δ/Δ*, *psd1Δ/Δpsd2Δ/Δ* and wild type (SC5314) strains; we expect a large decrease in available oxygen would limit free radical production. Strains grown under anoxic conditions and normoxic conditions could be subjected to TEM to determine if the *cho1Δ/Δ* mutants' cell wall defects are present even under limited oxygen conditions. It is expected that the further reducing oxygen levels in the environment may mitigate wall defects by reducing electron transport activity and thereby reducing reactive oxygen species. If our model is valid and the wall defects are due to activation of cell wall repair pathways induced by ROS, we would expect to see no differences in sensitivities to cell wall perturbing drugs calcofluor white and congo red between the *cho1Δ/Δ*, *psd1Δ/Δ psd2Δ/Δ*, and wild type strain upon neutralization of ROS in the presence of the scavengers 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or terbinafine.

The role of iron in mediating these processes remains to be tested. For example, does exogenous heme addition abrogate the cell wall defects in the mutant and what effect would addition of heme have on the cell wall phenotypes, response to hypoxia, or Als adhesin and other cell wall protein expression? It will be of interest to determine the role of *SEF1* in these processes by knocking out *SEF1* in *psd1Δ/Δpsd2Δ/Δ* and assaying if it has more severely altered cell walls like those observed in the *cho1Δ/Δ* strain

It would also be of interest to determine if deletion of *ERG11* may change the fluidity of the membrane and transcriptionally effect hypoxic response gene expression in *C. albicans*. Gene expression can be quantified and measured by RNA sequencing or qRT PCR. A more direct approach to measure membrane fluidity in the *cho1Δ/Δ* and *psd1Δ/Δpsd2Δ/Δ* strains would also be extremely valuable as inherent lipid polymorphisms (see chapter 1) most likely influence biochemical interactions between proteins and lipids and/or lipid-lipid interactions in membranes. This assay may be accomplished by generating protoplasts and using the dye 1,6-diphenyl-1,3,5-hexatriene that intercalates within the membrane and then measuring its change in optical absorbance (which is directly dependent on membrane fluidity). As we hypothesized that the *cho1Δ/Δ* strain has a less fluid membrane, we predict an increase in fluorescence of this dye in *cho1Δ/Δ* mutant compared to the wild type or *psd1Δ/Δpsd2Δ/Δ* strains.

In vitro phosphatidylserine (PS)/phosphatidylethanolamine (PE) liposome complexes may also be a valuable tool to test the hypothesis that phosphatidylserine (PS) directly interacts with components of the electron transport chain. Assays to determine the effects of phospholipid structural changes, including acyl chain length and saturation on the interactions of components of the electron transport chain such as the interaction of cytochrome c with heme in the mitochondrial inner membrane may indicate a previously unknown role for phosphatidylserine (PS) in facilitating correct orientation and proper protein-protein and/or protein-lipid interactions within these membranes.

The identity of the specific signaling pathway activated by loss of phosphatidylserine (PS) and/or *de novo* phosphatidylethanolamine (PE) may provide a direct mechanism for how these specific phospholipid changes cause defects in the *Candida albicans* cell wall. The role of the signaling pathways - Rim101, the major ion stress response pathway and Hog-1, an osmo-sensing mitochondrial activated pathway should be investigated (Davis et al., 2011, Alonso Monge et al., 2008). Western blotting can help elucidate if these pathways are activated and if

they are involved in the cell wall phenotypes in the *cho1* Δ/Δ strain. It would be of interest to test the effect of cell wall glycosylphosphatidylinositol (GPI) anchor proteins that are highly differentially expressed between the strains on the cell wall phenotypes by deleting genes such as *PGA31* and *ECM331* and assaying the effect these deletions have on cell wall ultrastructure and biochemical make up.

Conclusions

Taken together, these experiments should provide a better understanding of the role of PS in maintaining the cell wall and mitochondria in *C. albicans*. The diverse cellular processes that are influenced by phospholipids are just being uncovered and the ability to use phospholipid biosynthesis as a tool for elucidating drug targets and chemotherapies against the fungal pathogen *Candida albicans* is a promising area for future research.

APPENDICES

Supplementary Information and Tables

Abstract

The *cho1* Δ/Δ mutant is effectively cleared five days post-infection and therefore it may serve as an ideal live vaccine strain. The *cho1* Δ/Δ strain is however not viable as an anti-candidal vaccine strain against systematic candidiasis despite its lack of virulence and ability to initially colonize and grow in the mouse host. We also characterize the cell wall glycomic composition of the different strains and determine that unmasking of β -1,3-glucans may be responsible for the avirulence of the *cho1* Δ/Δ mutant.

Introduction

Vaccines against *Candida albicans* have been proposed to protect against both systemic and vulvovaginal candidiasis (Filler et al., 2010; Antonio C., 2000). We therefore hypothesized that since the *cho1* Δ/Δ mutant is able to grow in the host, but is avirulent in a murine model of disseminated candidiasis, it may be an ideal candidate for a live vaccine against *C. albicans*. The mutant is however effectively cleared five days post-infection and therefore it may serve as an ideal live vaccine strain. Cell surface components or wall proteins have been proposed as vaccine candidates (Bundle et al. 2012; Luo et al., 2012; Paulovicova et al. 2012; Filler et al., 2010). The cell wall changes observed in the *cho1* Δ/Δ mutant as well as the transcriptional increase in cell wall gene expression coupled with its hyper-filamentation suggested that the *cho1* Δ/Δ strain may present to the host an altered cell wall structure (Chapter 2 and Chen et al., 2010). We hypothesize that its unique cell wall phenotype via differential expression of Als proteins and Hwp1 may lead to enhanced recognition by host immune cells and effective clearance from host tissue. Thus this strain could confer resistance to re-infection by a virulent wild-type strain of *C. albicans* and represents a candidate for an experimental live, attenuated vaccine against systemic candidiasis and can be used as a tool to further decipher mechanisms of protective immunity during candidiasis. This section demonstrates that the *cho1* Δ/Δ mutant is not a viable vaccine candidate despite its lack of virulence and ability to initially colonize and grow in the mouse host.

As another analysis, we also analyzed the glycomic composition of these phospholipid mutants and delineated if the avirulence of the mutant is due to an auxotrophic growth defect. In Chapter 2, we showed that the phospholipid biosynthesis mutants have a defective cell wall. However, the biochemical make-up of the cell wall in these mutants has not been analyzed. Furthermore, the failure of the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ mutants to cause virulence in the$

host is not fully understood. We hypothesized that the lack of virulence may be due to an auxotrophic growth defect as the mutant may fail to grow by not acquiring essential phospholipid precursors, such as ethanolamine and choline, in the host environment. The resulting loss of virulence could be due to an inability to grow or due to a more effective clearance and response by the host immune system.

Materials and Methods

Strains and Growth conditions

All strains used in this study are listed in Table 2-1. Unless otherwise mentioned, the strains were grown in 2% yeast-extract 2 % peptone 2% dextrose (YPD) medium for 24 hrs at 30°C with shaking.

Growth in the mouse host

To examine growth in the host, the cells were stained with Alexa fluor 647 label. Cells were grown at 37°C overnight in YPD and were washed three times in phosphate buffered saline (PBS) and resuspended in PBS at a concentration of 1×10^9 /ml. Alexa fluor 647 (Molecular Probes) was dissolved and stored in DMSO at a concentration of 10 mg/ml. Prior to labeling, 11 μ L of 1 M NaCO_3 pH 10 was added to the *Candida* cells and then 2 μ L of Alexa fluor 647 was added and mixed briefly by vortexing. Cells were labeled for 1 hour at room temp, then washed five times with PBS, counted by hemacytometer, and used for infection (as described in the mice virulence section below). Viability of the cells used for injection was determined by plating the suspension on 2% yeast extract 2 % peptone 2% dextrose (YPD) agar plates. 6 h post-infection, mice kidneys were collected and homogenized in PBS, and suspensions were observed by fluorescence microscopy using Alex fluor and GFP specific filters. Images were taken with a MagnaFire Olympus digital camera and Olympus BX50 microscope (Olympus

America, Melville, NY,USA). Images were superimposed with PictureFrame software (Optronics, Goleta,CA).

β-Glucan exposure and Dectin-1 binding

The strains were sent to Dr.Robert Wheeler (University of Maine, Dept. of Molecular and Biomedical Sciences) to determine the unmasking of β-1,3-glucan and dectin-1 binding. Briefly, cells were grown overnight in YPD, washed, collected by centrifugation, resuspended in saline and then stained with anti-β-glucan antibody, specific for β1,3-glucan (Biosupplies, Inc., Australia), diluted 1:200 in 2%BSA /PBS overnight at 4°C on a rotator and washed three times with PBS. Then homogenates were stained for 1 hour at room temp with goat anti-mouse Cy3 antibody (Jackson ImmunoResearch) diluted 1:100 into 2%BSA/PBS with or without Alexa647-labeled Dectin-CRD (diluted 1:40 in 2%BSA/PBS). After three washes, cells were visualized by fluorescence microscopy. Live cells were identified based on GFP fluorescence.

Extraction of Cell Wall Polysaccharides and Glycosyl Composition Analysis

Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) at the Complex Carbohydrate Research Center by Dr.Parastoo Azadi. The per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. Between 100 and 400ug of sample was used for the analysis. 20ug of inositol was added to the samples. The samples were initially hydrolyzed using 4M HCL at 100 degrees for 2 hours. Methyl glycosides were then prepared from the samples by methanolysis in 1 M HCl in methanol at 80°C (18 hours), followed by re-*N*-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The samples were then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80°C (0.5 hours). These procedures were carried out as previously described in Merkle and Poppe (1994). GC/MS analysis of the TMS methyl glycosides was performed on an Agilent 6890N GC

interfaced to a 5975B MSD, using a Supelco EC-1 fused silica capillary column (30m × 0.25 mm ID).

Mouse virulence studies

For the infection studies, 5- to 6-week old male ICR mice from Harlan Laboratories were used. Five mice were housed per cage. *C.albicans* strains were grown overnight in 2% Yeast 2% Peptone 2% Dextrose (YPD) at 37°C with shaking and were washed three times in phosphate buffered saline. Cell numbers were determined by a haemocytometer and adjusted to a final concentration of 10^7 cells/ml in phosphate buffered saline. 100µL of the cell suspension (10^6 total cells) were injected into the tail veins of mice. Mice were monitored for three weeks for signs of infection. Moribund mice were euthanized. Each experiment was done in triplicate with five mice in each group. All experimental procedures were carried out in accordance with the NIH guidelines for the ethical treatment of animals. CFU numbers were determined from the weighed kidneys 3 and 5 days after infection by plating tissue homogenates on YPD agar. For the vaccine study, mice were first pre-inoculated with the *cho1*Δ/Δ or control strain using the same procedure and monitored for two weeks, after which the mice were challenged with 1×10^6 *Candida albicans* wild-type(SC5314) cells and monitored as described above.

Statistical Analysis

Prism 4.0 software (GraphPad) was used to determine the significance of differences for the mouse virulence studies. Statistically significant differences were determined using the Kaplan Meir survival curve test. Statistical significance was set at a *P* value of <0.05.

Results and Discussion

Lack of virulence of the *cho1*Δ/Δ and *psd1*Δ/Δ*psd2*Δ/Δ strains are not due to auxotrophic growth defects

We tested the hypothesis that the lack of virulence of the *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants is due to auxotrophic growth defects. In order to test if the strains could grow in vivo we injected Alexa fluor stained yeast form *C. albicans* cells expressing GFP and examined if they were able to form hyphae upon infection of the host by microscopy (see methods). We show that in the systemic model of infection that all the *C.albicans* strains are able to grow and form hyphae in mice kidneys 6 hours post infection (Fig:A-1). We also found that there are no differences in growth in mouse serum for all the strains tested (data not shown). This suggests that these strains are capable of obtaining ethanolamine or choline from the host and the avirulence of the mutants is not due to nutritional auxotrophies leading to starvation and death in the host.

Increased β -glucan exposure on *cho1* Δ/Δ mutant may enhance host immune recognition

β -1-3 glucan “unmasking” is the uncovering of inner cell wall layer, namely β -1-3-glucan, that is normally covered by mannoproteins. Masking of β -glucan avoids immune recognition through the Dectin-1 receptor on phagocytes (Brown et al., 2002; Gow et al., 2007; Netea et al., 2008). Recognition by Dectin-1 has been shown to activate defense mechanisms for the stimulation of pro-inflammatory cytokines (Le et al., 2012, Wheeler et al., 2008). In addition, *Candida* infection have been shown to be mitigated by IL-33 pretreatment; IL-33 mediates neutrophil recruitment to *Candida* infection sites via activation of Dectin-1 on host macrophages (Le et al., 2012). We propose that the cell wall defects of the *cho1* Δ/Δ mutant may cause an increase in immune-mediated clearance by increased immune recognition. This hypothesis is supported by an increased exposure of β -1-3 glucan on the fungal cell surface (Fig: A-2A) and increased binding of the soluble binding domain of Dectin-1 receptor on macrophages to the less virulent *cho1* Δ/Δ and *psd1* Δ/Δ *psd2* Δ/Δ mutants (Fig:A-2B). This is also consistent with the finding that the *cho1* Δ/Δ mutant has increased sensitivity to caspofungin (Chen et al., 2010), a strong inhibitor of β -1-3 glucan synthesis. Thus, increased exposure of β -1-3 glucans may be responsible for

increased host recognition and at least partially explain the lack of virulence observed in the *cho1Δ/Δ* strain.

Cell wall carbohydrate analysis reveals no differences in amounts of glucose or mannose residues between strains

We next analyzed the cell wall glycosyl composition by combined gas chromatography/mass spectrometry (GC/MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides collected from each strain by acid methanolysis. We observed no difference in wall carbohydrates suggesting that the total amount of β -1-3 glucans in the cell is not changed in the *cho1Δ/Δ* or *psd1Δ/Δpsd2Δ/Δ* mutant (Table A-1). This is similar to the observed cell wall polysaccharide structure in the mitochondria-defective *sam37Δ/Δ* strain that interestingly has similar phenotypes to *cho1Δ/Δ* (Qu et al., 2012, Chapter 2). We were unable to quantify chitin content in these strains but we did note an increase in Nacetyl glucosamine (a break down product of chitin) in the *cho1Δ/Δ* strain compared to the wild type. This is consistent with our fluorescent calcofluor white staining data that indicates much higher chitin content in the *cho1Δ/Δ* strain.

Pre-inoculation with *cho1Δ/Δ* mutant provides no protective effect against systemic candidiasis in mice.

The avirulence of the *cho1Δ/Δ* mutant and its clearance by the immune system after infection enabled us to examine its possible use as a live vaccine to prevent *Candida* infections. Isolated peptides against cell wall components and cell wall proteins have been utilized to show protective effects in *Candida albicans* systemic mouse infections such as the β -(Man)₃-Fba glycopeptide, mannosides, Hyr-1 recombinant vaccines (Cutler et al., 2011; Bar et al., 2012, Pietrella et al., 2012; Paulovicova et al., 2012; Luo et al., 2012). Anti- β -glucan antibodies have also shown to confer protective properties against vaginal candidiasis (Pietrella et al., 2010;

Torosantucci et al., 2009). In addition Filler et al. (2010), have found a vaccine to Als3p to be protective in mice, and we found Als3p be highly up-regulated in the *cho1Δ/Δ* strain in YPD (see Chapter 2). Since Als3 is highly expressed at the surface of the *cho1Δ/Δ* strain and β-1-3 glucan is highly exposed in this mutant, we hypothesized that the *cho1Δ/Δ* strain may be a good vaccine strain. To test our hypothesis, we performed *in vivo* infection assays to determine if pretreatment with *cho1Δ/Δ* cells leads to prolonged survival upon subsequent infection with wild type *Candida albicans* cells in a mouse model. We found that there was no protective effect in mice using this method suggesting that *cho1Δ/Δ* is not a viable vaccine candidate for the preventive treatment of invasive candidiasis despite its initial colonization of the host kidneys and its subsequent clearance from the host (Fig:A-3)(Chen et al., 2010).

No difference in virulence between the *psd2Δ/Δ* strain and the wild type strain in a mouse model of systemic infection.

The avirulence of the *cho1Δ/Δ* mutant and the severely reduced virulence of the *psd1Δ/Δpsd2Δ/Δ* mutant also led us to assay the virulence of the *psd2Δ/Δ* mutant alone. We had initially found that the *psd2Δ/Δ* strain may be hypervirulent in a mouse model of systemic infection as it had a higher fungal burden and more virulent survival curve data. We therefore tested the strain repeatedly to verify if our previous results were valid, and found that there is no difference in virulence between the wild type strain and the *psd2Δ/Δ* strain (Fig: A-4).

Summary

The data presented in this section suggests that although the *cho1Δ/Δ* strain is able to grow in the host kidney and form hyphae initially upon systemic infection, the *cho1Δ/Δ* strain is not an ideal vaccine candidate to prevent systemic infection as it did not provide any protection in our mouse model. We also propose that differences in virulence may be due to unmasking of β-1,3 glucans as we show that the *cho1Δ/Δ* strain has much more unmasking β-1,3 glucan and higher

Dectin-1 binding. Therefore, it is likely that alterations in the cell wall and immune recognition attenuates the virulence of the mutant and may modulate its response to hypoxia or iron limitation in the host. Recently it has been demonstrated that disruption of MAP kinase pathways in *C.albicans* causes enhanced β -glucan exposure, triggering more efficient immune responses (Galan-Diez et al., 2010). Thus, enhanced β -glucan exposure and altered cell wall organization (reflected in the transcriptional profile) in the *cho1 Δ/Δ* mutant may trigger activation of signaling pathways. Similar to the *cho1 Δ/Δ* mutant, these mutants that are defective in phosphorylation are hypersensitive to the cell wall perturbing drugs congo red and calcofluor white (Galan-Diez et al., 2010), suggesting that similar signaling pathways may be affected in the *cho1 Δ/Δ* mutant leading to unmasking of β -glucan and enhanced immune recognition in these strains.

Supplementary Figures

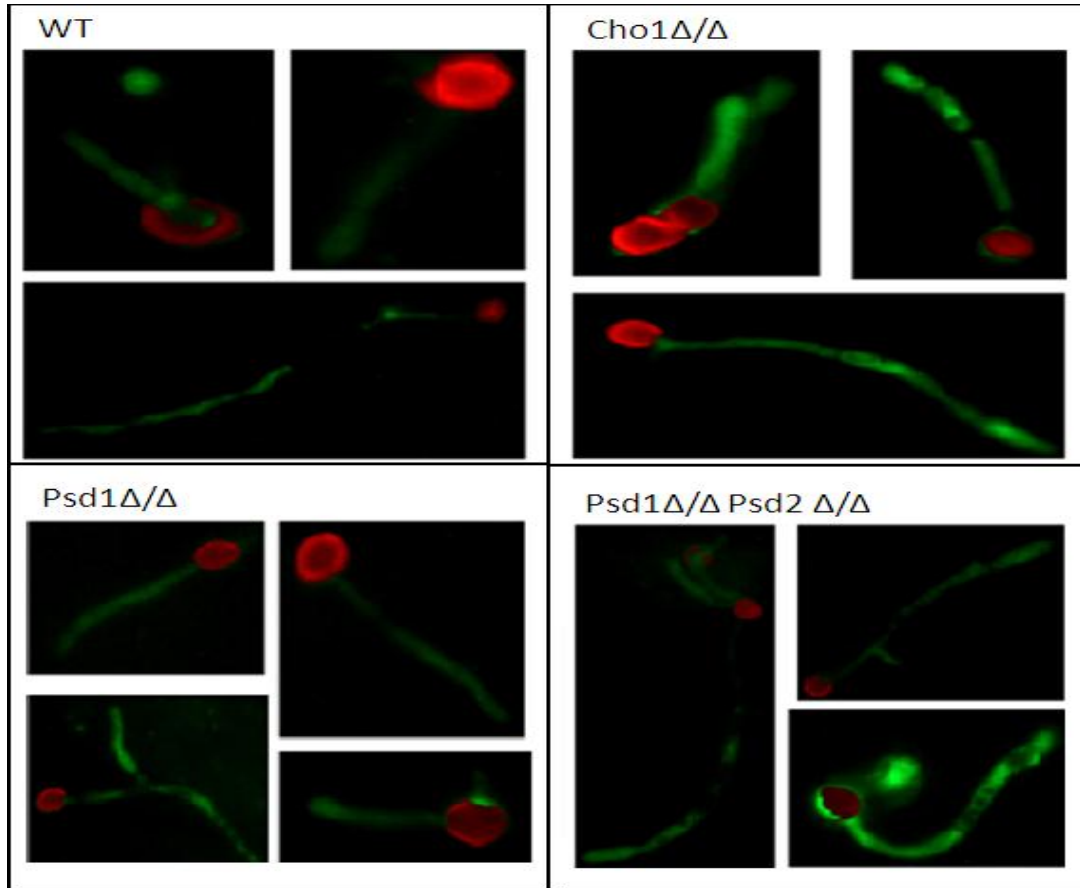


Figure A-1: Growth of the *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ strains in mice kidneys *in vivo*.$ The *cho1* Δ/Δ and *psd1* $\Delta/\Delta*psd2* Δ/Δ showed no defects in hyphal formation and growth 6 hours post infection. 1×10^6 Alexa fluor-labeled (RED) yeast cells were injected in the tail vein of mice, 6 h post-infection kidneys were harvested homogenized and viewed under a fluorescent microscope. Green fluorescence is indicative of new hyphal growth *in vivo*. Image is representative of 40 cells per strain. Sarah Davis collaborated on this figure and experiment.$

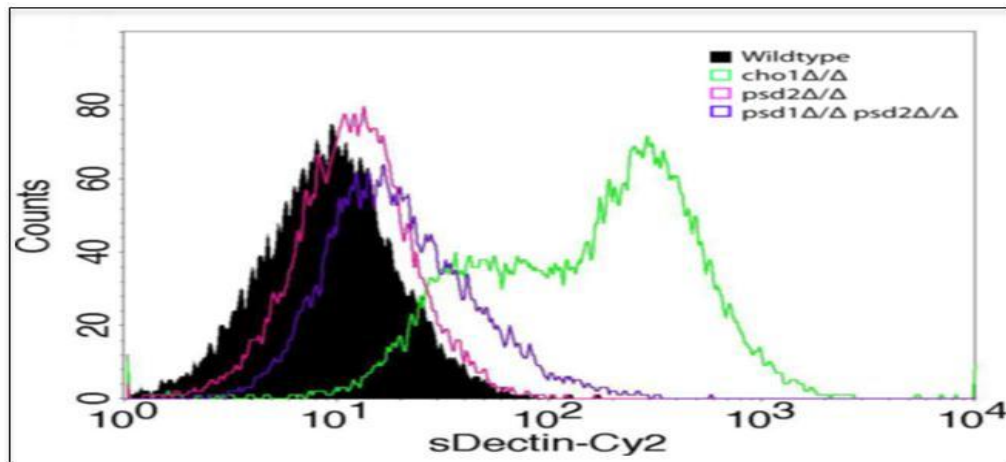


Figure A-2: Increased β -glucan exposure on the *cho1* Δ/Δ strain. (A) Cells were grown in YPD, washed, diluted and stained with anti- β -glucan antibody and Cy2-labeled secondary antibody. Images are representative of three independent experiments. Scale bar is 20 μ m. This experiment was done in collaboration with Robert Wheeler at the University of Maine.

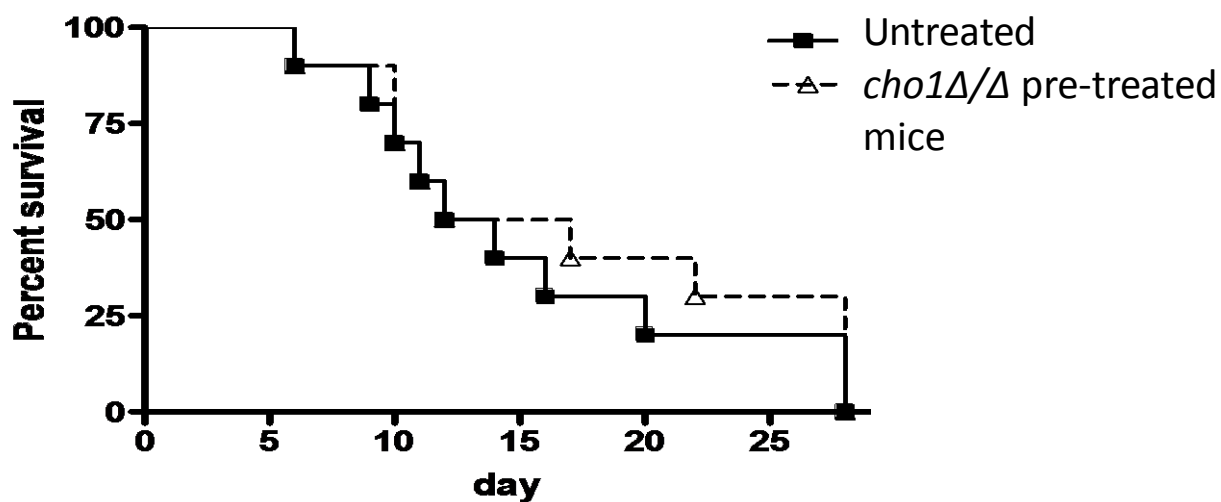
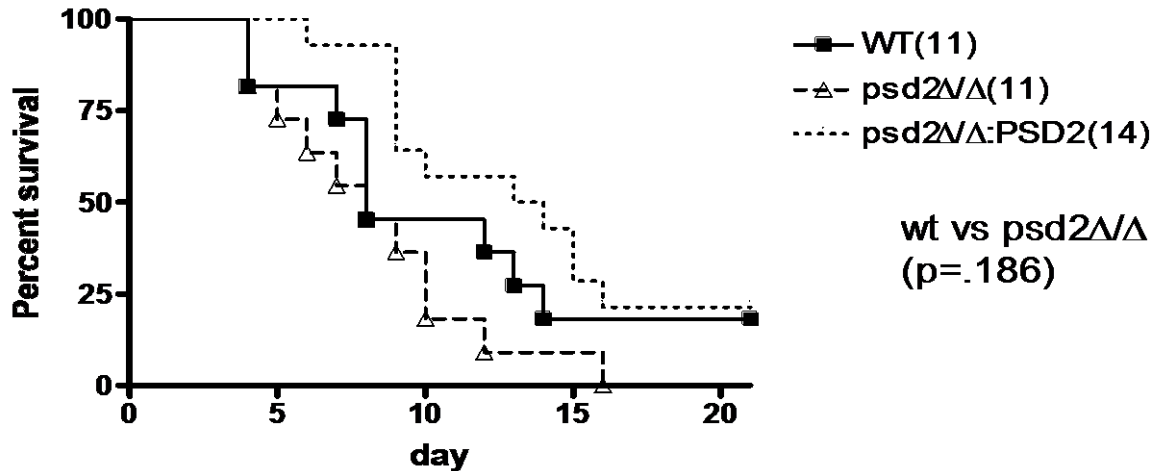


Figure A-3: Survival curves of the *cho1Δ/Δ* pre-treated and untreated mice challenged with the wild type strain. For the pre-treated group, mice were inoculated with 1×10^6 *Candida albicans cho1Δ/Δ* cells by tail vein injection. For the untreated group, saline was injected as a control. After two weeks, mice in both groups were challenged with 1×10^5 wild-type (SC5314) cells. Survival was monitored for four weeks. No difference in survival was observed between the two treatment groups. Number of mice used is indicated in parentheses. P value *cho1 Δ/Δ*-treated v/s untreated mice = 0.059 (no difference).

A



B

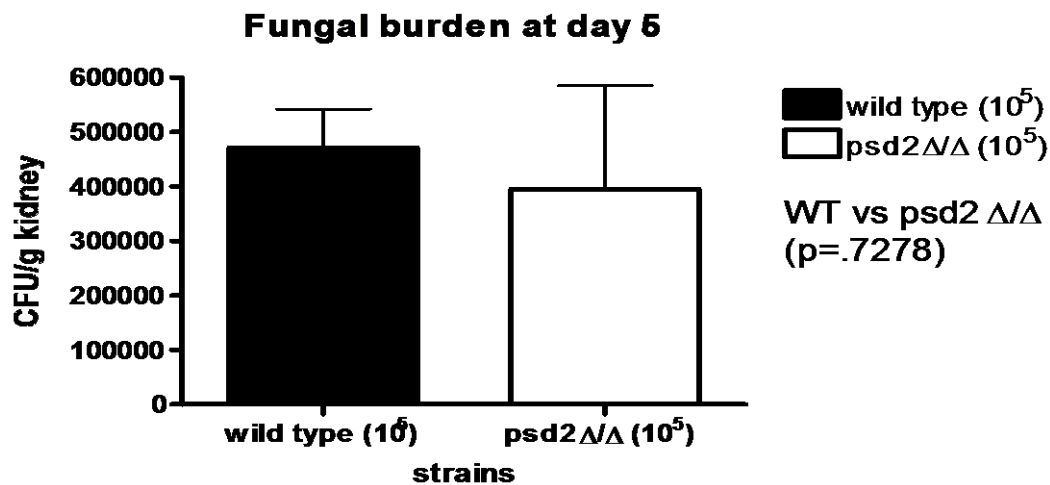


Figure A-4: (A) Survival curve of the *psd2Δ/Δ* strain in a mouse model of systemic infection. Mice were infected with 1×10^6 *Candida albicans* cells by tail vein injection and mice were monitored for 21 days. Survival curves for the wild type (SC5314), *psd2Δ/Δ* and *psd2Δ/Δ:PSD2* were examined. The number of mice used is indicated in parentheses. P value of wild type versus *psd2Δ/Δ* = 0.186 (no statistical difference). **(B) Fungal burden in the kidney of the *psd2Δ/Δ* strain compared to the wild type (SC5314) strain.** 1×10^6 *Candida albicans* cells were injected by tail vein injection. After five days post infection, kidneys were harvested, homogenized, and the fungal burden was measured by counting CFUs on YPD agar plates.

Table A-1: Glycosyl Composition analysis of *C.albicans* strains by GC/MS.

Glycosyl redidue	Mol % Wild-type	Mol% <i>cho1</i> Δ/Δ	Mol% <i>psd1</i> Δ/Δ	Mol% <i>psd1</i> Δ/Δ <i>psd2</i> Δ/Δ	Mol% <i>cho1</i> Δ/Δ:: <i>CHO1</i>
Ribose	-	-	-	-	-
Arabinose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Fucose	-	-	-	-	-
Xylose	-	2.0	10.6	2.6	-
Glucuronic acid	-	-	-	-	-
Galacturonic acid	-	-	-	-	-
Mannose	48.1	40.6	24.6	43.3	39.0
Galactose	-	0.2	0.5	0.4	-
Glucose	51.9	53.8	63.5	52.0	60.2
N-acetyl galactosamine	-	-	-	-	-
N-acetyl glucosamine	-	3.3	0.8	1.7	0.9
N-acetyl mannosamine	-	-	-	-	-

Table A-2: Expression of glucan and chitin synthases genes

Gene Name	CGD Function	<i>cho1Δ/Δ</i>	<i>psd1Δ/Δpsd2Δ/Δ</i>
<i>GLS1</i>	Glucan Synthase 1	1.39	1.41
<i>GLS2</i>	Glucan Synthase 2	2.14	2.83
<i>CHS1</i>	Chitin Synthase 1	1.35	2.82
<i>CHS2</i>	Chitin Synthase 2	2.83	2.46
<i>CHS3</i>	Chitin Synthase 3	1.52	1.83
<i>CHS4</i>	Chitin Synthase 4	1.23	2.22
<i>CHS5</i>	Chitin Synthase 5	1.40	1.14
<i>CHS6</i>	Chitin Synthase 6	1.41	1.35
<i>CHS7</i>	Chitin Synthase 7	1.44	1.74
<i>CHS8</i>	Chitin synthase 8	2.00	1.74

Table A-3: List of all the genes up-regulated in the *cho1*Δ/Δ mutant compared to the wild-type strain.

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0005124	125	Secreted Protein	1.00E-300
CAL0004244	86.6	Uncharacterized ORF	1.00E-300
CAL0000215	50.1	Inositol-1-phosphate synthase	1.00E-300
CAL0001195	33.16	Putative GPI-anchored protein	1.00E-300
CAL0000162	31.48	GPI-anchored cell wall protein required for filamentous growth at acidic pH	4.64E-70
CAL0004933	30.16	Immunogenic stress-associated protein	1.00E-300
CAL0003753	19.11	Putative transcription factor; required for wild-type resistance to cell wall perturbation	1.00E-300
CAL0002828	15.10	Uncharacterized ORF	1.09E-76
CAL0000413	14.78	General amino acid permease	4.54E-166
CAL0000986	13.30	Putative GPI-anchored protein	1.19E-158
CAL0001735	10.76	Putative ribonucleoside diphosphate reductase	1.00E-300
CAL0001866	10.69	Member of the secreted aspartyl proteinase family	1.59E-225
CAL0002992	10.40	Basic amino acid permease	1.00E-300
CAL0000567	9.77	Verified ORF; Protein required for cohesion, adhesion, and biofilm formation	1.14E-39
CAL0001974	9.63	Urea amidolyase	1.00E-300
CAL0000715	9.48	Plasma membrane protein of heme-iron utilization upregulated by Rim101p	7.82E-98
CL0000259	9.01	GPI-anchored protein	1.50E-200
CAL0004374	8.34	Uncharacterized ORF; Ortholog(s) have tubulin binding activity, role in microtubule cytoskeleton organization, mitochondrion inheritance	1.00E-300
CAL0000188	8.34	Plasma-membrane-localized protein; filament induced; Hog1p-, and hypoxia-induced	1.00E-300
CAL0001283	8.10	ALS family protein	1.00E-300
CAL0120975	7.94	Uncharacterized ORF	1.00E-300
CAL0120924	7.39	Putative adhesin-like protein	2.99E-05
CAL0001538	6.70	Putative peroxisomal copper amine oxidase	1.08E-189
CAL0000427	6.68	Protein similar to plant pathogenesis-related proteins; required for virulence in mouse systemic and rabbit corneal infections	1.00E-300
CAL0004799	6.43	Uncharacterized ORF; Hap43p-repressed gene	6.26E-56
CAF0006989	6.25	Cytoplasmic protein expressed specifically in white phase yeast cells	6.03E-146
CAL0002282	6.24	Unchara Putative ceramide hydroxylase; predicted enzyme of sphingolipid Tsa1Bp	1.00E-300

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0000377	6.15	Uncharacterized ORF; Planktonic growth-induced gene	5.38E-14
CAL0001605	5.98	ALS family cell wall adhesin	1.00E-300
CAL0001407	5.91	Putative DNA-binding transcription factor	1.01E-53
CAL0003337	5.75	Putative ARP2/3 complex subunit	4.63E-42
CAL0002165	5.71	GPI-anchored protein	1.00E-300
CAL0005195	5.64	Predicted ORF in retrotransposon Tca8	1.45E-113
CAL0000023	5.61	Uncharacterized ORF	5.67E-222
CAL0006337	5.60	Ammonium permease	1.00E-300
CAL0005305	5.55	Uncharacterized ORF	1.00E-300
CAL0001738	5.36	Uncharacterized ORF	4.52E-39
CAF0007042	5.32	Uncharacterized ORF	2.29E-15
CAL0004418	5.29	Putative protein of unknown function; Hap43p-repressed gene	1.00E-300
CAL0000494	5.24	Essential protein involved in cytokinesis	4.62E-50
CAL0000285	5.20	Uncharacterized ORF	5.88E-88
CAL0004349	5.15	Putative DNA cross-link repair protein	4.11E-124
CAL0000177	5.05	Putative aminopeptidase	6.07E-183
CAL0004191	4.99	Uncharacterized ORF	1.86E-55
CAL0003075	4.94	Uncharacterized ORF	5.56E-09
CAL0003484	4.94	Uncharacterized ORF; Hap43p-induced gene	6.46E-75
CAL0002632	4.92	G1 cyclin	3.45E-144
CAL0003166	4.70	Uncharacterized ORF	1.00E-300
CAL0005585	4.68	Uncharacterized ORF; Biofilm-induced gene	9.17E-40
CAL0004553	4.63	Essential transcription factor	1.00E-300
CAL0006272	4.60	Acyl CoA:sterol acyltransferase	3.27E-220
CAL0000709	4.59	Basic amino acid permease	1.45E-90
CAL0003763	4.57	Uncharacterized ORF	1.00E-300
CAL0004442	4.55	Uncharacterized ORF	7.80E-46
CAL0005876	4.51	Putative peptide:N-glycanase	1.00E-300
CAL0000443	4.45	Cell wall acid trehalase	1.41E-176
CAL0000739	4.43	Uncharacterized ORF	3.41E-70
CAL0003828	4.42	Secreted lipase	1.00E-300
CAL0004833	4.40	Uncharacterized ORF	2.40E-258
CAL0000503	4.39	Uncharacterized ORF	1.68E-11
CAL0004522	4.36	Predicted membrane transporter	2.54E-12
CAL0000209	4.32	Putative transcription factor	1.00E-300
CAL0002724	4.26	Member of a family encoded by FGR6-related genes	2.35E-32
CAL0002389	4.25	Uncharacterized ORF	3.99E-45
CAL0005923	4.24	Uncharacterized ORF	2.79E-44
CAL0001357	4.19	Uncharacterized ORF;role in mitochondrial genome maintenance	2.33E-191

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0004258	4.16	Putative GPI-anchor	7.02E-180
CAL0002907	4.12	Putative phosphomethylpyrimidine kinase	1.92E-53
CAL0001619	4.11	Uncharacterized ORF; ATP sulfurlyase of sulfate assimilation	5.84E-80
CAL0003020	4.02	Zinc finger transcriptional regulator of nitrogen utilization	2.61E-105
CAL0006302	4.00	Mannose-1-phosphate guanyltransferase	1.13E-132
CAL0000359	3.97	Uncharacterized ORF	1.51E-113
CAL0005075	3.97	Hypha-specific G1 cyclin-related protein	1.91E-37
CAL0005323	3.96	Secreted lipase	2.14E-28
CAL0006398	3.94	Hyphal-induced GPI-anchored cell wall protein	1.36E-50
CAL0002156	3.94	Uncharacterized ORF	3.89E-74
CAL0001358	3.93	Putative mitochondrial leucyl-tRNA synthetase	1.00E-300
CAL0120845	3.90	Uncharacterized ORF	0.0013996
CAL0002630	3.90	Putative mitochondrial translation initiation factor	1.00E-300
CAL0001625	3.87	Zinc cluster DNA-binding transcription factor	1.69E-30
CAL0002161	3.87	Uncharacterized ORF	1.00E-300
CAL0002939	3.86	UDP-glucose:ceramide glucosyltransferase	9.80E-36
CAL0002789	3.85	ALS family protein	1.00E-300
CAL0005541	3.84	Squalene epoxidase	1.00E-300
CAL0002798	3.82	Uncharacterized ORF	5.03E-15
CAL0006312	3.82	Putative DNA mismatch repair factor	1.57E-92
CAL0003055	3.80	Phosphofructokinase alpha subunit	1.00E-300
CAL0002550	3.80	Predicted zinc-finger protein	1.00E-300
CAL0000318	3.74	Uncharacterized ORF; Hap43p-induced gene	8.91E-177
CAL0001295	3.73	Uncharacterized ORF; Hap43p-repressed gene	1.18E-35
CAL0004519	3.71	Putative mitochondrial biogenesis protein	1.00E-300
CAL0006341	3.71	Biofilm- and planktonic growth-induced gene	3.14E-72
CAL0003546	3.70	Uncharacterized ORF; Ortholog(s) have role in mitochondrial genome maintenance	1.56E-22
CAL0001212	3.68	DNA binding protein with Cys2-His2 zinc-finger	1.00E-300
CAL0002964	3.66	Uncharacterized ORF	2.68E-06
CAF0007422	3.65	Uncharacterized ORF; Ortholog(s) have role in mitochondrial genome maintenance and integral to mitochondrial inner membrane localization	4.17E-267
CAL0001762	3.60	Putative transporter of ATP-binding cassette (ABC) superfamily	1.00E-300
CAL0005303	3.60	Uncharacterized ORF	2.97E-71
CAL0000374	3.59	Putative protein of GPI synthesis	3.26E-48

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0000998	3.56	Uncharacterized ORF	1.31E-183
CAL0003593	3.55	Uncharacterized ORF	8.47E-37
CAL0002878	3.54	Member of a family encoded by FGR6-related genes	1.57E-62
CAL0000151	3.54	Predicted zinc-finger protein	1.22E-16
CAL0001515	3.52	Uncharacterized ORF	1.56E-32
CAL0004602	3.52	Uncharacterized ORF; Ortholog(s) have cytosol localization	1.15E-39
CAL0002404	3.47	Uncharacterized ORF; Protein related to arginases	1.63E-14
CAL0002930	3.44	Uncharacterized OR; induced by hypoxia	4.42E-209
CAL0006342	3.44	Verified ORF; Hap43p-repressed gene	1.20E-216
CAL0001440	3.43	ALS family of cell-surface glycoproteins	1.00E-300
CAL0005593	3.41	Verified ORF; Putative mitochondrial protein with a predicted role in respiratory growth; mutants display a strong defect in biofilm formation; fluconazole-induced; ketoconazole-repressed	1.00E-300
CAL0000040	3.41	Uncharacterized ORF; Putative acyl-CoA oxidase	2.83E-22
CAL0000601	3.38	Uncharacterized ORF	1.00E-300
CAL0003697	3.35	Uncharacterized ORF; Hap43p-induced gene	1.00E-300
CAL0003797	3.33	Uncharacterized ORF	1.13E-192
CAL0003896	3.33	Uncharacterized ORF	6.32E-130
CAL0005961	3.32	Putative glucokinase	1.00E-300
CAL0004071	3.32	Uncharacterized ORF	4.55E-142
CAL0002158	3.32	Uncharacterized ORF	6.76E-14
CAL0001776	3.31	Uncharacterized ORF	2.28E-33
CAL0000212	3.31	Secreted aspartyl protease	1.00E-300
CAL0005531	3.31	Predicted extracellular or membrane-associated glucoamylase	9.16E-31
CAL0002488	3.31	Uncharacterized ORF; gene induced by hypoxia	1.79E-44
CAL0004528	3.30	Inositol phosphoryl transferase	1.04E-41
CAL0006186	3.30	Mannosyltransferase	5.73E-27
CAL0004027	3.30	Chitin synthase	1.73E-148
CAL0002852	3.29	Uncharacterized ORF	1.86E-19
CAL0004944	3.29	Zn2-Cys6 transcriptional regulator of ergosterol biosynthetic genes and sterol uptake	1.00E-300
CAL0003435	3.28	Plasma membrane-localized protein; repressed by nitric oxide; Hap43p-repressed gene	1.13E-11
CAL0005027	3.25	Coproporphyrinogen III oxidase	1.00E-300
CAL0000800	3.23	Putative GPI-anchored protein	9.83E-07
CAL0002549	3.23	Uncharacterized ORF	0.000571786
CAL0003463	3.22	Putative transcription factor with zinc cluster DNA-binding motif	2.45E-25

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0003217	3.21	Putative stretch-activated Ca ²⁺ channel	2.25E-20
CAL0001985	3.21	Putative ceramide hydroxylase	1.00E-300
CAL0003499	3.21	Uncharacterized ORF	6.94E-14
CAL0004275	3.20	Uncharacterized ORF	1.75E-06
CAL0003180	3.18	Uncharacterized ORF	1.00E-300
CAL0004436	3.16	Uncharacterized ORF; role in endosome transport	1.00E-300
CAL0001911	3.16	Putative adhesin-like protein	3.68E-22
CAL0003637	3.15	Uncharacterized ORF	1.00E-300
CAL0001478	3.15	Uncharacterized ORF; Ortholog(s) have role in mitochondrial translation and mitochondrion localization	2.10E-216
CAL0001393	3.13	Trehalose-6-phosphate (Tre6P) phosphatase	1.00E-300
CAL0004601	3.12	Uncharacterized ORF	4.22E-09
CAL0004745	3.12	Purine-cytosine permease of pyrimidine salvage;	2.62E-179
CAL0004169	3.12	Uncharacterized ORF	1.00E-300
CAL0005203	3.11	Putative GPI-anchored protein	6.42E-09
CAL0003532	3.11	Uncharacterized ORF	1.41E-172
CAL0000344	3.10	Uncharacterized ORF	3.76E-95
CAL0005136	3.09	Putative transcription factor	2.03E-112
CAL0005640	3.07	Predicted membrane transporter	1.38E-26
CAL0004499	3.07	N-linked mannoprotein of cell wall and membrane	4.19E-54
CAL0004417	3.06	Putative peroxisomal 3-ketoacyl CoA thiolase	6.86E-106
CAL0001962	3.06	Aldo-keto reductase family member	3.01E-245
CAL0002117	3.06	Transcriptional regulator of the CTR1 copper transporter	1.28E-81
CAL0003915	3.03	Translation factor eRF3	1.00E-300
CAL0005752	3.02	Putative nicotinic acid mononucleotide adenyltransferase	7.81E-284
CAL0005674	3.01	Neutral trehalase	2.74E-173
CAL0005400	3.01	Uncharacterized ORF	1.31E-16
CAL0004456	3.00	Uncharacterized ORF	1.00E-300
CAL0000422	2.99	Cytochrome oxidase assembly protein	1.00E-300
CAL0004319	2.96	Putative choline/ethanolamine transporter	1.00E-300
CAL0004678	2.96	Uncharacterized ORF	5.97E-232
CAL0004368	2.96	Uncharacterized ORF	8.43E-27
CAL0000899	2.94	Uncharacterized ORF	3.19E-11
CAL0001558	2.94	DNA ligase	2.62E-86
CAL0000390	2.94	Uncharacterized ORF;	8.20E-131
CAL0000808	2.93	Uncharacterized ORF; Protein similar to isoleucyl-tRNA synthetase	1.00E-300
CAL0000041	2.93	Uncharacterized ORF; Hap43p-repressed gene	2.92E-42
CAL0002752	2.93	Uncharacterized ORF	6.25E-285

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0004026	2.92	Putative cystathionine gamma-synthase	6.41E-165
CAL0004624	2.92	Uncharacterized ORF; Putative actin interacting protein;	4.86E-58
CAL0001294	2.91	Uncharacterized ORF	3.68E-17
CAL0003069	2.91	Uncharacterized ORF	2.02E-11
CAL0005571	2.91	Uncharacterized ORF; Protein similar to GTPase regulators	8.13E-177
CAL0001564	2.90	Uncharacterized ORF; Hap43p-repressed gene	1.02E-109
CAL0000820	2.90	Putative cytochrome c lysine methyltransferase	1.00E-300
CAL0004892	2.90	Uncharacterized ORF; Hap43p-repressed gene	3.45E-13
CAL0000430	2.89	Putative mitochondrial translation elongation factor	2.76E-260
CAL0000779	2.88	Putative GPI-anchored protein	6.45E-12
CAL0002436	2.88	Putative prolyl-tRNA synthetase	2.94E-156
CAL0003968	2.87	Phosphomannose isomerase	1.25E-15
CAL0004017	2.87	Uncharacterized ORF	5.14E-92
CAL0001729	2.86	Uncharacterized ORF; Possible stress protein	1.61E-75
CAL0004146	2.86	Putative RNA polymerase II subunit B150	6.71E-56
CAF0007014	2.85	Uncharacterized ORF	9.28E-85
CAL0002758	2.85	Uncharacterized ORF	7.38E-96
CAL0003714	2.85	Uncharacterized ORF	4.35E-252
CAL0005643	2.84	Putative zinc transporter	1.00E-300
CAL0005848	2.82	Uncharacterized ORF	1.30E-262
CAL0005127	2.82	Uncharacterized ORF; role in oxidation-reduction process	2.25E-58
CAL0003889	2.81	Uncharacterized ORF; Biofilm-induced gene	2.86E-42
CAL0000588	2.81	Uncharacterized ORF	1.71E-09
CAL0003612	2.81	Uncharacterized ORF; Hap43p-repressed gene	7.81E-15
CAL0000782	2.81	Uncharacterized ORF; Putative Pol Protein	1.44E-224
CAL0005050	2.80	Putative amino acid permease	2.38E-13
CAL0004394	2.80	Uncharacterized ORF; Putative low-affinity copper transporter	8.35E-193
CAL0002471	2.79	Uncharacterized ORF; Hap43p-repressed gene	1.79E-233
CAL0005937	2.79	Uncharacterized ORF	5.76E-09
CAF0006940	2.79	Uncharacterized ORF	4.10E-05
CAL0006344	2.79	Glucosamine-6-phosphate synthase	1.00E-300
CAL0002198	2.79	Uncharacterized ORF; Ortholog(s) have fungal-type vacuole membrane, mitochondrion localization	1.75E-89
CAL0004847	2.77	Protein similar to <i>S. cerevisiae</i> Ybr075wp	0.000482213
CAL0004976	2.76	Uncharacterized ORF	1.00E-73

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0005306	2.76	Uncharacterized ORF	3.12E-58
CAL0004351	2.76	Uncharacterized ORF	1.00E-300
CAL0003611	2.75	Uncharacterized ORF	1.12E-07
CAL0000416	2.75	Uncharacterized ORF; Putative transmembrane protein	6.05E-11
CAL0001905	2.74	C-5 sterol desaturase	1.00E-300
CAL0002781	2.74	Predicted membrane transporter	7.72E-72
CAL0001123	2.72	Uncharacterized ORF	4.60E-195
CAF0006926	2.72	Uncharacterized ORF	9.51E-08
CAL0004652	2.72	Putative glutamine-dependent NAD synthetase	7.08E-44
CAL0004120	2.72	Uncharacterized ORF; Predicted ribosomal protein	1.45E-08
CAL0001720	2.71	Ribosomal protein of the large subunit, mitochondrial;	1.00E-300
CAL0005236	2.71	Glycerol 3-phosphatase	1.00E-300
CAL0005673	2.70	Uncharacterized ORF; Putative carboxypeptidase	1.43E-18
CAL0002049	2.70	Putative mitochondrial carrier protein	1.10E-117
CAL0001970	2.70	Putative glycogen phosphorylase	1.00E-300
CAL0000985	2.69	Putative D-xylulose reductase	1.00E-300
CAL0005762	2.69	Isocitrate lyase	5.19E-47
CAL0001734	2.69	Putative malate permease	9.88E-12
CAL0001803	2.69	Putative glucokinase	1.00E-300
CAL0001919	2.69	Uncharacterized ORF	2.95E-89
CAL0004952	2.69	Uncharacterized ORF	2.35E-33
CAL0002546	2.68	Uncharacterized ORF; Putative transporter	6.08E-14
CAL0003251	2.68	Uncharacterized ORF	8.28E-112
CAL0000727	2.68	Cysteine transporter	5.35E-150
CAL0003477	2.68	Dynein heavy chain	3.08E-14
CAL0003007	2.68	Mitochondrial methionyl-tRNA synthetase (MetRS)	6.10E-113
CAL0002531	2.67	Uncharacterized ORF; Putative adhesin-like protein	1.28E-170
CAL0001380	2.67	Uncharacterized ORF	1.71E-30
CAL0001310	2.67	Putative zinc-finger transcription factor	2.33E-35
CAL0001963	2.67	Uncharacterized ORF; role in mitochondrial aspartyl-tRNA aminoacylation and mitochondrion localization	5.43E-201
CAL0003709	2.66	Putative transcription factor with zinc cluster DNA-binding motif	1.12E-21
CAL0004444	2.66	Uncharacterized ORF; role in mitochondrial inner boundary membrane	1.44E-31
CAL0003627	2.66	Protein required for normal filamentous growth	1.00E-300
CAL0004521	2.65	Putative transcription factor with zinc cluster DNA-binding motif	4.88E-22
CAL0005579	2.65	Uncharacterized ORF	1.68E-19
CAL0005583	2.66	Uncharacterized ORF	8.95E-31

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0004434	2.66	Uncharacterized ORF	9.18E-87
CAL0004938	2.65	Uncharacterized ORF	5.96E-15
CAL0003100	2.64	Uncharacterized ORF	3.14E-245
CAL0001160	2.64	Putative phospholipase B	7.55E-17
CAL0005805	2.63	Uncharacterized ORF; Hap43p-repressed gene	6.39E-17
CAL0005121	2.63	Cirt family transposase	7.84E-62
CAL0004496	2.63	Predicted exocyst subunit	0.00768509
CAL0003364	2.63	Putative alcohol dehydrogenase	7.94E-07
CAL0001987	2.63	Uncharacterized ORF	7.74E-244
CAL0004048	2.62	Putative transcription factor with zinc cluster DNA-binding motif	6.20E-91
CAL0000475	2.61	Predicted serine/threonine protein kinase	1.51E-246
CAL0002874	2.61	Uncharacterized ORF; Early-stage biofilm-induced gene	7.50E-98
CAL0002448	2.61	Amino acid permeases	7.77E-152
CAL0002772	2.61	Uncharacterized ORF	1.14E-26
CAL0005944	2.60	Dubious ORF	2.54E-05
CAL0005550	2.60	Uncharacterized ORF	3.88E-120
CAL0001861	2.60	Uncharacterized ORF	1.67E-21
CAL0005146	2.60	Uncharacterized ORF	1.43E-135
CAL0001663	2.60	Uncharacterized ORF; Hap43p-repressed gene	1.22E-150
CAL0002008	2.59	Uncharacterized ORF	0.000145466
CAL0001534	2.59	Uncharacterized ORF; Hap43p-repressed gene	6.00E-31
CAL0004987	2.59	Uncharacterized ORF; Hap43p-repressed gene	3.28E-123
CAL0000884	2.58	Uncharacterized ORF	2.44E-11
CAL0006085	2.58	Uncharacterized ORF	3.19E-118
CAL0006226	2.58	Uncharacterized ORF	1.60E-71
CAL0004509	2.57	Uncharacterized ORF	1.23E-75
CAL0004476	2.57	Uncharacterized ORF	6.52E-109
CAL0001907	2.56	Uncharacterized ORF	3.73E-120
CAL0002764	2.56	Putative ion transporter	1.35E-113
CAL0005175	2.56	Uncharacterized ORF	1.49E-116
CAL0004219	2.56	Putative 2-deoxyglucose-6-phosphatase	1.87E-197
CAL0000304	2.56	Putative GPI-anchored protein	1.13E-174
CAL0006161	2.56	Uncharacterized ORF	2.55E-107
CAL0006370	2.55	Putative transcription factor with zinc cluster DNA-binding motif	2.39E-34
CAL0000780	2.55	Uncharacterized ORF	2.59E-261
CAL0001309	2.55	Uncharacterized ORF	8.30E-27
CAL0001898	2.55	Uncharacterized ORF	6.33E-31
CAL0003133	2.55	B-type mitotic cyclin	1.00E-300
CAL0001047	2.55	Uncharacterized ORF	2.11E-55

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0003604	2.55	Uncharacterized ORF; Hap43p-induced gene	1.91E-41
CAL0004443	2.55	Secreted protein required for normal cell wall structure	1.00E-300
CAL0004447	2.55	Uncharacterized ORF	3.03E-08
CAL0005563	2.54	Uncharacterized ORF	2.35E-45
CAL0005693	2.54	Uncharacterized ORF	0.000497533
CAL0003608	2.54	Uncharacterized ORF	3.41E-215
CAL0006262	2.54	Uncharacterized ORF	9.63E-121
CAL0005544	2.54	Uncharacterized ORF	1.68E-45
CAL0005574	2.53	Uncharacterized ORF	1.19E-110
CAL0000712	2.53	Ammonium permease and regulator of nitrogen starvation	3.77E-30
CAL0001981	2.53	Uncharacterized ORF	3.94E-74
CAL0004473	2.53	Uncharacterized ORF	4.04E-298
CAL0005031	2.53	Uncharacterized ORF	7.26E-35
CAL0005771	2.52	Uncharacterized ORF	5.31E-45
CAL0003522	2.52	Dubious ORF	1.33E-111
CAL0001415	2.52	Uncharacterized ORF	1.53E-32
CAL0004802	2.51	Potential role in beta-1,6 glucan biosynthesis	7.01E-19
CAL0002799	2.51	Putative vacuolar transporter of large neutral amino acids	3.27E-61
CAL0001431	2.51	Putative patatin-like phospholipase	1.00E-300
CAL0004432	2.51	Uncharacterized ORF	1.00E-300
CAL0002507	2.51	Uncharacterized ORF	6.47E-60
CAL0001591	2.50	Protein similar to <i>S. cerevisiae</i> Kar9p	6.16E-34
CAL0006406	2.50	Uncharacterized ORF	2.61E-12
CAL0004429	2.50	N-acetylglucosamine (GlcNAc)-specific transporter	1.37E-190
CAL0002996	2.49	Uncharacterized ORF	5.72E-05
CAL0004609	2.49	Putative glucanase; induced during cell wall regeneration	5.54E-24
CAL0000703	2.49	Uncharacterized ORF; Hap43p-repressed gene	2.80E-58
CAL0000402	2.49	Putative dynamin-related GTPase	1.52E-296
CAL0004733	2.49	Uncharacterized ORF	5.71E-74
CAL0005604	2.49	Uncharacterized ORF	1.87E-44
CAL0006261	2.48	Secreted aspartyl proteinase	2.56E-06
CAL0003232	2.48	Protein kinase C	4.23E-09
CAL0005369	2.48	Transcriptional activator	2.16E-11
CAF0006954	2.48	Uncharacterized ORF	1.79E-30
CAL0001744	2.48	Heat shock protein	1.00E-300
CAL0003154	2.48	Regulator of nitrogen utilization	8.25E-44
CAL0006027	2.48	Putative ferric reductase	4.58E-11
CAL0000707	2.47	Uncharacterized ORF	1.31E-18
CAL0004107	2.47	Uncharacterized ORF	1.00E-300

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0003873	2.47	Putative adhesin-like protein	6.55E-144
CAL0003395	2.47	Putative UDP glucose/starch glucosyltransferase	3.80E-45
CAL0004704	2.46	Uncharacterized ORF; Hap43p-repressed gene	1.60E-07
CAL0005204	2.45	Putative GPI-anchored protein	1.35E-35
CAL0001161	2.45	Putative glycerol 3-P dehydrogenases)	1.00E-300
CAL0002626	2.45	Uncharacterized ORF	0.000985931
CAF0006965	2.45	Flavin-linked sulfhydryl oxidase activity and role in cellular iron ion homeostasis	6.24E-111
CAL0002283	2.45	NAD(P)H oxidoreductase family protein; induced by nitric oxide	3.37E-41
CAL0000393	2.45	Ortholog(s) have mitochondrion localization	8.65E-38
CAL0003208	2.44	Ortholog(s) have thiol oxidase activity	4.77E-124
CAL0004615	2.44	Srb9p transcription mediator	1.43E-18
CAL0003229	2.44	Uncharacterized ORF; Similar to Rab GTPase	3.84E-32
CAL0003874	2.44	Uncharacterized ORF	1.51E-23
CAL0001980	2.44	Uncharacterized ORF	3.86E-88
CAL0003844	2.44	Putative RNA-binding protein	9.35E-124
CAL0003011	2.44	Uncharacterized ORF; Ortholog(s) have phosphatidylserine decarboxylase activity,	1.24E-108
CAL0005953	2.43	Putative class III aminotransferase	7.43E-07
CAL0003062	2.43	Plasma membrane-localized protein	5.53E-84
CAL0001158	2.43	Aldo-keto reductase family member	1.57E-36
CAL0000894	2.42	Probable protein kinase	6.32E-176
CAL0003267	2.42	Uncharacterized ORF	2.20E-48
CAL0002010	2.42	Putative mitochondrial respiratory protein	8.89E-50
CAL0000008	2.42	Uncharacterized ORF	1.58E-64
CAL0002917	2.41	Uncharacterized ORF	1.66E-235
CAL0000997	2.41	Uncharacterized ORF	7.80E-22
CAL0005126	2.41	Putative mitochondrial RNA polymerase	1.00E-300
CAL0006340	2.41	Putative transcription factor with a role in colony morphology	1.64E-52
CAL0004070	2.40	Putative thiamin-phosphate pyrophosphorylase	2.74E-29
CAL0002453	2.40	Putative phosphatidylinositol kinase	7.28E-111
CAL0002529	2.40	Cell wall adhesin-like protein;repressed by Rim101p	3.68E-36
CAL0005002	2.40	Sef1p-, Sfu1p-, and Hap43p regulated gene	1.67E-05
CAL0002087	2.40	Uncharacterized ORF	7.42E-10
CAL0005933	2.39	structural constituent of ribosome and localizes to mitochondrial small ribosomal subunit	1.00E-300
CAL0000530	2.39	Putative beta-mannosyltransferase,	5.53E-124
CAL0005365	2.39	Putative RNA-binding protein	3.78E-128
CAL0004261	2.38	Uncharacterized ORF	3.31E-29

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0005727	2.38	Regulated by Sef1p-, Sfu1p-, and Hap43p	2.17E-124
CAL0003673	2.38	Putative translation initiation factor	1.61E-49
CAL0003274	2.38	Putative Rho1p GTPase activating protein	9.53E-133
CAL0005434	2.38	Ortholog(s) have phosphatidylinositol-4-phosphate phosphatase activity	1.48E-157
CAL0003363	2.37	Uncharacterized ORF; Putative fungal-specific transmembrane protein	1.00E-300
CAL0005212	2.37	Putative patatin-like phospholipase	1.45E-19
CAL0005009	2.37	Uncharacterized ORF	1.19E-33
CAL0002807	2.36	Uncharacterized ORF	8.61E-52
CAL0004422	2.36	Uncharacterized ORF	1.00E-300
CAL0003054	2.36	Putative cell wall protein, member of the CRH family	5.91E-52
CAL0005800	2.36	Uncharacterized ORF	6.64E-06
CAL0005015	2.36	Putative serine/threonine/tyrosine (dual-specificity) kinase	2.13E-111
CAL0000621	2.35	Putative processing peptidase	7.39E-147
CAL0005588	2.35	Uncharacterized ORF	3.04E-50
CAL0003243	2.35	Imidazoleglycerol-phosphate dehydratase	2.81E-33
CAL0004910	2.35	Protein involved in sphingolipid biosynthesis	4.97E-111
CAL0002160	2.35	Protein similar to asparagine and glutamine permease	1.00E-300
CAL0004555	2.35	Putative transcription factor with zinc cluster DNA-binding motif	1.34E-42
CAL0005224	2.34	ERK-family protein kinase	3.82E-89
CAL0002154	2.34	Uncharacterized ORF	2.07E-15
CAL0120529	2.34	Uncharacterized ORF	0.285184
CAL0005343	2.34	involved in ubiquitin-mediated protein degradation	3.73E-263
CAF0007423	2.34	Rho GTPase activator activity	1.00E-300
CAL0005683	2.34	Verified ORF;mutation affects filamentous growth	1.53E-14
CAL0005653	2.34	Protein mannosyltransferase	1.00E-300
CAL0004518	2.34	Ortholog(s) have poly(A)-specific ribonuclease activity	3.47E-198
CAL0002941	2.33	Uncharacterized ORF	5.21E-34
CAL0003907	2.33	Uncharacterized ORF;regulated upon white-opaque switching	2.41E-65
CAL0006309	2.33	Protein with a Gal4p-like DNA-binding domain	3.88E-139
CAL0004511	2.33	Putative oxidoreductas	1.00E-300
CAL0003712	2.33	Uncharacterized ORF	2.61E-294
CAL0002210	2.33	Uncharacterized ORF	1.91E-13
CAL0001069	2.33	Putative membrane protein with a predicted role in membrane fusion during mating	3.61E-20
CAL0001600	2.32	Protein involved in heme uptake	2.22E-134

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0001725	2.32	Putative protein kinase with a role in cell wall regulation	1.45E-82
CAL0004577	2.32	Protein involved in synthesis of the thiamine precursor hydroxymethylpyrimidine	1.00E-300
CAL0005580	2.31	Role in manganese ion transport and mitochondrion localization	2.69E-72
CAL0001210	2.31	Putative phosphoglycerate mutase	3.16E-222
CAL0002602	2.31	Uncharacterized ORF	1.00E-300
CAL0002765	2.31	Protein with a predicted role in docking and fusion of post-Golgi vesicles with the plasma membrane	8.20E-174
CAL0000064	2.31	Required for homologous DNA recombination	1.54E-62
CAL0005516	2.30	Possible regulatory protein	1.29E-89
CAL0000818	2.30	Uncharacterized ORF	1.00E-300
CAL0003539	2.30	Dubious ORF	1.07E-93
CAL0000429	2.30	Uncharacterized ORF	2.80E-37
CAL0003065	2.30	3-hydroxyacyl-CoA epimerase	3.11E-113
CAL0001565	2.30	Putative cytochrome b2 precursor protein	4.50E-37
CAL0006330	2.30	Uncharacterized ORF	2.44E-07
CAL0005320	2.29	Uncharacterized ORF	4.00E-16
CAL0006120	2.29	Uncharacterized ORF	1.44E-70
CAL0000099	2.29	Protein similar to Yta6p ATPase	3.99E-76
CAL0002790	2.29	Uncharacterized ORF	1.16E-269
CAL0005497	2.29	Uncharacterized ORF	1.96E-51
CAL0002655	2.29	Uncharacterized ORF	0.000290448
CAL0005239	2.29	Uncharacterized ORF	2.46E-46
CAL0000417	2.29	Uncharacterized ORF; Hap43p-repressed gene	9.40E-69
CAL0000807	2.29	Uncharacterized ORF; Putative adhesin-like protein	1.53E-27
CAL0000816	2.29	Putative tRNA-Pro synthetase	2.46E-235
CAL0004665	2.28	Uncharacterized ORF	1.00E-300
CAL0001498	2.28	Putative glucose transporter of the major facilitator superfamily	1.19E-22
CAL0000139	2.28	Uncharacterized ORF	7.93E-42
CAL0003557	2.28	Putative enzyme with predicted indole-3-glycerol-phosphate synthase	8.74E-61
CAL0002003	2.28	Putative vacuolar transporter of large neutral amino acids	1.08E-47
CAL0001629	2.27	Uncharacterized ORF	1.05E-20
CAL0005560	2.27	Protein mannosyltransferase	1.00E-300
CAL0006399	2.27	Uncharacterized ORF	2.06E-39
CAL0001441	2.27	ALS family cell-surface glycoprotein	2.72E-34
CAL0004068	2.27	Uncharacterized ORF; mitochondrion localization	4.63E-142
CAL0001828	2.26	Heat shock protein	1.00E-300
CAF0006969	2.26	Gene induced by hypoxia	5.23E-55

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0006390	2.26	Uncharacterized ORF; Hap43p-induced gene	7.66E-59
CAL0001098	2.26	Uncharacterized ORF	3.11E-17
CAL0000244	2.25	Putative oligopeptide transporter	1.00E-300
CAL0003239	2.25	Uncharacterized ORF; Hap43p-induced gene; induced upon biofilm formation	5.71E-114
CAL0005620	2.25	Uncharacterized ORF	1.76E-06
CAL0003766	2.25	Uncharacterized ORF	3.24E-92
CAL0005021	2.25	Putative glucose transporter of the major facilitator superfamily;	7.93E-28
CAL0003571	2.25	Putative sphingosine kinase	6.49E-19
CAL0001179	2.25	Uncharacterized ORF; Hap43p-repressed gene	1.64E-112
CAL0001213	2.25	Uncharacterized ORF; Putative serine/threonine protein kinase	4.63E-78
CAL0000075	2.25	Similar to <i>S. cerevisiae</i> Afg3p, a subunit of the mitochondrial inner membrane m-AAA protease	1.00E-300
CAL0005852	2.25	Uncharacterized ORF	4.23E-88
CAL0001131	2.24	Uncharacterized ORF	8.23E-47
CAL0005119	2.24	Late-stage biofilm-induced gene	8.70E-19
CAL0002530	2.24	Uncharacterized ORF	7.17E-22
CAL0002548	2.24	Uncharacterized ORF; Hap43p-repressed gene	9.91E-111
CAL0005698	2.24	Uncharacterized ORF	9.99E-29
CAL0005471	2.24	Uncharacterized ORF	1.49E-41
CAL0004790	2.24	Uncharacterized ORF	1.35E-09
CAL0002761	2.24	Uncharacterized ORF	4.01E-156
CAL0003737	2.24	Uncharacterized ORF	1.00E-300
CAL0001944	2.24	Uncharacterized ORF	5.76E-24
CAL0006276	2.23	role in mitochondrial translation and mitochondrion localization	1.00E-300
CAL0003151	2.23	Putative oxidized purine base lesion DNA N-glycosylase activity	0.00207077
CAL0002954	2.23	Putative GTPase-activating protein	1.00E-300
CAL0005141	2.23	Ortholog(s) have protein kinase regulator activity	6.25E-37
CAL0004072	2.23	Uncharacterized ORF	2.24E-07
CAF0006932	2.22	Putative copper metallochaperone; Hap43p-repressed gene	3.27E-44
CAL0004575	2.22	Uncharacterized ORF	1.40E-253
CAL0004606	2.22	Predicted membrane transporter	3.65E-30
CAL0000789	2.22	Uncharacterized ORF	4.27E-07
CAL0004832	2.22	Ortholog(s) have ATP-dependent RNA helicase activity	8.53E-141
CAL0004600	2.22	Uncharacterized ORF; Adenine phosphoribosyltransferase; flucytosine induced; repressed by nitric oxide; protein level decreased in stationary phase yeast cultures	1.46E-43
CAL0004042	2.21	Putative type-1 protein phosphatase targeting subunit	2.98E-57

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0006244	2.21	Uncharacterized ORF	4.31E-26
CAL0003485	2.21	Uncharacterized ORF	2.11E-45
CAL0005202	2.21	Putative pyruvate decarboxylase	1.00E-300
CAL0006036	2.20	Putative dicarboxylic amino acid permease	1.00E-300
CAL0002245	2.20	Ribonucleoside-diphosphate reductase	1.00E-300
CAL0004789	2.20	Uncharacterized ORF	1.04E-18
CAL0000249	2.20	Uncharacterized ORF; Predicted zinc-finger protein	1.99E-07
CAL0005453	2.20	Ortholog(s) have metalloendopeptidase activity	1.43E-159
CAL0003949	2.20	Uncharacterized ORF	1.56E-161
CAL0004457	2.20	Copper- and zinc-containing superoxide dismutase	1.62E-146
CAL0004587	2.20	Uncharacterized ORF	1.00E-300
CAL0002979	2.20	Protein involved in the negative control of pseudohyphal growth	2.05E-139
CAL0003016	2.20	Protein with role in resistance to host antimicrobial peptides	1.00E-300
CAL0003838	2.19	Putative RSC chromatin remodeling complex component	5.64E-65
CAL0003199	2.19	Cytosolic chaperonin Cct ring complex	4.33E-32
CAL0005655	2.19	Uncharacterized ORF; Hap43p-induced gene	6.02E-13
CAL0003178	2.19	Uncharacterized ORF	3.17E-17
CAL0006182	2.19	Uncharacterized ORF	1.96E-58
CAL0002565	2.19	Putative biotin synthase	1.03E-14
CAL0005976	2.19	Uncharacterized ORF; Protein similar to DNA helicase	1.03E-36
CAL0002649	2.19	Uncharacterized ORF	1.52E-22
CAL0005517	2.19	Uncharacterized ORF; Hap43p-repressed gene	1.46E-112
CAL0001427	2.19	Putative Arf3p GTPase activating protein	6.30E-71
CAL0002041	2.18	Secreted aspartyl protease	1.61E-12
CAL0002725	2.18	Uncharacterized ORF	3.33E-17
CAL0003641	2.18	Uncharacterized ORF	1.00E-300
CAL0005854	2.18	Uncharacterized ORF; Hap43p-repressed gene	3.99E-210
CAL0001120	2.18	Uncharacterized ORF; Hap43p-repressed gene	3.22E-53
CAL0002025	2.18	Protein involved in proteolytic activation of Rim101p	4.85E-38
CAL0004709	2.18	Protein with predicted role in beta-1,6-glucan synthesis	4.43E-63
CAL0005745	2.18	Putative PDR-subfamily ABC transporter	4.63E-95
CAL0001297	2.18	Uncharacterized ORF	0.00081435
CAL0001575	2.18	Putative termination and polyadenylation protein	1.01E-158
CAL0001136	2.18	Putative mitochondrial carrier protein	1.00E-300
CAL0005112	2.18	Member of a family encoded by FGR6-related genes	8.41E-13
CAL0003852	2.18	Uncharacterized ORF	3.17E-13

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0000385	2.18	oxidoreductase activity, role in cytochrome c-heme linkage, mitochondrial membrane organization and extrinsic to mitochondrial inner membrane localization	6.79E-92
CAL0006152	2.17	Verified ORF; Putative RNA helicase	9.78E-45
CAL0004999	2.17	mRNA binding activity, role in mitochondrion organization	8.60E-12
CAL0120801	2.17	Uncharacterized ORF	8.90E-114
CAL0004816	2.17	Ortholog(s) have DNA-directed DNA polymerase activity mitochondrion, nuclear chromatin localization	3.39E-29
CAL0002229	2.16	Uncharacterized ORF	2.29E-11
CAL0005461	2.16	Uncharacterized ORF	1.00E-300
CAL0005737	2.16	Ortholog(s) have ubiquitin-specific protease activity	9.93E-127
CAL0002777	2.16	Putative secreted aspartyl protease	0.000631581
CAL0003073	2.16	Uncharacterized ORF; induced by nitric oxide	5.01E-06
CAL0002942	2.16	Putative GTPase-activating protein (GAP) for Rho-type GTPase Cdc42p	1.03E-132
CAL0004311	2.15	Predicted ribosomal protein	2.58E-35
CAL0000861	2.15	Putative GINS complex subunit	7.61E-10
CAL0003131	2.15	Uncharacterized ORF	1.14E-142
CAL0000137	2.15	Uncharacterized ORF	1.28E-64
CAL0000012	2.14	Uncharacterized ORF	2.54E-05
CAL0000489	2.14	Uncharacterized ORF	1.83E-31
CAL0003370	2.14	Cell wall protein	7.77E-39
CAL0003919	2.14	Ortholog(s) have role in ubiquinone biosynthetic process, aerobic respiration and mitochondrial inner membrane	6.49E-170
CAF0007419	2.14	Putative adhesin-like protein	5.21E-33
CAL0000184	2.14	Protein lacking an ortholog in <i>S. cerevisiae</i> ; transposon mutation affects filamentous growth	3.69E-106
CAL0001237	2.14	Uncharacterized ORF	1.75E-11
CAL0005436	2.14	VH1 family MAPK phosphatase	8.33E-39
CAL0004905	2.13	Putative GPI-anchored adhesin-like protein	2.55E-20
CAL0000235	2.13	Monooxygenase of the cytochrome P450	6.85E-09
CAL0000885	2.13	Uncharacterized ORF	2.79E-22
CAL0000116	2.13	Uncharacterized ORF	1.00E-300
CAL0003638	2.13	Uncharacterized ORF	7.40E-116
CAL0001553	2.13	Cell wall protein; putative GPI-anchor	9.41E-211
CAL0004810	2.13	Uncharacterized ORF	1.49E-217
CAL0005353	2.13	Malate synthase; glyoxylate cycle enzyme	3.47E-144
CAL0005948	2.12	Uncharacterized ORF	1.00E-11
CAL0120947	2.12	Uncharacterized ORF	0.130919

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0001758	2.12	Putative methionyl-tRNA transformylase	1.49E-39
CAL0001396	2.12	Predicted acyl-CoA oxidase	9.03E-36
CAL0003804	2.12	Uncharacterized ORF	6.11E-11
CAL0002434	2.12	Plasma membrane, hyphal cell wall protein	4.89E-236
CAL0005443	2.12	Uncharacterized ORF	1.72E-152
CAL0001496	2.12	Uncharacterized ORF	1.05E-52
CAL0000122	2.12	Uncharacterized ORF	1.48E-128
CAL0000180	2.11	Uncharacterized ORF; Putative mRNA maturation factor	8.06E-242
CAL0005908	2.11	Uncharacterized ORF; Hap43p-repressed gene	2.41E-46
CAL0002933	2.11	Transcriptional repressor	2.00E-19
CAL0001799	2.11	Putative Rho GTPase activating protein	1.52E-202
CAL0005187	2.11	Heme oxygenase	3.10E-129
CAL0006202	2.11	Uncharacterized ORF; Putative MFS transporter	6.17E-15
CAL0004874	2.11	Uncharacterized ORF; Hap43p-repressed gene	2.14E-24
CAL0005238	2.11	Uncharacterized ORF	2.71E-20
CAL0000305	2.11	Putative allantoate permease	2.42E-17
CAL0003619	2.11	Uncharacterized ORF	1.00E-300
CAL0005019	2.11	Uncharacterized ORF	1.82E-23
CAL0003351	2.11	Uncharacterized ORF	1.00E-300
CAL0002509	2.11	Alcohol dehydrogenase	7.64E-267
CAL0003264	2.10	Putative histone acetyltransferase complex subunit	1.00E-300
CAL0003448	2.10	Voltage-gated Ca ²⁺ channel of the calcium uptake system;	9.05E-43
CAL0006303	2.10	Uncharacterized ORF	1.64E-21
CAL0004451	2.10	Cell wall adhesin required for cell-cell adhesion, GPI anchored	5.41E-50
CAL0001139	2.10	Uncharacterized ORF	1.11E-28
CAL0002621	2.105	Protein similar to <i>S. cerevisiae</i> Bck1p MAP kinase kinase kinase of cell integrity pathway	6.08E-40
CAL0005999	2.10	Subunit of beta-1,3-glucan synthase	1.00E-300
CAL0005832	2.103	Uncharacterized ORF	6.30E-05
CAL0003148	2.10	Uncharacterized ORF	3.07E-83
CAL0001560	2.10	Uncharacterized ORF	8.95E-101
CAL0000729	2.10	Uncharacterized ORF	5.16E-61
CAL0001943	2.10	Uncharacterized ORF	4.02E-31
CAL0005421	2.10	ALS family protein	1.29E-24
CAL0000198	2.09	Uncharacterized ORF; Hexokinase II	1.00E-300
CAL0004675	2.09	Uncharacterized ORF	2.44E-26
CAL0002088	2.09	Uncharacterized ORF	4.58E-15
CAL0004157	2.09	Uncharacterized ORF	1.55E-73
CAL0000038	2.09	Uncharacterized ORF	2.01E-72

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0005977	2.09	Pyruvate kinase	1.00E-300
CAL0000651	2.09	Uncharacterized ORF; Hap43p-repressed	1.02E-170
CAL0003413	2.08	Uncharacterized ORF; histone demethylase activity	1.50E-19
CAL0002370	2.08	Role in heme a biosynthetic process and mitochondrion localization	6.82E-199
CAL0005685	2.08	C-14 sterol reductase	6.75E-140
CAL0003516	2.08	Uncharacterized ORF	1.12E-82
CAL0000518	2.08	Uncharacterized ORF	1.36E-261
CAL0003880	2.08	Transcription factor involved in alkaline pH response	8.70E-12
CAL0003394	2.08	Dubious ORF	1.00E-300
CAL0003139	2.08	Uncharacterized ORF	4.73E-58
CAL0000858	2.07	Uncharacterized ORF	1.11E-05
CAL0000614	2.07	Ferric reductase	2.43E-05
CAL0001626	2.07	Uncharacterized ORF	5.49E-31
CAL0006048	2.07	Uncharacterized ORF	1.90E-64
CAL0003728	2.07	Uncharacterized ORF	8.55E-87
CAL0000438	2.07	Uncharacterized ORF	4.57E-18
CAL0005901	2.07	Glucosaminyl-phosphatidylinositol O-acyltransferase activity	9.11E-65
CAL0005404	2.07	Phosphatidylinositol 3-phosphate 5-kinase	1.79E-64
CAL0001834	2.07	Uncharacterized ORF	3.43E-91
CAL0004653	2.07	Uncharacterized ORF	3.25E-192
CAL0002276	2.07	Uncharacterized ORF; Putative glycerol-3-phosphate acyltransferase; fungal-specific (no human or murine homolog)	1.77E-70
CAL0004490	2.07	Uncharacterized ORF; Hap43p-repressed gene	1.01E-149
CAL0002184	2.07	Putative glucose-6-phosphate isomerase, enzyme of glycolysis;	1.00E-300
CAL0004695	2.07	Uncharacterized ORF	3.71E-52
CAL0000445	2.07	Uncharacterized ORF	4.65E-49
CAL0005341	2.06	Uncharacterized ORF; Hap43p-repressed gene	3.07E-110
CAL0001865	2.06	Putative mitochondrial large subunit ribosomal protein	6.62E-93
CAL0005273	2.06	Uncharacterized ORF	2.78E-38
CAL0001902	2.06	Putative Golgi alpha-1,2-mannosyltransferase	7.80E-141
CAL0004450	2.06	Uncharacterized ORF	2.18E-38
CAL0004425	2.06	Uncharacterized ORF	7.10E-51
CAL0003326	2.06	Uncharacterized ORF	1.08E-37
CAL0003492	2.06	Uncharacterized ORF	4.56E-47
CAL0000052	2.06	Uncharacterized ORF	1.86E-211
CAL0003572	2.06	Uncharacterized ORF; induced by nitric oxide	1.64E-15
CAL0006266	2.06	Uncharacterized ORF; oxidoreductase	1.82E-39
CAL0002993	2.06	Possible oxidoreductase	1.51E-05

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0001941	2.05	Uncharacterized ORF; Putative golgi GTPase-activating protein	1.49E-108
CAL0003528	2.05	Uncharacterized ORF	2.80E-127
CAL0006090	2.05	Uncharacterized ORF	2.07E-18
CAL0002950	2.05	Uncharacterized ORF; Ortholog(s) have peptidase activity	1.00E-300
CAL0004580	2.05	Protein with a role in phosphate transport	1.00E-300
CAL0004949	2.05	Protein similar to <i>S. cerevisiae</i> Ecm29p	2.72E-153
CAF0006924	2.05	Uncharacterized ORF	2.38E-33
CAL0005656	2.05	Uncharacterized ORF; Putative proteinase	1.00E-300
CAL0001630	2.05	Putative transcription factor with zinc finger DNA-binding motif	4.07E-34
CAL0004808	2.05	protein involved in regulation of SCF complex	9.38E-104
CAL0002479	2.05	Uncharacterized ORF	0.00195682
CAL0005679	2.05	Uncharacterized ORF; Ortholog of <i>C. glabrata</i> CBS138<i> : CAGL0L12628g	5.77E-37
CAL0004963	2.04	Uncharacterized ORF	3.51E-05
CAL0000444	2.04	Uncharacterized ORF	1.00E-300
CAL0002334	2.04	Putative RNA binding protein	4.85E-28
CAL0006143	2.04	Predicted membrane transporter,	8.71E-25
CAL0001524	2.04	Ribonucleotide reductase large subunit;	1.00E-300
CAL0005233	2.04	Protein involved in the pH response pathway;	2.73E-34
CAL0005308	2.04	Scaffold protein for the mitogen-activated protein (MAP) kinase	1.88E-16
CAL0001752	2.04	Uncharacterized ORF	1.28E-12
CAL0004037	2.04	Protein with putative zinc finger	1.09E-10
CAL0006311	2.04	Uncharacterized ORF	6.91E-48
CAL0000549	2.04	Uncharacterized ORF	3.21E-223
CAL0002936	2.040	Major carnitine acetyl transferase localized in peroxisomes and mitochondria	3.79E-151
CAL0005024	2.03	Lysine histone methyltransferase; methylates K4 of histone H3	1.06E-42
CAL0005328	2.03	Uncharacterized ORF	2.32E-56
CAL0005010	2.03	Hyphal growth regulator	1.00E-300
CAL0004775	2.03	Adhesin-like protein;Rim101p-repressed	3.27E-142
CAL0003640	2.03	Putative high affinity methionine permease; upregulated by Rim101p	5.61E-242
CAL0000583	2.03	Putative 1,4-glucan branching enzyme	2.71E-240
CAL0002321	2.03	Uncharacterized ORF	1.47E-29
CAL0000547	2.029	Uncharacterized ORF	1.68E-10
CAF0006896	2.028	Ribosomal protein S27	4.73E-14
CAL0003161	2.028	Uncharacterized ORF; Hap43p-induced gene	3.42E-34
CAL0000141	2.025	Predicted zinc-finger protein	5.33E-61
CAL0005260	2.023	Putative kynurenine 3-monooxygenase	3.36E-206

Table A-3 continued

Gene ID	Fold up-regulated	CGD annotated Function	P-value
CAL0005696	2.022	Uncharacterized ORF	6.75E-137
CAL0004102	2.021	Uncharacterized ORF	9.82E-89
CAL0004468	2.021	Uncharacterized ORF; Putative transcription factor with zinc finger DNA-binding motif	1.57E-28
CAL0002900	2.020	Essential protein involved in endocytosis and polarized growth;	1.00E-300
CAL0001871	2.020	Uncharacterized ORF	1.00E-300
CAL0004405	2.019	Uncharacterized ORF	6.18E-173
CAL0003306	2.018	Putative ribosomal protein;	2.59E-83
CAL0000720	2.018	Uncharacterized ORF	1.50E-270
CAL0004028	2.017	Sphingolipid delta-8 desaturase	4.87E-184
CAL0003072	2.017	Predicted subunit of the exocyst complex,	1.01E-44
CAL0003196	2.017	Uncharacterized ORF	8.16E-177
CAL0003800	2.016	Carboxypeptidase Y	4.03E-110
CAL0002787	2.015	Uncharacterized ORF	1.71E-53
CAL0003545	2.015	Uncharacterized ORF	2.43E-87
CAL0001802	2.014	Uncharacterized ORF	6.95E-24
CAL0003613	2.014	Uncharacterized ORF	1.03E-25
CAL0002988	2.014	Uncharacterized ORF	4.25E-63
CAL0002002	2.011	Glycosidase of cell surface	1.00E-300
CAL0002890	2.010	Uncharacterized ORF	4.98E-24
CAL0002238	2.010	Uncharacterized ORF	5.74E-08
CAL0000516	2.009	Putative glutamate synthase	1.00E-300
CAF0006928	2.009	Uncharacterized ORF	3.06E-12
CAL0005962	2.009	Uncharacterized ORF; hypoxia induced	1.29E-97
CAL0003703	2.008	Uncharacterized ORF; role in mitochondrial translational termination and mitochondrion localization	0.0019543
CAL0120967	2.008	Uncharacterized ORF	0.000246561
CAL0003185	2.006	Master regulator (activator) of a-type mating	1.45E-42
CAL0000015	2.005	Uncharacterized ORF	7.82E-115
CAL0004900	2.004	Uncharacterized ORF	3.35E-09
CAL0000796	2.004	Uncharacterized ORF t	1.72E-65
CAL0000004	2.004	Uncharacterized ORF	1.66E-80
CAL0003029	2.000	Protein similar to <i>S. cerevisiae</i> Cdc20p	1.09E-52

Table A-4: List of all the genes down-regulated in the *cho1* Δ/Δ mutant compared to the wild-type strain

Gene ID	Fold down-regulated	Function	P-value
CAL0003238	2.01	Putative dihydrolipoamide acetyltransferase	7.16E-70
CAL0003246	2.02	Ortholog(s) have role in protein import into mitochondrial outer membrane, phospholipid transport, mitochondrial outer membrane translocase complex assembly	1.25E-36
CAL0004170	2.02	GPI-anchored cell wall transglycosylase	6.84E-50
CAL0001598	2.02	Uncharacterized ORF	5.01E-142
CAL0005139	2.02	Uncharacterized ORF; Hap43p-repressed gene	1.00E-300
CAL0001250	2.05	Uncharacterized ORF	1.34E-110
CAL0000435	2.08	Putative 30 kda heat shock protein	1.16E-69
CAL0000520	2.08	Protein with homology to peroxisomal membrane proteins; Sef1p-, Sfu1p-, and Hap43p-regulated gene	2.47E-18
CAL0001881	2.08	Putative heme-binding stress-related protein	4.04E-190
CAL0005617	2.09	Uncharacterized ORF	1.00E-300
CAL0004738	2.11	Uncharacterized ORF; regulated by Sef1p-, Sfu1p-, and Hap43p	1.27E-63
CAL0005454	2.12	Putative mitochondrial inner membrane transporter	2.20E-92
CAL0000815	2.14	Uncharacterized ORF	2.36E-46
CAL0003028	2.17	Uncharacterized ORF	0.0472951
CAL0003510	2.17	Putative 3-hydroxyacyl-[acyl-carrier-protein] dehydratase activity	0.000408543
CAL0001041	2.17	Uncharacterized ORF	4.30E-185
CAL0004846	2.18	Uncharacterized ORF; Hap43p-induced gene	7.94E-147
CAL0005956	2.18	Dubious ORF	2.72E-36
CAL0004997	2.19	Uncharacterized ORF	4.67E-247
CAL0000267	2.23	Putative nucleolar protein	1.37E-61
CAL0002986	2.23	Ferric reductase	1.96E-11
CAL0004548	2.24	Uncharacterized ORF	1.00E-300
CAL0004552	2.25	Alternative oxidase	3.08E-22
CAL0002995	2.25	Transcriptional repressor	6.36E-288
CAF0006908	2.26	Hap43p-repressed gene; hypoxia downregulated	1.50E-57
CAL0000921	2.26	Putative RNase MRP and nuclear RNase P component	5.43E-83
CAL0004612	2.28	Uncharacterized ORF	0.0411878
CAL0004328	2.28	Uncharacterized ORF; Protein not essential for viability	2.06E-55
CAL0005065	2.28	Putative nucleolar protein with role in ribosomal assembly	6.46E-49
CAL0003742	2.29	a ubiquitin hydrolase	1.97E-07
CAL0002023	2.31	Ortholog(s) have telomerase inhibitor activity	4.97E-130
CAL0001782	2.32	Putative inducible acid phosphatase	1.00E-300
CAL0001718	2.34	Secreted protein	6.49E-176
CAL0002504	2.38	Uncharacterized ORF	4.78E-108

Table A-4 continued

Gene ID	Fold down-regulated	Function	P-value
CAL0003860	2.38	Uncharacterized ORF	2.32E-27
CAL0001507	2.46	Putative transcription factor with bZIP DNA-binding motif	3.74E-196
CAL0001053	2.47	Putative nucleolar protein	6.97E-130
CAL0005460	2.48	Putative ATP-dependent DEAD-box RNA helicase	4.05E-67
CAL0003683	2.50	Ortholog(s) have phosphatidylinositol-3-phosphate binding, phosphatidylinositol-5-phosphate binding activity	3.75E-14
CAL0000014	2.52	Uncharacterized ORF; Hap43p-induced gene	1.06E-62
CAL0000479	2.56	Uncharacterized ORF; Ortholog(s) have rRNA binding activity	8.11E-67
CAL0006057	2.57	Putative constituent of 66S pre-ribosomal particles	1.08E-144
CAL0004872	2.58	Uncharacterized ORF	1.08E-144
CAL0000754	2.65	Putative zinc finger transcription factor	2.91E-29
CAL0002350	2.68	Response regulator of a two-component system involved in stress	7.32E-220
CAL0001258	2.69	Uncharacterized ORF; Hap43p-induced gene	3.48E-27
CAL0002743	2.74	Similar to catabolic serine/threonine dehydratases;	2.69E-268
CAL0003582	2.78	Putative RNA polymerase III transcription initiation factor complex subunit	1.84E-38
CAL0002189	2.89	Uncharacterized ORF	9.61E-20
CAL0001847	2.92	Protein of unknown function; Plc1p-regulated	1.20E-08
CAL0006076	2.93	Uncharacterized ORF	7.76E-81
CAL0005712	2.96	Uncharacterized ORF	1.76E-42
CAL0001467	3.00	Glycerol permease involved in glycerol uptake	3.86E-41
CAL0006301	3.05	Dubious ORF	1.01E-163
CAF0007089	3.08	Ortholog(s) have electron carrier activity	1.56E-27
CAL0001013	3.12	Uncharacterized ORF; Biofilm-induced gene	2.24E-174
CAL0120807	3.14	Putative adhesin-like protein	8.61E-11
CAL0005860	3.15	Predicted hydrolase activity	1.00E-209
CAL0003136	3.35	Ortholog(s) have role in cytoplasmic translation	4.70E-19
CAL0003776	3.43	Cell wall protein	1.66E-113
CAL0004550	3.87	Alternative oxidase	0.000190087
CAL0000982	3.89	Formate dehydrogenase	2.04E-207
CAL0004487	4.02	Putative cytosolic iron-sulfur (FeS) protein assembly machinery protein; induced by nitric oxide	8.97E-156
CAL0000550	5.15	Glycerophosphoinositol permease	1.03E-78
CAL0003313	5.23	Putative TFIID and SAGA complex subunit	1.00E-300
CAL0005695	5.88	Uncharacterized ORF	1.01E-129
CAL0001883	7.40	Uncharacterized ORF	1.00E-300
CAL0120803	7.86	Uncharacterized ORF	1.00E-300
CAL0000501	8.37	Putative potassium transporter	3.25E-32
CAL0005929	8.61	Putative oxidoreductase	2.22E-62
CAL0002987	9.03	Putative oxidoreductase, iron utilization	6.73E-43
CAL0000931	20.80	Uncharacterized ORFe	4.50E-153

Table A-5: List of all the genes up-regulated in the *psd1*Δ/*psd2*Δ mutant compared to the wild-type strain

Gene ID	Fold up-regulated	Function	P-value
CAL0002907	17.62	Putative phosphomethylpyrimidine kinase	1.00E-300
CAL0004215	14.21	Putative tRNA splicing endonuclease subunit; Hap43p-induced	3.01E-261
CAL0004374	11.99	NADP-glutamate dehydrogenase	1.00E-300
CAL0004601	9.51	Uncharacterized ORF; Ortholog(s) have cytosol localization	1.82E-32
CAL0001974	9.22	Urea amidolyase	1.00E-300
CAL0005400	9.14	Protein similar to <i>S. cerevisiae</i> Hcm1p; Hap43p-induced gene	2.20E-88
CAL0002939	8.90	UDP-glucose:ceramide glucosyltransferase	4.54E-108
CAL0000318	8.83	Uncharacterized ORF; Hap43p-induced gene	1.00E-300
CAL0002632	8.53	Verified ORF; G1 cyclin	1.63E-259
CAL0000377	8.33	Uncharacterized ORF; Planktonic growth-induced gene	2.44E-16
CAL0004068	8.12	High affinity spermidine transporter	1.00E-300
CAL0004528	7.71	Putative transporter	6.72E-143
CAL0001357	7.54	Uncharacterized ORF	1.00E-300
CAL0003064	7.45	Uncharacterized ORF	4.01E-127
CAL0003075	7.42	Dubious ORF	7.39E-12
CAL0000215	7.18	Inositol-1-phosphate synthase; inositol biosynthesis	8.84E-59
CAL0002798	7.00	Uncharacterized ORF	7.71E-30
CAL0004602	6.93	Putative transporter	1.94E-98
CAL0000494	6.79	Essential protein involved in cytokinesis	1.73E-55
CAL0003463	6.78	Uncharacterized ORF; Cyclin homolog	3.41E-73
CAL0003477	6.47	Uncharacterized ORF	1.23E-58
CAL0005136	6.34	Putative transcription factor; oxidative stress-induced	1.00E-300
CAL0004833	6.31	Uncharacterized ORF; mitochondrion localization	1.00E-300
CAL0000998	6.30	Uncharacterized ORF	1.00E-300
CAL0001407	6.25	Putative DNA-binding transcription factor	3.81E-42
CAF0007042	6.09	Uncharacterized ORF	4.64E-14
CAL0004275	6.08	Uncharacterized ORF; Hap43p-repressed gene	4.37E-12
CAL0004519	6.06	Putative GPI-anchored cell wall adhesin-like protein	1.00E-300
CAL0003555	5.77	Putative ATP-binding protein with a predicted role in DNA replication	2.47E-89
CAL0000707	5.77	Uncharacterized ORF; Ortholog of <i>S. cerevisiae</i> : PRM5	1.91E-79
CAL0000371	5.74	Predicted membrane transporter; member of the proton coupled folate transporter/heme carrier protein (PCFT/HCP) family	1.90E-70
CAL0002531	5.70	Putative adhesin-like protein	1.00E-300
CAL0120975	5.66	Uncharacterized ORF	3.44E-155
CAL0005322	5.65	Uncharacterized ORF	8.14E-07
CAL0000344	5.54	Ortholog(s) have exonuclease II activity, role in RNA catabolic process and mitochondrial matrix localization	2.00E-218
CAL0004436	5.50	Putative DEAD-box protein required for efficient splicing of mitochondrial Group I and II introns; Hap43p-induced gene	1.00E-300

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000259	5.40	GPI-anchored protein; hypoxia, Hap43p-induced	1.22E-62
CAL0005923	5.36	Uncharacterized ORF	7.91E-48
CAL0003499	5.30	Uncharacterized ORF; Hap43p-repressed gene	1.09E-25
CAL0001605	5.27	ALS family cell wall adhesin	3.09E-177
CAL0001358	5.22	Putative mitochondrial leucyl-tRNA synthetase	1.00E-300
CAL0004847	5.21	Protein affects filamentous growth	5.85E-09
CAL0000413	5.10	General amino acid permease	2.53E-24
CAL0002161	5.06	Ortholog(s) have metalloendopeptidase activity, cellular iron ion homeostasis and mitochondrion localization	1.00E-300
CAL0005574	5.01	Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit localization	1.00E-300
CAL0005771	5.01	Ortholog(s) have role in mitochondrial tRNA threonylcarbamoyladenosine modification and mitochondrion localization	4.33E-146
CAL0006360	4.98	Uncharacterized ORF	1.24E-28
CAL0001415	4.96	Uncharacterized ORF	1.67E-102
CAL0005693	4.94	Uncharacterized ORF	2.68E-10
CAL0000885	4.91	Uncharacterized ORF	1.02E-114
CAL0004840	4.89	Dubious ORF	4.90E-05
CAL0000601	4.88	Uncharacterized ORF	1.00E-300
CAL0003267	4.86	Nucleoside permease	6.01E-167
CAL0004522	4.86	Putative transcription factor with zinc cluster DNA-binding motif	7.30E-11
CAL0001440	4.81	Adhesin	1.00E-300
CAL0002354	4.81	Putative ribonucleotide reductase large subunit	1.73E-82
CAL0001629	4.80	Uncharacterized ORF	2.25E-82
CAL0002549	4.75	Uncharacterized ORF	1.38E-05
CAL0004976	4.75	Ortholog(s) have 1-acylglycerophosphocholine O-acyltransferase activity	1.61E-168
CAL0004789	4.69	Uncharacterized ORF	3.99E-78
CAL0002550	4.67	Predicted zinc-finger protein	1.00E-300
CAL0000151	4.66	Predicted zinc-finger protein	4.92E-20
CAL0004952	4.66	Uncharacterized ORF	1.82E-77
CAL0000028	4.65	Putative RNA-binding protein	2.42E-126
CAL0003637	4.62	Uncharacterized ORF	1.00E-300
CAL0002630	4.61	Putative mitochondrial translation initiation factor	1.00E-300
CAL0005203	4.60	Putative GPI-anchored protein	2.05E-13
CAL0001515	4.60	Uncharacterized ORF	8.01E-39
CAL0003797	4.55	Amino acid permease	1.70E-255
CAL0005710	4.52	Putative transporter	5.69E-29
CAL0002049	4.51	Putative mitochondrial carrier protein	1.01E-258
CAL0003166	4.40	Putative vacuolar protease	1.00E-300
CAL0000858	4.38	Uncharacterized ORF	1.99E-22
CAL0003697	4.37	Putative mitochondrial membrane protein	1.00E-300
CAL0005633	4.37	Uncharacterized ORF; Putative transcription factor with zinc finger DNA-binding motif; Hap43p-induced	4.56E-59

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000188	4.35	Plasma-membrane-localized protein; filament induced, hypoxia-induced	1.64E-200
CAL0005320	4.35	Uncharacterized ORF	2.33E-50
CAL0005583	4.35	Uncharacterized ORF	5.76E-64
CAL0000430	4.35	Putative mitochondrial translation elongation factor	1.00E-300
CAL0002964	4.34	Protein involved in nuclear membrane fusion during karyogamy	4.06E-06
CAL0001309	4.34	Uncharacterized ORF	3.66E-62
CAL0000374	4.33	Putative protein of GPI synthesis	1.09E-48
CAL0000782	4.31	Uncharacterized ORF; Putative Pol protein of retrotransposon Tca2	1.00E-300
CAL0000285	4.29	Uncharacterized ORF	1.27E-42
CAL0002139	4.29	Uncharacterized ORF	3.58E-47
CAL0002992	4.28	Uncharacterized ORF; Basic amino acid permease	1.37E-116
CAL0003611	4.21	Uncharacterized ORF; Hap43p-repressed gene	6.26E-13
CAL0001720	4.16	Ribosomal protein of the large subunit, mitochondrial	1.00E-300
CAL0000808	4.16	Protein similar to isoleucyl-tRNA synthetase	1.00E-300
CAL0000715	4.13	Plasma membrane protein of heme-iron utilization	2.42E-17
CAL0001591	4.12	Protein has role in mitotic spindle positioning	6.88E-75
CAL0005579	4.10	Uncharacterized ORF	1.03E-29
CAL0120984	4.10	Uncharacterized ORF	2.08E-18
CAL0000023	4.10	Uncharacterized ORF; transcription is induced upon yeast-hyphal transition	2.58E-84
CAL0001478	4.09	Uncharacterized ORF	1.05E-261
CAL0000709	4.08	Basic amino acid permease	2.18E-49
CAL0001283	4.08	ALS family protein	1.12E-65
CAF0007422	4.05	Putative role in mitochondrial genome maintenance and integral to mitochondrial inner membrane localization	2.99E-226
CAF0006931	4.02	Uncharacterized ORF	1.10E-15
CAL0006161	3.99	Protein induced upon induction of hyphal growth	2.75E-212
CAL0001294	3.97	Uncharacterized ORF	5.57E-23
CAL0004745	3.95	Purine-cytosine permease of pyrimidine salvage	2.08E-205
CAL0001919	3.89	Uncharacterized ORF	3.38E-144
CAL0003673	3.89	Uncharacterized ORF	1.90E-113
CAL0002117	3.88	Transcriptional regulator of the CTR1 copper transporter	4.59E-94
CAL0003035	3.86	NADPH oxidoreductase	3.22E-42
CAL0004790	3.86	Uncharacterized ORF	6.21E-15
CAL0000929	3.86	Uncharacterized ORF	6.30E-135
CAL0000209	3.85	Putative transcription factor with zinc cluster DNA-binding motif	9.09E-211
CAL0001735	3.84	Putative ribonucleoside diphosphate reductase	6.67E-120
CAL0000390	3.82	Protein similar to mitochondrial tryptophanyl-tRNA synthetase	4.81E-161
CAL0005937	3.81	Uncharacterized ORF	3.22E-12
CAL0005126	3.81	Uncharacterized ORF; Putative mitochondrial RNA polymerase	1.00E-300
CAL0003199	3.79	G1 cyclin; required for hyphal growth	4.01E-89
CAL0000443	3.78	Cell wall acid trehalase	1.05E-85

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0005563	3.77	Uncharacterized ORF	1.07E-79
CAL0004832	3.76	Ortholog(s) have ATP-dependent RNA helicase activity	1.00E-300
CAL0005442	3.75	Transcriptional regulator of iron-responsive genes	8.04E-149
CAL0002996	3.74	Uncharacterized ORF	1.09E-07
CAL0004594	3.74	Protein similar to <i>S. cerevisiae</i> Esc4p, which represses transposition	4.46E-75
CAL0002565	3.74	Putative biotin synthase, Hap43p-repressed	2.68E-38
CAL0003435	3.73	Uncharacterized ORF	1.70E-10
CAL0004394	3.73	Uncharacterized ORF	4.06E-256
CAL0003968	3.72	Uncharacterized ORF; Hap43p-repressed gene	4.49E-19
CAL0004100	3.71	Uncharacterized ORF	7.25E-73
CAL0003188	3.69	Uncharacterized ORF	3.47E-37
CAL0001087	3.68	Uncharacterized ORF	1.23E-24
CAL0003714	3.66	Uncharacterized ORF; Hap43p-repressed gene	1.00E-300
CAL0005306	3.66	Uncharacterized ORF	1.41E-76
CAL0001776	3.62	Uncharacterized ORF	1.80E-27
CAL0004447	3.60	Uncharacterized ORF; Putative SH3-domain-containing protein	2.19E-12
CAL0000618	3.59	GPI-anchored cell wall protein involved in hemoglobin utilization	7.08E-55
CAL0003484	3.56	Uncharacterized ORF	1.16E-26
CAL0002282	3.55	Putative ceramide hydroxylase	1.00E-300
CAL0005948	3.54	Uncharacterized ORF	1.02E-29
CAL0002890	3.53	Uncharacterized ORF	7.30E-78
CAL0006398	3.52	Hyphal-induced GPI-anchored cell wall protein	2.61E-30
CAL0003880	3.50	Uncharacterized ORF	1.66E-31
CAL0006303	3.50	Uncharacterized ORF	1.58E-56
CAL0003338	3.49	Predicted zinc-finger protein	7.45E-52
CAL0003133	3.49	Putative mitochondrial acidic matrix protein;	1.00E-300
CAL0003532	3.48	Putative GPI-anchored protein; hyphal induced; Hog1p-downregulated	3.57E-149
CAL0005195	3.48	Uncharacterized ORF; Predicted ORF in retrotransposon	4.82E-30
CAL0002436	3.47	Putative prolyl-tRNA synthetase	2.12E-163
CAL0003149	3.46	Uncharacterized ORF; Hap43p-induced gene	1.33E-26
CAL0003984	3.45	Putative DNA damage checkpoint protein	6.29E-26
CAL0005876	3.45	Putative peptide:N-glycanase	1.83E-160
CAL0001213	3.44	Putative serine/threonine protein kinase	5.97E-162
CAL0002156	3.42	Uncharacterized ORF	5.50E-37
CAL0000393	3.42	Uncharacterized ORF	3.18E-59
CAL0002229	3.40	Uncharacterized ORF	1.17E-24
CAL0000351	3.40	Uncharacterized ORF	7.70E-32
CAL0002383	3.38	Uncharacterized ORF	2.55E-15
CAL0005700	3.38	Putative polysome-associated RNA binding protein	1.08E-32
CAL0002283	3.36	NAD(P)H oxidoreductase family protein; induced by nitric oxide	9.23E-62
CAL0003705	3.36	Similar to CCAAT-binding transcription factor that regulates respiration	4.38E-14

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0002467	3.36	Uncharacterized ORF	7.50E-48
CAL0006315	3.35	Uncharacterized ORF	8.70E-37
CAL0000807	3.33	Putative adhesin-like protein	4.28E-49
CAL0005323	3.31	Secreted lipase	2.06E-13
CAL0002540	3.28	Putative zinc cluster protein	8.99E-95
CAL0004624	3.28	Putative GPI-anchored protein	7.99E-51
CAL0004933	3.27	Immunogenic stress-associated protein; regulated by filamentous growth	4.09E-59
CAL0005593	3.27	Putative mitochondrial protein with a predicted role in respiratory growth	1.00E-300
CAL0005544	3.26	Uncharacterized ORF	6.18E-57
CAL0003073	3.26	Dubious ORF; Protein induced by nitric oxide	2.14E-11
CAL0001524	3.24	Ribonucleotide reductase large subunit; regulated by Sef1p, Sfu1p, and Hap43p	1.00E-300
CAL0002799	3.22	Putative vacuolar transporter of large neutral amino acids	1.10E-76
CAL0004709	3.22	Protein with predicted role in beta-1,6-glucan synthesis	2.49E-123
CAL0000422	3.22	Cytochrome oxidase assembly protein	1.00E-300
CAL0003007	3.22	Mitochondrial methionyl-tRNA synthetase	2.67E-118
CAL0000621	3.22	Putative processing peptidase	4.83E-224
CAL0005145	3.21	Uncharacterized ORF	3.18E-122
CAL0005785	3.21	Predicted Zn2-Cys6 zinc-finger protein	1.13E-197
CAL0006202	3.21	Putative MFS transporter	4.08E-31
CAL0006237	3.20	Uncharacterized ORF	2.57E-44
CAL0003627	3.19	Lanosterol 14-alpha-demethylase; hypoxia regulated	1.00E-300
CAL0001075	3.17	Uncharacterized ORF	1.75E-91
CAL0000906	3.17	Transcriptional repressor; required for white-phase cell type; hyphal growth, metabolism, cell-wall gene regulator	1.83E-289
CAL0003631	3.17	Putative exodeoxyribonuclease	7.36E-191
CAL0001968	3.16	Uncharacterized ORF	2.18E-197
CAF0006940	3.16	Uncharacterized ORF	6.42E-05
CAL0001860	3.16	Uncharacterized ORF	0.00063809
CAL0003614	3.15	Uncharacterized ORF; Hap43p-induced gene	1.04E-32
CAL0005303	3.15	Uncharacterized ORF	5.27E-35
CAL0001656	3.15	Protein similar to Ste3p, the receptor for a-factor mating pheromone	1.06E-12
CAL0004994	3.15	Uncharacterized ORF; Hap43p-induced gene	1.34E-48
CAL0006312	3.14	Putative DNA mismatch repair factor	1.30E-39
CAL0004031	3.14	Protein similar to <i>S. cerevisiae</i> Smc3p, which is an	6.03E-74
CAL0001744	3.13	Heat shock protein	1.00E-300
CAL0000721	3.13	Putative urea permease	7.34E-22
CAL0003557	3.13	Uncharacterized ORF; Protein of major facilitator superfamily	2.26E-95
CAL0003896	3.13	Putative cell wall integrity and stress response subunit 4 precursor	1.34E-73
CAL0005933	3.12	Uncharacterized ORF	1.00E-300
CAL0000997	3.12	Uncharacterized ORF	3.06E-28
CAL0004675	3.11	component of RNA polymerase II holoenzyme	1.15E-53

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004339	3.11	Uncharacterized ORF	5.06E-16
CAL0001963	3.10	Uncharacterized ORF	1.78E-193
CAL0001123	3.10	Putative integral inner mitochondrial membrane protein	2.10E-176
CAL0003443	3.10	Uncharacterized ORF	2.35E-264
CAL0001036	3.10	Protein involved in regulation of DNA-damage-induced filamentous growth	4.42E-137
CAL0003069	3.09	Uncharacterized ORF	5.21E-09
CAL0003834	3.09	High-affinity iron permease	1.00E-300
CAL0004027	3.08	Sphingolipid delta-8 desaturase	5.54E-82
CAL0005069	3.08	Uncharacterized ORF	3.53E-98
CAL0006272	3.08	Acyl CoA:sterol acyltransferase	1.05E-60
CAL0005620	3.07	Uncharacterized ORF	2.46E-09
CAL0004064	3.06	Uncharacterized ORF	7.85E-13
CAL0002008	3.06	Uncharacterized ORF	0.000116314
CAL0003082	3.06	Uncharacterized ORF	3.06E-13
CAL0006390	3.06	Uncharacterized ORF; Hap43p-induced gene	4.15E-89
CAL0002724	3.05	Protein member of a family encoded by FGR6-related genes	5.13E-11
CAL0005805	3.05	Uncharacterized ORF; Hap43p-repressed gene	3.63E-16
CAL0001145	3.05	Uncharacterized ORF	4.48E-05
CAL0006305	3.05	Uncharacterized ORF; Protein involved in mismatch repair	5.44E-223
CAL0001687	3.04	Uncharacterized ORF	4.88E-10
CAL0004368	3.04	Member of a family encoded by FGR6-related genes	5.31E-19
CAL0004107	3.04	Uncharacterized ORF; Ortholog(s) have ATPase activator activity	1.00E-300
CAL0005580	3.04	Uncharacterized ORF; Ortholog(s) have metallochaperone activity	5.44E-100
CAL0002835	3.04	Verified ORF; Putative ammonium transporter	9.19E-166
CAL0004999	3.04	Uncharacterized ORF	2.75E-19
CAL0001931	3.04	Uncharacterized ORF	1.91E-06
CAL0000652	3.03	Uncharacterized ORF; Hap43p-induced gene	5.60E-146
CAL0006262	3.03	Uncharacterized ORF	1.33E-125
CAL0006204	3.03	Putative Ribonuclease H (RNase H); hyphal-induced	9.71E-123
CAL0005519	3.03	Ste20p family Ser/Thr kinase required for wild-type filamentous growth	1.26E-128
CAL0000884	3.03	Uncharacterized ORF	2.68E-11
CAL0000324	3.02	Predicted zinc ion binding activity	1.15E-119
CAL0005767	3.01	Dubious ORF	3.42E-07
CAL0004443	3.00	Uncharacterized ORF	1.00E-300
CAL0003193	3.00	Uncharacterized ORF	3.39E-28
CAL0001534	3.00	Uncharacterized ORF; Hap43p-repressed gene	1.52E-29
CAL0001655	3.00	Uncharacterized ORF	1.43E-81
CAL0002184	2.99	Putative glucose-6-phosphate isomerase	1.00E-300
CAL0003671	2.99	Uncharacterized ORF	1.83E-48
CAL0005558	2.99	Uncharacterized ORF	2.26E-20
CAL0005696	2.98	Uncharacterized ORF	2.70E-292

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000561	2.98	Uncharacterized ORF	8.86E-102
CAL0002790	2.98	Uncharacterized ORF; Has domain(s) with predicted structural molecule activity	1.00E-300
CAL0006337	2.97	Ammonium permease	7.51E-137
CAL0003251	2.97	Uncharacterized ORF	1.31E-94
CAL0003161	2.97	Protein required for virulence in reconstituted human epithelium (RHE) model of ex vivo infection	5.59E-70
CAL0005031	2.97	Uncharacterized ORF	6.81E-35
CAL0004892	2.96	Uncharacterized ORF; Hap43p-repressed gene	1.66E-09
CAL0002431	2.96	Putative DNA directed DNA polymerase alpha	4.78E-224
CAL0002878	2.96	Member of a family encoded by FGR6-related genes	5.84E-27
CAL0001746	2.95	Putative RNA polymerase I subunit	1.53E-39
CAL0000369	2.95	Protein involved in telomere maintenance	7.87E-09
CAL0004072	2.94	Uncharacterized ORF	3.88E-10
CAL0005941	2.94	Putative Argonaute protein involved in RNA silencing	6.07E-164
CAL0120845	2.94	Uncharacterized ORF	0.0526201
CAL0001396	2.93	Predicted acyl-CoA oxidase	2.03E-59
CAL0001936	2.93	Putative homeodomain-containing transcriptional repressor	7.49E-65
CAL0006244	2.93	Uncharacterized ORF	1.67E-37
CAL0000499	2.93	Uncharacterized ORF	1.26E-113
CAL0003229	2.92	Putative multidrug resistance protein; upregulated by Efg1p	3.33E-34
CAL0001481	2.92	Protein similar to <i>S. cerevisiae</i> Smc5p, which is involved in DNA repair	6.44E-96
CAL0004412	2.92	Putative GPI-anchored protein	3.40E-15
CAL0001158	2.92	Aldo-keto reductase family member	3.95E-39
CAL0006363	2.91	Uncharacterized ORF	6.27E-15
CAL0004048	2.91	Component of the general transcription factor for RNA polymerase III	8.26E-78
CAL0003781	2.91	Putative transcription factor	1.00E-300
CAL0005187	2.91	Heme oxygenase	9.54E-218
CAL0004987	2.91	Uncharacterized ORF; Hap43p-repressed gene	1.74E-108
CAL0000244	2.91	Putative oligopeptide transporter	1.00E-300
CAL0003239	2.91	Ortholog(s) have rRNA (guanine-N1-)-methyltransferase activity	1.37E-152
CAL0001295	2.91	Uncharacterized ORF; Hap43p-repressed gene	1.46E-13
CAL0003154	2.91	Verified ORF; Hap43p-repressed gene	1.73E-43
CAL0000464	2.91	Uncharacterized ORF	2.03E-145
CAL0000614	2.91	Ferric reductase	1.38E-08
CAL0003304	2.90	Uncharacterized ORF	5.35E-36
CAL0004977	2.90	Uncharacterized ORF	2.56E-63
CAL0003638	2.90	Uncharacterized ORF	2.96E-185
CAL0003737	2.90	Uncharacterized ORF	1.00E-300
CAL0001318	2.89	Uncharacterized ORF	0.00284226
CAL0003641	2.89	Uncharacterized ORF	1.00E-300
CAL0000780	2.89	Putative Gag protein of retrotransposon Tca2	8.01E-234

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0001380	2.88	Uncharacterized ORF	3.33E-24
CAL0003370	2.87	Protein similar to beta-1,3-glucan synthase	3.64E-59
CAL0000729	2.87	Uncharacterized ORF	2.41E-100
CAL0001169	2.86	Uncharacterized ORF	1.84E-105
CAL0005779	2.86	Putative mismatch repair protein	2.15E-21
CAL0005652	2.86	Uncharacterized ORF	8.21E-31
CAL0002712	2.86	Uncharacterized ORF; Major facilitator superfamily protein	6.86E-53
CAL0002807	2.85	Uncharacterized ORF	2.52E-56
CAL0000075	2.85	A subunit of the mitochondrial inner membrane m-AAA protease	1.00E-300
CAL0006245	2.85	Uncharacterized ORF; Hap43p-repressed gene	1.38E-11
CAL0005653	2.84	Protein mannosyltransferase with roles in hyphal growth	1.00E-300
CAL0001828	2.84	Heat shock protein	1.00E-300
CAL0000273	2.83	Uncharacterized ORF	4.35E-62
CAL0005752	2.83	Putative nicotinic acid mononucleotide adenylyltransferase	9.50E-154
CAL0006182	2.83	Uncharacterized ORF	1.95E-79
CAL0003874	2.83	Putative GPI-anchored protein	7.66E-23
CAL0000890	2.83	Uncharacterized ORF	5.05E-24
CAL0005385	2.82	Uncharacterized ORF	2.93E-90
CAL0005976	2.82	Uncharacterized ORF	2.14E-49
CAL0003619	2.82	Putative fructose-bisphosphate aldolase, enzyme of glycolysis	1.00E-300
CAL0002087	2.81	Uncharacterized ORF	6.44E-10
CAL0001215	2.81	Zn2-Cys6 transcription factor; regulates iron uptake	1.52E-64
CAL0004463	2.81	Uncharacterized ORF; Hap43p-induced gene	1.74E-42
CAL0001117	2.81	Uncharacterized ORF	1.50E-11
CAL0000116	2.81	Uncharacterized ORF	1.00E-300
CAL0000955	2.80	Uncharacterized ORF	1.59E-44
CAL0000429	2.80	Uncharacterized ORF	7.82E-42
CAL0001753	2.80	Uncharacterized ORF	5.80E-46
CAL0000811	2.80	Role in mitochondrial translation and mitochondrial inner membrane localization	9.26E-46
CAL0005560	2.79	Protein mannosyltransferase required for normal cell wall composition	1.00E-300
CAL0002663	2.79	Protein required for chlamydospore formation	1.07E-133
CAL0003196	2.79	Uncharacterized ORF	1.00E-300
CAL0005365	2.79	Putative RNA-binding protein	2.54E-126
CAL0001139	2.78	Uncharacterized ORF	1.95E-42
CAL0002874	2.78	Uncharacterized ORF	5.03E-74
CAL0001080	2.77	Uncharacterized ORF	2.80E-100
CAL0002245	2.77	Ribonucleoside-diphosphate reductase	1.00E-300
CAL0005977	2.77	Pyruvate kinase	1.00E-300
CAL0000180	2.77	Uncharacterized ORF; Putative mRNA maturation factor	1.00E-300
CAL0002758	2.77	Uncharacterized ORF	1.62E-56
CAL0002127	2.76	Uncharacterized ORF	9.41E-173

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0003243	2.76	Uncharacterized ORF	1.10E-33
CAL0000402	2.76	Putative dynamin-related GTPase	2.76E-251
CAL0001564	2.76	Uncharacterized ORF; Hap43p-repressed gene	2.13E-61
CAL0001595	2.76	Class V myosin	1.00E-300
CAL0004060	2.76	Uncharacterized ORF	1.01E-99
CAL0006036	2.76	Putative dicarboxylic amino acid permease; upregulated by Rim101p	1.00E-300
CAL0006143	2.75	Predicted membrane transporter	2.47E-39
CAL0001981	2.75	Ortholog(s) have role in vacuole organization, endocytosis,	1.73E-59
CAL0005901	2.75	Glucosaminyl-phosphatidylinositol O-acyltransferase activity, role in GPI anchor biosynthetic process	6.90E-99
CAL0000530	2.75	Putative beta-mannosyltransferase	5.03E-117
CAL0004042	2.74	Protein required for normal filamentous growth	5.78E-69
CAL0000873	2.74	Putative transcription factor with zinc finger DNA-binding motif	5.59E-24
CAL0004669	2.74	Uncharacterized ORF; tubulin-dependent ATPase activity	1.27E-58
CAL0003055	2.74	Phosphofructokinase alpha subunit	1.00E-300
CAL0004069	2.74	Putative thiamin-phosphate pyrophosphorylase	2.36E-40
CAL0000385	2.73	Uncharacterized ORF; oxidoreductase activity	3.47E-116
CAL0000486	2.73	Uncharacterized ORF	1.93E-64
CAL0001160	2.73	Putative phospholipase B	3.58E-12
CAL0001319	2.73	Putative fatty acid elongase with a predicted role in sphingolipid biosynthesis	1.62E-116
CAL0004557	2.73	Putative dihydroxyacetone kinase	1.00E-300
CAL0003940	2.72	Uncharacterized ORF	1.69E-17
CAL0001136	2.72	Putative mitochondrial carrier protein	1.00E-300
CAL0000969	2.71	Uncharacterized ORF	1.51E-80
CAL0001865	2.71	Putative mitochondrial large subunit ribosomal protein	2.79E-137
CAL0002507	2.70	Uncharacterized ORF; Role in osmosensory signaling pathway	9.99E-47
CAL0002602	2.70	Uncharacterized ORF	1.00E-300
CAL0000712	2.70	Ammonium permease and regulator of nitrogen starvation-induced filamentation	5.14E-23
CAL0000720	2.70	Uncharacterized ORF	1.00E-300
CAL0000861	2.69	Uncharacterized ORF; Putative GINS complex subunit	4.92E-12
CAL0006048	2.69	Uncharacterized ORF	7.06E-92
CAL0005024	2.69	Lysine histone methyltransferase	1.02E-64
CAL0000842	2.69	Predicted membrane transporter	3.45E-40
CAL0005453	2.68	Ortholog(s) have metalloendopeptidase activity	3.56E-183
CAL0004828	2.68	Protein involved in regulation of filamentous growth and virulence	3.36E-163
CAL0006276	2.68	Ortholog(s) have role in mitochondrial translation and localization	1.00E-300
CAL0002488	2.68	Uncharacterized ORF; Hap43p-repressed gene; gene induced by hypoxia	2.66E-17
CAL0002211	2.68	Uncharacterized ORF; Hap43p-induced gene	1.49E-62
CAL0005516	2.67	Possible regulatory protein; putative adhesin-like protein;	3.46E-87
CAL0004799	2.67	Uncharacterized ORF; Hap43p-repressed gene	6.91E-07

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0003178	2.67	Uncharacterized ORF	1.78E-19
CAL0003761	2.66	Uncharacterized ORF	9.98E-21
CAL0004810	2.66	Uncharacterized ORF	6.82E-274
CAL0001202	2.66	Dubious ORF	0.00734558
CAL0006399	2.66	Uncharacterized ORF	7.21E-40
CAL0004281	2.66	Putative transcription factor with zinc finger DNA-binding motif	2.34E-34
CAL0001498	2.66	Putative glucose transporter of the major facilitator superfamily	2.58E-22
CAL0004071	2.66	Uncharacterized ORF; Stationary phase enriched protein	3.43E-51
CAL0003878	2.65	Uncharacterized ORF	8.94E-73
CAL0005112	2.65	Protein member of a family encoded by FGR6-related genes	1.79E-14
CAF0006928	2.64	Uncharacterized ORF	3.49E-18
CAL0000249	2.64	Predicted zinc-finger protein	4.55E-08
CAL0003703	2.64	Protein required for damage to oral epithelial cells and for normal hyphal growth and stress resistance	0.000649097
CAL0000703	2.64	Uncharacterized ORF; Hap43p-repressed gene;	2.47E-43
CAL0004889	2.64	Uncharacterized ORF	1.37E-48
CAL0002334	2.63	Putative RNA binding protein	3.14E-39
CAL0003255	2.63	Putative structural maintenance of chromosomes protein; Hap43p-induced gene	5.18E-99
CAL0006010	2.63	Uncharacterized ORF	2.51E-55
CAL0001166	2.62	Uncharacterized ORF; Protein of RGS superfamily	6.31E-10
CAL0005675	2.62	Role in DNA repair, replication fork arrest	3.32E-72
CAL0004934	2.62	Dubious ORF	0.0259515
CAL0002933	2.61	Transcriptional repressor; regulator of filamentation	1.63E-23
CAL0005764	2.61	Uncharacterized ORF	5.27E-56
CAL0004575	2.61	Corticosteroid binding protein	3.75E-260
CAL0005537	2.61	Cyclin homolog	4.73E-32
CAL0004555	2.61	Uncharacterized ORF; Ortholog(s) have protein kinase activator activity	1.00E-36
CAL0005055	2.61	Putative flavodoxin	3.91E-83
CAL0003249	2.60	Membrane insertase activity and role in protein insertion into mitochondrial membrane from inner side	1.00E-300
CAL0001985	2.60	Putative ceramide hydroxylase; regulated by iron	1.62E-278
CAL0000894	2.60	Probable protein kinase involved in determination of morphology during the cell cycle of both yeast-form and hyphal cells	4.19E-135
CAL0005058	2.60	Uncharacterized ORF	9.22E-60
CAL0000375	2.60	Thioredoxin reductase	1.00E-300
CAL0004518	2.59	Putative mitochondrial biogenesis protein	4.95E-169
CAL0004521	2.59	Predicted membrane transporter	2.20E-13
CAL0000796	2.59	Rab guanyl-nucleotide exchange factor activity	2.13E-95
CAL0003227	2.59	Similar to Rab GTPase activators; Hap43p-induced gene	1.14E-45
CAL0000134	2.59	Putative high-affinity glutamine permease	1.32E-24
CAL0000846	2.58	Uncharacterized ORF	1.04E-25
CAL0005338	2.58	Uncharacterized ORF; GTPase activity	9.68E-103
CAL0001987	2.58	Uncharacterized ORF	2.25E-144

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000863	2.58	Uncharacterized ORF	1.21E-79
CAL0000816	2.58	Uncharacterized ORF; Putative tRNA-Pro synthetase	4.84E-212
CAL0005944	2.58	Dubious ORF	0.000959105
CAL0004026	2.58	Chitin synthase	6.85E-74
CAL0001441	2.58	ALS family cell-surface glycoprotein	7.25E-32
CAL0003151	2.57	Dubious ORF	0.0032933
CAL0001245	2.57	Plasma membrane protein; negatively regulated by Rim101p	1.28E-81
CAL0002662	2.57	Uncharacterized ORF; ubiquitin-protein ligase activity	1.94E-160
CAL0004490	2.57	Cytochrome oxidase assembly protein	3.76E-187
CAL0120672	2.56	Uncharacterized ORF	8.49E-08
CAL0003502	2.56	Uncharacterized ORF	2.07E-49
CAL0003947	2.56	Uncharacterized ORF; Hap43p-induced gene	3.11E-23
CAL0005202	2.56	Putative pyruvate decarboxylase	1.00E-300
CAL0004148	2.55	Putative mannosyltransferase	6.07E-122
CAL0002787	2.55	Uncharacterized ORF; L-glutamate transmembrane transporter activity	1.39E-72
CAL0001575	2.55	Putative termination and polyadenylation protein	1.06E-161
CAL0003828	2.55	Secretory protein	1.25E-64
CAL0004938	2.55	Uncharacterized ORF	7.05E-09
CAL0005685	2.55	C-14 sterol reductase	3.54E-166
CAL0001578	2.55	Uncharacterized ORF; mitochondrion localization	4.04E-68
CAL0005823	2.55	Uncharacterized ORF	2.06E-27
CAL0006186	2.54	Mannosyltransferase	2.31E-09
CAL0004577	2.54	Putative copper transporter; Hap43p-repressed gene	1.20E-252
CAL0004586	2.54	Uncharacterized ORF	3.86E-152
CAL0002917	2.54	Uncharacterized ORF	6.40E-171
CAL0000278	2.54	Manganese transporter required for normal filamentous growth	2.01E-213
CAL0004816	2.54	Uncharacterized ORF; DNA-directed DNA polymerase activity	9.16E-30
CAL0000616	2.54	possible GPI-anchor; induced by Rim101p, cell wall regeneration-induced	6.51E-06
CAL0005541	2.54	Squalene epoxidase	1.00E-300
CAL0001313	2.53	Uncharacterized ORF	4.40E-233
CAL0002789	2.53	ALS family protein	1.27E-77
CAL0006287	2.53	mRNA-binding protein that localizes specific mRNAs to daughter yeast-form cells and to hyphal tips	4.38E-171
CAL0005009	2.53	Uncharacterized ORF	9.14E-26
CAL0006342	2.53	Protein similar that affects filamentous growth; Hap43p-repressed gene	5.08E-62
CAL0000015	2.53	Putative role in regulation of mitochondrion organization, signal peptide processing and mitochondrial inner membrane localization	6.15E-155
CAL0006191	2.53	Putative protein with a predicted role in establishment and maintenance of sister chromatid condensation and cohesion	1.62E-88
CAL0001119	2.53	Predicted GPI-anchor, role in 1,6-beta-D-glucan biosynthesis	1.68E-06
CAL0002448	2.52	Amino acid permease	2.14E-85
CAL0003398	2.52	Glucose and galactose sensor	3.36E-18
CAL0005819	2.52	Outwardly rectifying, noisily gated potassium channel	1.67E-33

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004120	2.52	Uncharacterized ORF; Hap43p-repressed gene	6.54E-05
CAL0003218	2.52	Uncharacterized ORF	8.31E-10
CAF0006965	2.52	flavin-linked sulfhydryl oxidase activity and role in cellular iron ion homeostasis	2.11E-74
CAL0005524	2.51	Uncharacterized ORF	3.71E-27
CAL0005657	2.51	NAD-linked glyceraldehyde-3-phosphate dehydrogenase	1.00E-300
CAL0006044	2.51	Uncharacterized ORF	4.04E-47
CAL0004468	2.51	Enzyme regulator involved in phospholipid metabolism	3.92E-36
CAL0004875	2.51	GABA/polyamine transporter	1.32E-07
CAL0000727	2.51	Cysteine transporter	1.10E-76
CAL0000173	2.50	Uncharacterized ORF; Hap43p-induced gene	1.85E-20
CAL0004672	2.50	Uncharacterized ORF	1.70E-75
CAL0001047	2.50	Uncharacterized ORF	1.10E-32
CAL0005999	2.50	Subunit of beta-1,3-glucan synthase	1.00E-300
CAL0000350	2.50	Transcription factor that positively regulates rRNA and ribosomal protein gene transcription	4.65E-73
CAL0000093	2.50	Dubious ORF	7.39E-07
CAL0001239	2.50	Transcriptional activator, required for hyphal growth	5.25E-44
CAF0006924	2.49	Uncharacterized ORF	2.99E-39
CAL0003261	2.49	Putative endosomal transmembrane protein; Hap43p-induced	3.25E-65
CAL0003907	2.49	Uncharacterized ORF	1.17E-49
CAL0005775	2.49	Uncharacterized ORF	4.44E-24
CAL0006074	2.49	Uncharacterized ORF	2.33E-13
CAL0003275	2.49	Putative SH3-domain-containing protein	7.87E-126
CAL0005141	2.49	Uncharacterized ORF	3.69E-32
CAL0001241	2.49	Putative pre-replication complex helicase subunit	3.04E-61
CAL0005040	2.48	Uncharacterized ORF	2.70E-44
CAL0003148	2.48	Putative mitochondrial lysine-tRNA synthetase	1.06E-88
CAL0003431	2.48	Putative glucose transporter of the major facilitator superfamily	2.73E-15
CAL0000304	2.48	Putative GPI-anchored protein	7.07E-99
CAL0002655	2.48	Uncharacterized ORF	0.00136654
CAL0003029	2.48	Protein member of conserved Mcm1p regulon	3.46E-67
CAL0004231	2.48	Uncharacterized ORF; Ortholog(s) have role in invasive growth in response to glucose limitation	3.51E-155
CAL0005986	2.47	Mitochondrial 60S ribosomal protein subunit; Hap43p-repressed gene	1.00E-300
CAL0005745	2.47	Putative PDR-subfamily ABC transporter	2.79E-87
CAL0004733	2.47	Uncharacterized ORF	5.08E-45
CAL0005199	2.47	DNA topoisomerase II	9.72E-177
CAL0004882	2.47	Uncharacterized ORF	0.00010797
CAL0003260	2.47	Putative iron transporter	4.51E-52
CAL0005204	2.47	Putative GPI-anchored protein	9.63E-23
CAL0005038	2.47	Uncharacterized ORF	5.93E-21
CAL0002525	2.47	Uncharacterized ORF	9.34E-24

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0002687	2.46	Protein kinase involved in growth control, normal cell size, prevents hypha formation under hypoxia at high CO ₂ levels	1.78E-56
CAL0004874	2.46	Putative transcription factor with zinc finger DNA-binding motif; Hap43p-repressed gene	6.59E-25
CAL0001311	2.46	Putative serine transporter with possible role in assimilation of sulfur	4.74E-21
CAL0001054	2.46	Autophosphorylated kinase	7.72E-151
CAL0004706	2.46	Uncharacterized ORF	3.55E-44
CAL0005239	2.46	Uncharacterized ORF	8.80E-36
CAL0002321	2.46	Uncharacterized ORF	1.74E-34
CAL0000889	2.46	Amino acid permease	5.54E-185
CAL0004778	2.46	Putative subunit of the Nucleotide Excision Repair Factor 4	2.23E-46
CAL0001514	2.45	Dubious ORF	0.0572537
CAL0002494	2.45	Putative DNA replication protein	8.61E-128
CAL0002812	2.45	Uncharacterized ORF	1.00E-300
CAL0006175	2.45	Uncharacterized ORF	8.12E-168
CAL0004826	2.45	Uncharacterized ORF	2.97E-78
CAL0000957	2.45	Uncharacterized ORF	3.31E-47
CAF0006932	2.45	Putative copper metallochaperone	7.48E-37
CAL0005042	2.45	Polo-like kinase	1.17E-195
CAL0001758	2.45	Putative methionyl-tRNA transformylase	1.73E-38
CAL0000546	2.44	Verified ORF; Putative calcium-transporting ATPase	1.00E-300
CAL0003256	2.44	Putative sensor/transporter protein with a predicted role in cell wall biogenesis	1.51E-216
CAL0002841	2.44	CCAAT-binding factor regulates low-iron induction of FRP1	2.06E-21
CAL0005540	2.44	Uncharacterized ORF	1.20E-10
CAL0005874	2.43	Uncharacterized ORF	2.64E-71
CAL0006391	2.43	Chitinase	4.70E-36
CAL0000087	2.43	Uncharacterized ORF	3.27E-14
CAL0003574	2.42	Phosphoglycerate mutase	1.00E-300
CAF0006926	2.42	Uncharacterized ORF	0.000470138
CAL0005084	2.42	Putative mitochondrial ATP-dependent RNA helicase	1.00E-300
CAL0002487	2.42	Putative GPI-linked phospholipase B	6.87E-27
CAL0001448	2.42	Uncharacterized ORF	2.07E-261
CAL0004837	2.42	Golgi membrane GDPase, required for wild-type O-mannosylation	1.89E-207
CAL0000639	2.42	Uncharacterized ORF	1.61E-10
CAL0001553	2.42	Cell wall protein; putative GPI-anchor	4.52E-194
CAL0004016	2.41	Monopolar spindle protein, a putative kinase	8.90E-33
CAL0001905	2.41	C-5 sterol desaturase	3.35E-168
CAL0001962	2.41	Aldo-keto reductase family member	2.59E-78
CAL0005794	2.41	Uncharacterized ORF	1.00E-300
CAL0005800	2.41	Uncharacterized ORF	0.000211114
CAL0003752	2.41	Putative transcription factor; required for resistance to cell wall perturbation	1.17E-38
CAL0004243	2.41	Uncharacterized ORF	2.45E-59

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0002370	2.41	Putative role in heme a biosynthetic process and mitochondrion localization	1.66E-195
CAL0005713	2.40	Uncharacterized ORF	1.50E-192
CAL0004509	2.40	Aldo-keto reductase family protein	3.40E-38
CAL0000363	2.40	Putative role in protein N-linked glycosylation and endoplasmic reticulum membrane localization	2.24E-10
CAL0001854	2.40	Uncharacterized ORF	0.000202792
CAL0006365	2.40	Uncharacterized ORF	1.86E-08
CAL0002909	2.40	Uncharacterized ORF	1.10E-74
CAL0003716	2.40	Uncharacterized ORF	7.58E-50
CAL0000820	2.40	Putative cytochrome c lysine methyltransferase	4.38E-206
CAL0002088	2.39	Uncharacterized ORF; Has domain(s) with predicted catalytic activity	2.15E-14
CAL0000642	2.39	Uncharacterized ORF	4.90E-30
CAL0000542	2.39	Uncharacterized ORF	1.20E-68
CAL0004598	2.39	Uncharacterized ORF	1.38E-28
CAL0000099	2.39	Protein similar to <i>S. cerevisiae</i> Yta6p ATPase	6.86E-53
CAL0005683	2.39	Verified ORF; Protein that affects filamentous growth	7.45E-10
CAL0000378	2.39	Uncharacterized ORF	1.86E-36
CAL0000432	2.39	Uncharacterized ORF	1.56E-27
CAL0005781	2.38	Uncharacterized ORF; Has domain(s) with predicted binding activity	1.47E-89
CAL0000141	2.38	Predicted zinc-finger protein	6.01E-65
CAL0004953	2.38	Enolase, enzyme of glycolysis and gluconeogenesis	1.00E-300
CAL0003854	2.38	Uncharacterized ORF	1.76E-08
CAL0005542	2.38	Uncharacterized ORF	3.54E-17
CAL0001426	2.38	ALS family adhesin	1.48E-30
CAL0003341	2.38	Uncharacterized ORF	8.65E-22
CAL0001614	2.38	Uncharacterized ORF; Hap43p-induced gene	2.22E-22
CAL0004511	2.38	Alpha-tubulin	1.00E-300
CAL0000685	2.38	Putative ketol-acid reductoisomerase	1.00E-300
CAL0003282	2.37	Uncharacterized ORF	2.07E-18
CAL0001940	2.37	Putative mitochondrial ribosomal protein	1.26E-174
CAL0002946	2.37	Putative mitochondrial ribosomal protein of the small subunit	4.96E-213
CAL0005223	2.37	Putative DNA primase	3.92E-34
CAL0003220	2.36	Uncharacterized ORF	3.69E-19
CAL0003572	2.36	Uncharacterized ORF	4.52E-15
CAL0004802	2.36	Protein with a potential role in beta-1,6 glucan biosynthesis	3.98E-10
CAL0001279	2.36	Uncharacterized ORF	1.00E-47
CAL0004944	2.36	Zn2-Cys6 transcriptional regulator of ergosterol biosynthetic genes and sterol uptake	3.43E-145
CAL0004434	2.36	Uncharacterized ORF	1.11E-37
CAL0001977	2.36	Uncharacterized ORF	9.64E-27
CAL0003593	2.36	Putative pyruvate decarboxylase	9.64E-09
CAL0003799	2.36	Carboxypeptidase Y	5.21E-210

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0002735	2.36	Neutral arginine-, alanine-, leucine-specific metallo-aminopeptidase	1.00E-300
CAL0000620	2.36	Component of actomyosin ring	2.12E-88
CAL0000963	2.35	Putative plasma membrane transporter of the ATP-binding cassette family	2.95E-93
CAL0006226	2.35	Uncharacterized ORF	1.82E-33
CAL0003571	2.35	Uncharacterized ORF	1.29E-13
CAL0000936	2.35	Cell surface mannoprotein; cell-wall glucan metabolism	1.00E-300
CAL0004102	2.34	Predicted UDP-glucose:glycoprotein glucosyltransferase	4.40E-90
CAL0002343	2.34	Uncharacterized ORF	1.01E-16
CAL0002619	2.34	Uncharacterized ORF	1.19E-137
CAL0002122	2.34	Uncharacterized ORF	3.58E-19
CAL0004864	2.34	Na ⁺ /H ⁺ antiporter	4.35E-109
CAL0003540	2.34	Uncharacterized ORF	7.40E-63
CAL0004759	2.33	Uncharacterized ORF; role in DNA replication	5.89E-68
CAL0002588	2.33	Uncharacterized ORF	1.51E-35
CAL0001055	2.33	protein channel activity and role in protein import into mitochondrial inner membrane, protein import into mitochondrial matrix	1.00E-300
CAL0004340	2.33	Uncharacterized ORF	8.57E-248
CAL0000130	2.33	Probable subunit of DNA polymerase II	1.69E-39
CAL0003132	2.33	B-type mitotic cyclin	7.47E-65
CAL0005885	2.32	Uncharacterized ORF; Ortholog(s) have acetylglucosaminyltransferase activity	5.52E-43
CAL0000041	2.32	Uncharacterized ORF; Hap43p-repressed gene	4.57E-14
CAL0005930	2.32	Uncharacterized ORF	5.36E-97
CAL0002406	2.32	Uncharacterized ORF	1.00E-300
CAL0001496	2.32	Uncharacterized ORF; Has domain(s) with predicted binding activity	4.75E-43
CAL0003323	2.32	Uncharacterized ORF	1.56E-88
CAL0004155	2.32	Protein similar to a mitochondrial complex I intermediate-associated protein	5.58E-33
CAL0001260	2.31	Putative mannosyltransferase; transcriptionally regulated by iron	2.46E-11
CAL0002772	2.31	Uncharacterized ORF	8.44E-12
CAL0004474	2.31	Uncharacterized ORF	1.42E-27
CAL0005080	2.31	Transcriptional regulator of filamentous growth and hyphal genes	4.86E-37
CAL0002685	2.31	Uncharacterized ORF	1.71E-227
CAL0004446	2.31	Uncharacterized ORF	0.0516619
CAL0003287	2.31	Putative NADPH dehydrogenase	8.97E-07
CAL0002266	2.31	Uncharacterized ORF	3.22E-12
CAL0005826	2.30	Uncharacterized ORF	9.33E-18
CAL0002857	2.30	GATA transcription factor involved in regulation of filamentous growth induced by nitrogen starvation	3.44E-59
CAL0000800	2.30	Putative GPI-anchored protein	0.0184641
CAL0005604	2.30	Uncharacterized ORF	1.46E-21
CAL0005434	2.30	Uncharacterized ORF; Ortholog(s) have phosphatidylinositol-4-phosphate phosphatase activity	4.24E-85
CAL0002875	2.30	GDP-mannose transporter	2.27E-184
CAL0002053	2.30	Putative pre-tRNA processing protein	2.70E-63

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004678	2.29	Uncharacterized ORF	1.64E-67
CAL0001551	2.29	Mannosyltransferase of glycosylphosphatidylinositol (GPI) biosynthesis	5.52E-24
CAL0005455	2.29	Role in regulation of cell budding, protein monoubiquitination	5.57E-46
CAL0001538	2.29	Putative peroxisomal copper amine oxidase	4.08E-12
CAL0005436	2.29	VH1 family MAPK phosphatase	9.47E-30
CAL0005945	2.29	Uncharacterized ORF; Putative kinase	2.67E-41
CAL0004988	2.29	Uncharacterized ORF	1.43E-17
CAL0003326	2.29	Uncharacterized ORF	1.34E-32
CAL0002941	2.29	Uncharacterized ORF	1.19E-19
CAL0002636	2.29	Uncharacterized ORF	9.09E-98
CAL0004564	2.29	Uncharacterized ORF; Putative DNA replication factor A	1.54E-197
CAL0002777	2.29	Putative secreted aspartyl protease	0.00289642
CAL0001352	2.29	Uncharacterized ORF	3.55E-17
CAL0003053	2.28	Uncharacterized ORF	3.13E-14
CAF0006892	2.28	Uncharacterized ORF	2.60E-28
CAL0000595	2.28	Uncharacterized ORF	4.61E-105
CAL0005015	2.28	Uncharacterized ORF	3.70E-60
CAL0000101	2.28	Uncharacterized ORF; Putative heat shock protein	7.58E-42
CAL0005740	2.28	Uncharacterized ORF	3.29E-28
CAF0006989	2.28	Cytoplasmic protein expressed specifically in white phase yeast cells	2.09E-10
CAL0004499	2.28	Uncharacterized ORF	4.51E-15
CAL0003800	2.28	NAD-dependent histone deacetylase	6.78E-102
CAL0003915	2.27	1,3-beta-glucan-linked structural cell wall protein	1.82E-188
CAL0004861	2.27	Triose-phosphate isomerase	1.00E-300
CAL0001039	2.27	Uncharacterized ORF	8.49E-62
CAL0006092	2.26	Role in mitochondrial respiratory chain complex III assembly	4.79E-143
CAL0001476	2.26	Uncharacterized ORF; Putative protein	4.20E-85
CAL0000235	2.26	Monooxygenase of the cytochrome P450 family	6.70E-07
CAL0000237	2.26	Uncharacterized ORF; Ortholog of <i>C. glabrata</i> CBS138 : CAGL0C02871g	2.26E-59
CAL0003812	2.26	Uncharacterized ORF	2.12E-54
CAL0004007	2.26	Uncharacterized ORF; Predicted ORF in Assemblies 19, 20 and 21; Hog1p-downregulated	3.51E-37
CAL0004981	2.26	Putative beta-mannosyltransferase	7.94E-82
CAL0000072	2.26	Uncharacterized ORF	7.39E-55
CAL0001812	2.26	Uncharacterized ORF	2.04E-49
CAL0004476	2.26	Zinc finger transcription factor	2.16E-44
CAL0005210	2.26	Uncharacterized ORF; mitochondrion localization	1.07E-116
CAL0001359	2.25	Protein involved in cell polarity	7.27E-194
CAL0000328	2.25	Uncharacterized ORF; Putative RNA-binding protein	4.05E-148
CAL0002621	2.25	Protein with a role in MAP kinase kinase kinase of cell integrity pathway	1.42E-30
CAL0003545	2.25	Uncharacterized ORF; role in mitochondrial fusion, mitochondrial genome maintenance	5.17E-78

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000044	2.25	Putative glucosyltransferase involved in cell wall mannan biosynthesis	2.39E-31
CAL0006009	2.25	Uncharacterized ORF	1.62E-262
CAL0006397	2.25	Delta(24)-sterol C-methyltransferase, converts zymosterol to fecosterol in ergosterol biosynthesis	1.00E-300
CAL0003343	2.25	Protein involved in telomere maintenance	0.00042065
CAL0001625	2.25	Zinc cluster DNA-binding transcription factor required for wild-type hyphal extension	1.34E-05
CAL0005082	2.25	Uncharacterized ORF	1.46E-196
CAL0005961	2.25	Putative glucokinase	1.90E-72
CAL0004777	2.25	Putative component of the U1 snRNP, involved in splicing	7.21E-21
CAL0000789	2.25	Uncharacterized ORF	6.74E-05
CAL0004347	2.24	Uncharacterized ORF; Ortholog(s) have tRNA adenylyltransferase activity	1.43E-85
CAL0001681	2.24	Uncharacterized ORF; Putative mitochondrial protein	1.32E-32
CAL0003512	2.24	Uncharacterized ORF	1.15E-19
CAL0002825	2.24	Uncharacterized ORF; Hap43p-induced gene	9.81E-45
CAL0000801	2.24	Uncharacterized ORF	1.32E-18
CAL0003486	2.24	Uncharacterized ORF	1.06E-18
CAL0000111	2.24	Uncharacterized ORF	1.55E-20
CAL0004238	2.24	Uncharacterized ORF; role in protein import into mitochondrial inner membrane	6.37E-53
CAL0001732	2.24	Uncharacterized ORF	3.61E-139
CAL0004553	2.24	Uncharacterized ORF; Hap43p-repressed gene	6.44E-48
CAL0003779	2.23	Putative transcription factor with C3HC4 zinc finger DNA-binding motif	4.68E-179
CAL0006340	2.23	Putative transcription factor with a role in colony morphology and virulence	3.48E-25
CAL0003180	2.23	Phosphofructokinase beta subunit	1.00E-300
CAL0002570	2.23	Putative magnesium ion transporter, mitochondrial	7.73E-34
CAL0003790	2.23	Putative membrane transporter; Hap43p-induced gene	7.33E-25
CAL0005423	2.23	Uncharacterized ORF	3.11E-94
CAL0002649	2.23	Uncharacterized ORF	9.82E-15
CAL0006027	2.23	Protein with similarity to ferric reductases	1.82E-05
CAL0002629	2.23	Putative Cdc7p-Dbf4p kinase complex regulatory subunit	4.38E-52
CAL0005441	2.23	Uncharacterized ORF	1.37E-42
CAL0004808	2.23	Protein similar Cullin-Associated Nedd8-Dissociated protein involved in regulation of SCF complexes	4.93E-83
CAL0005947	2.23	Putative GPI-anchored adhesin-like protein	0.0103604
CAL0001554	2.23	Uncharacterized ORF; Transcriptionally regulated by iron	1.81E-77
CAL0000270	2.23	Kinesin-like microtubule motor protein	4.35E-18
CAL0003306	2.23	3-Keto sterol reductase of ergosterol biosynthesis	1.13E-70
CAL0004640	2.23	Uncharacterized ORF	4.70E-38
CAL0000537	2.23	Uncharacterized ORF	3.73E-116
CAL0000549	2.22	Uncharacterized ORF	3.99E-180
CAL0004070	2.22	Uncharacterized ORF	3.60E-14
CAL0000444	2.22	Uncharacterized ORF	1.00E-300

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000462	2.22	Catalase; resistance to oxidative stress	3.73E-246
CAL0004647	2.22	Activator of Chs3p chitin synthase	5.33E-155
CAL0001930	2.22	Uncharacterized ORF	1.07E-113
CAL0000768	2.22	DNA polymerase epsilon	4.90E-70
CAL0003016	2.22	Protein with role in resistance to host antimicrobial peptides	5.24E-257
CAL0005175	2.22	Uncharacterized ORF	5.86E-45
CAL0002392	2.22	Uncharacterized ORF	4.99E-17
CAL0000668	2.22	Protein similar to ferric reductase Fre10p	8.74E-10
CAL0004525	2.22	Uncharacterized ORF	2.46E-86
CAL0004177	2.22	Uncharacterized ORF	3.74E-213
CAL0004663	2.21	Putative allantoin permease	2.20E-08
CAL0004305	2.21	Uncharacterized ORF	2.38E-42
CAL0000090	2.21	Uncharacterized ORF; Putative transporter	8.32E-21
CAL0003062	2.21	Plasma membrane-localized protein of unknown function; Hap43p-repressed gene	2.15E-37
CAL0002010	2.21	Putative mitochondrial respiratory protein	1.33E-22
CAL0001212	2.21	DNA binding protein with Cys2-His2 zinc-finger	2.60E-58
CAL0005569	2.21	Putative U3 snoRNP protein	8.07E-202
CAL0000217	2.21	Protein kinase of Ste20p/p65PAK family, required for wild-type mating efficiency	7.51E-64
CAL0005422	2.21	Protein required for chlamydospore formation	7.91E-44
CAL0005518	2.20	Ubiquitin-activating enzyme	1.00E-300
CAL0000415	2.20	Phosphoglycerate kinase	1.00E-300
CAL0000516	2.20	Putative glutamate synthase	1.06E-267
CAL0005212	2.20	Putative patatin-like phospholipase	9.64E-10
CAL0004496	2.20	Dubious ORF	0.0828716
CAL0001593	2.20	Phosphoacetylglucosamine mutase	1.15E-83
CAL0000849	2.20	Putative GPI-anchored protein	1.76E-19
CAL0005623	2.20	Putative bud formation and morphogenesis protein	3.27E-34
CAL0005443	2.20	Uncharacterized ORF	7.29E-102
CAL0005185	2.20	Essential protein with similarity to <i>S. cerevisiae</i> Cdc13p, involved in telomere maintenance	1.43E-21
CAL0001227	2.20	Uncharacterized ORF	1.99E-05
CAL0004414	2.20	Putative diacylglycerol kinase activity	3.90E-57
CAL0001388	2.19	Member of RNase L inhibitor (RLI) subfamily of ABC family	3.79E-233
CAL0002640	2.19	Putative mannoprotein of cell wall with role in response to stress	6.51E-32
CAL0005578	2.19	Uncharacterized ORF	0.000351824
CAL0005862	2.19	Endoplasmic reticulum (ER) protein-translocation complex subunit	7.82E-219
CAL0005255	2.19	Uncharacterized ORF	6.29E-14
CAF0007077	2.19	Putative nuclear protein	1.28E-15
CAL0002673	2.19	Uncharacterized ORF; Similar to thioredoxins	0.00722364
CAL0003775	2.19	Cell wall protein, regulated by Sef1p, Sfu1p, Hap43p	3.44E-64
CAL0003698	2.19	Uncharacterized ORF	4.04E-21
CAL0004726	2.19	Uncharacterized ORF	1.40E-170

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000338	2.19	Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization	1.55E-256
CAL0003091	2.18	Uncharacterized ORF	1.01E-13
CAL0006264	2.18	Uncharacterized ORF, role in phosphatidylinositol dephosphorylation	1.69E-18
CAL0004677	2.18	Uncharacterized ORF	0.0164763
CAL0000438	2.18	Uncharacterized ORF	4.79E-13
CAL0000406	2.18	Copper transporter	1.13E-122
CAL0003535	2.18	Mitochondrial ribosomal protein S9	3.92E-239
CAL0004983	2.18	Uncharacterized ORF	0.0120357
CAL0002198	2.18	Uncharacterized ORF	9.00E-26
CAL0003835	2.18	Uncharacterized ORF	6.17E-55
CAL0004788	2.18	Uncharacterized ORF	5.32E-18
CAL0003142	2.17	Putative role in protein import into mitochondrial matrix and mitochondrial inner membrane presequence translocase complex localization	7.31E-94
CAL0006105	2.17	Uncharacterized ORF	8.47E-68
CAL0006372	2.17	Uncharacterized ORF	2.68E-05
CAL0003001	2.17	Delta-12 fatty acid desaturase	4.27E-247
CAL0003916	2.17	Uncharacterized ORF	2.36E-27
CAL0005285	2.17	Activator of CDR1 induction by antifungal drugs	5.11E-146
CAL0005804	2.17	Putative role in mitochondrial translation and mitochondrion localization	1.10E-47
CAF0007117	2.17	Role in protein refolding and mitochondrial matrix localization	5.14E-263
CAL0002012	2.17	Uncharacterized ORF	1.17E-12
CAL0005490	2.16	Uncharacterized ORF	2.77E-48
CAL0001022	2.16	Uncharacterized ORF	1.33E-42
CAL0005830	2.16	Putative mitochondrial ribosomal protein	8.03E-262
CAL0005224	2.16	ERK-family protein kinase	1.50E-40
CAL0002858	2.16	Verified ORF; Protein required for respiratory growth	4.27E-65
CAL0004372	2.16	Ortholog(s) have tubulin binding activity, role in microtubule cytoskeleton organization, mitochondrion inheritance	1.48E-112
CAL0004422	2.16	Protein with a possible role in ER to Golgi transport	1.00E-300
CAL0000739	2.16	Putative heat shock protein	6.77E-08
CAL0001772	2.16	Uncharacterized ORF	2.20E-19
CAL0005975	2.16	Uncharacterized ORF	4.06E-14
CAL0006341	2.16	Biofilm- and planktonic growth-induced gene	1.55E-23
CAL0005820	2.15	Uncharacterized ORF	8.99E-254
CAL0003344	2.15	Histidine kinase involved in a two-component signaling pathway that regulates cell wall biosynthesis	1.54E-46
CAL0001255	2.15	Uncharacterized ORF	1.19E-16
CAL0001626	2.15	Uncharacterized ORF	4.31E-21
CAL0005406	2.15	Putative transporter	1.21E-177
CAL0005404	2.15	Phosphatidylinositol 3-phosphate 5-kinase	1.46E-43
CAL0003518	2.15	Putative RNA exonuclease	1.52E-11
CAL0002210	2.15	Uncharacterized ORF	9.16E-07

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004475	2.15	Uncharacterized ORF	1.30E-145
CAL0005260	2.15	Putative kynurenine 3-monooxygenase	3.30E-150
CAL0000261	2.15	Integrin-like protein	1.60E-85
CAL0006159	2.14	Uncharacterized ORF	5.80E-38
CAL0003115	2.14	Similar to Snu114p, which is an RNA helicase involved in pre-mRNA splicing	9.83E-27
CAL0002681	2.14	Uncharacterized ORF	3.55E-60
CAL0002861	2.14	Uncharacterized ORF	5.85E-122
CAL0000869	2.14	Uncharacterized ORF	2.30E-128
CAL0002071	2.14	Uncharacterized ORF	2.94E-25
CAL0006185	2.14	Uncharacterized ORF	1.42E-08
CAL0000959	2.14	Copper-transporting P-type ATPase of Golgi	2.32E-24
CAL0000615	2.14	Uncharacterized ORF	8.83E-61
CAL0001739	2.14	kinesin from the bipolar family; involved in formation of mitotic spindle	8.18E-15
CAL0000463	2.14	Septin	1.56E-83
CAL0005619	2.14	Uncharacterized ORF	6.61E-38
CAL0005953	2.14	Putative class III aminotransferase	0.00281208
CAL0005231	2.14	Putative role in cellular zinc ion homeostasis and mitochondrial matrix localization	2.10E-56
CAL0001612	2.14	Uncharacterized ORF	3.92E-08
CAL0000192	2.13	Uncharacterized ORF	1.02E-38
CAL0002960	2.13	Uncharacterized ORF	1.13E-69
CAL0003350	2.13	Putative component of the microtubule-nucleating gamma-tubulin complex	2.92E-19
CAL0002441	2.13	Verified ORF; Protein with similarity to <i>S. cerevisiae</i> Pin4p	7.83E-64
CAL0004671	2.13	Uncharacterized ORF	1.03E-55
CAL0004773	2.13	Putative acyl CoA synthetase	2.98E-08
CAL0004150	2.13	Component of mitochondrial ribosome	2.88E-209
CAL0003753	2.13	Hyphal cell wall protein	4.38E-05
CAL0002204	2.13	GPI-linked chitinase required for normal filamentous growth	1.00E-300
CAL0001958	2.13	Mannosyltransferase	4.92E-39
CAL0003546	2.13	Uncharacterized ORF	0.000580363
CAL0006152	2.13	Putative RNA helicase	1.20E-24
CAL0000977	2.13	Role in ER-associated protein catabolic process	1.90E-50
CAL0006130	2.13	Role in mitochondrial threonyl-tRNA aminoacylation and mitochondrion localization	1.44E-75
CAL0005698	2.13	Uncharacterized ORF	1.40E-14
CAL0002725	2.13	Uncharacterized ORF	1.23E-09
CAL0005346	2.12	Putative hexameric MCM complex subunit with a predicted role in control of cell division	3.98E-68
CAL0005954	2.12	Putative transcriptional repressor	4.04E-36
CAL0002606	2.12	Essential transcriptional activator that regulates ribosomal protein genes and the rDNA locus	1.95E-52
CAL0000070	2.12	Uncharacterized ORF	5.86E-34
CAL0002529	2.12	Cell wall adhesin-like protein	8.21E-15

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000527	2.12	Uncharacterized ORF	4.19E-08
CAL0004337	2.12	Ortholog(s) have protein binding, bridging activity	9.23E-58
CAL0004782	2.12	Putative transcription factor with zinc cluster DNA-binding motif	0.000378361
CAL0000437	2.12	Uncharacterized ORF	2.84E-16
CAL0005448	2.12	Putative mitochondrial RNA polymerase specificity factord	8.09E-68
CAL0001479	2.12	Member of PDR subfamily of ABC family	9.21E-64
CAL0004753	2.12	Zinc-finger protein; regulator of MDR1 transcription	1.87E-24
CAL0004400	2.12	Putative component of the MBF and SBF transcription complexes involved in G1/S cell-cycle progression	9.71E-50
CAL0003395	2.11	Putative GPI-anchored adhesin-like protein	1.27E-16
CAL0002781	2.11	Predicted membrane transporter	1.48E-19
CAL0006077	2.11	Putative transcription factor with zinc finger DNA-binding motif	7.02E-24
CAL0006238	2.11	Uncharacterized ORF	3.59E-15
CAL0003451	2.11	Putative protein of unknown function	8.38E-19
CAL0000288	2.11	Uncharacterized ORF	5.85E-26
CAL0004686	2.11	Uncharacterized ORF	2.77E-217
CAL0004712	2.11	Protein of beta-1,6-glucan synthesis	2.69E-72
CAL0002610	2.11	Uncharacterized ORF; Hap43p-repressed gene	8.97E-76
CAL0001819	2.11	Putative oxysterol-binding protein	5.47E-103
CAL0003329	2.11	DNA topoisomerase type I activity	4.39E-18
CAL0004461	2.11	Uncharacterized ORF	8.66E-11
CAL0002014	2.10	Uncharacterized ORF	5.70E-76
CAL0002015	2.10	Putative heteropentameric replication factor C subunit	6.47E-39
CAL0003708	2.10	Putative transcription factor with zinc cluster DNA-binding motif	1.12E-34
CAL0000074	2.10	Uncharacterized ORF	3.07E-17
CAL0000186	2.10	Uncharacterized ORF	4.21E-07
CAL0000531	2.10	Uncharacterized ORF	2.25E-68
CAL0003492	2.10	Uncharacterized ORF	1.36E-29
CAL0002327	2.10	Uncharacterized ORF	3.46E-158
CAL0001082	2.10	Uncharacterized ORF	8.89E-14
CAL0001228	2.10	Member of MDR subfamily of ABC family	3.29E-41
CAL0005369	2.10	Transcriptional activator; regulates filamentous growth	3.99E-05
CAL0005596	2.10	CNT family H(+)/nucleoside symporter	4.23E-183
CAL0004656	2.10	Putative 3'-5' RNA exonuclease	6.17E-48
CAL0006201	2.10	Involved in acidification of the vacuole	1.92E-57
CAL0002234	2.10	Uncharacterized ORF	4.13E-12
CAL0004910	2.09	Protein involved in sphingolipid biosynthesis	3.26E-44
CAL0002186	2.09	Uncharacterized ORF	2.09E-08
CAL0005742	2.09	Role in chaperone-mediated protein complex assembly	2.00E-108
CAL0005679	2.09	Uncharacterized ORF	2.33E-23
CAL0003791	2.09	Protein with homology to mitochondrial intermembrane space proteins; regulated by Sef1p-, Sfu1p-, and Hap43p	3.09E-47
CAL0000346	2.09	Uncharacterized ORF	2.07E-38

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0003516	2.09	Putative GPI transamidase	2.29E-48
CAL0005087	2.09	Uncharacterized ORF	3.43E-15
CAL0003695	2.09	Uncharacterized ORF	3.53E-70
CAL0005435	2.08	Uncharacterized ORF	4.98E-39
CAL0004523	2.08	Putative nuclear pore membrane glycoprotein	1.21E-72
CAL0005915	2.08	Putative thymidylate synthase	5.61E-42
CAL0003522	2.08	Predicted role in sphingolipid-mediated signaling pathway	4.00E-35
CAL0005039	2.08	Ortholog(s) have ubiquitin binding activity	4.00E-231
CAL0004451	2.08	Putative phosphatidylinositol 3-phosphate (PI3P) phosphatase	5.50E-28
CAL0003511	2.08	NADPH-cytochrome P450 reductase	5.19E-243
CAL0005246	2.08	Uncharacterized ORF	1.59E-27
CAL0005461	2.08	Uncharacterized ORF	2.66E-166
CAL0002620	2.08	Putative NAD-dependent (R,R)-butanediol dehydrogenase	0.0546733
CAL0005144	2.08	Putative role in regulation of biogenesis of the cell wall	3.00E-24
CAL0003872	2.08	Putative adhesin-like protein	3.75E-25
CAL0000767	2.08	Uncharacterized ORF	9.89E-16
CAL0002954	2.07	Putative GTPase-activating protein	1.34E-283
CAL0004925	2.07	Acts in regulation of glycolytic genes	2.01E-144
CAL0003049	2.07	Uncharacterized ORF; Putative DNA polymerase III subunit	3.87E-50
CAL0001944	2.07	Uncharacterized ORF	2.45E-11
CAL0001642	2.07	Putative adhesin-like protein involved in cell wall maintenance	1.00E-300
CAL0004886	2.07	Uncharacterized ORF; Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit localization	2.00E-99
CAL0006136	2.07	Putative nuclear polyadenylated RNA-binding protein	1.04E-70
CAL0004962	2.07	Ortholog(s) have phospholipid-translocating ATPase activity and role in intracellular protein transport	1.25E-54
CAL0003054	2.07	Putative cell wall protein	8.06E-20
CAL0006042	2.07	Uncharacterized ORF	1.22E-33
CAL0005725	2.07	Putative pre-mRNA processing RNA-helicase	2.15E-36
CAL0006099	2.07	ER protein-translocation complex subunit	1.00E-300
CAL0003713	2.07	Uncharacterized ORF	9.99E-14
CAL0000105	2.07	Role in ER to Golgi vesicle-mediated transport	7.56E-254
CAL0004157	2.07	Role in mitochondrial asparaginyl-tRNA aminoacylation and mitochondrion localization	1.22E-40
CAL0003413	2.06	Beta-mannosyltransferase	1.94E-11
CAL0006031	2.06	Uncharacterized ORF; Putative cyclin-like protein	3.68E-191
CAL0000084	2.06	Uncharacterized ORF	1.16E-22
CAL0005879	2.06	Role in protein import into mitochondrial matrix	4.04E-83
CAL0004527	2.06	Inositol phosphoryl transferase	1.43E-25
CAL0004063	2.06	Predicted membrane transporter	1.73E-20
CAL0002306	2.06	Role in mitotic cell cycle spindle orientation checkpoint	3.54E-09
CAL0002583	2.06	Uncharacterized ORF; Putative multidrug resistance factor	6.41E-21
CAL0004535	2.06	Uncharacterized ORF	3.51E-12

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004924	2.06	Putative translation initiation factor	1.56E-297
CAL0000073	2.06	Uncharacterized ORF; Hap43p-repressed gene	3.63E-15
CAL0005807	2.06	Ortholog(s) have role in protein targeting to vacuole	2.82E-12
CAL0003539	2.06	Rhomboid-like protein	7.58E-37
CAL0000177	2.06	Putative aminopeptidase	2.34E-12
CAL0004387	2.06	Uncharacterized ORF	0.0397405
CAL0004319	2.06	Putative regulatory subunit of trehalose-phosphate synthase	4.47E-103
CAL0002873	2.06	Uncharacterized ORF; Ortholog(s) have mitochondrion localization	1.12E-25
CAL0002555	2.06	Uncharacterized ORF	1.09E-12
CAL0006314	2.05	Protein similar to quinone oxidoreductases	9.60E-11
CAL0005655	2.05	Uncharacterized ORF; Hap43p-induced gene	1.46E-06
CAL0004985	2.05	Putative ammonia transport protein	1.97E-72
CAL0001871	2.05	Uncharacterized ORF	1.92E-236
CAL0004219	2.05	Predicted phosphotransferase activity	2.16E-55
CAL0002638	2.05	Uncharacterized ORF	8.55E-120
CAL0003727	2.05	Uncharacterized ORF	6.89E-09
CAL0001431	2.05	Putative phospholipase	3.09E-97
CAL0004037	2.05	Uncharacterized ORF;	8.39E-07
CAL0000342	2.05	Putative vesicular transport protein	3.66E-116
CAL0005647	2.05	Putative DNA-binding transcription factor	2.94E-12
CAL0001274	2.05	Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization	3.64E-38
CAL0004781	2.05	Putative transcription factor with zinc finger DNA-binding motif	2.42E-12
CAL0004606	2.05	Uncharacterized ORF	1.21E-13
CAL0003093	2.05	Uncharacterized ORF	0.00152667
CAL0003032	2.05	Uncharacterized ORF	3.99E-71
CAL0002576	2.05	Role in protein import into mitochondrial inner membrane and mitochondrial inner membrane protein insertion complex localization	4.33E-195
CAL0003873	2.05	Putative vacuolar membrane protein with a predicted role in sphingolipid metabolism	1.76E-44
CAL0006212	2.05	Putative casein kinase	3.60E-23
CAL0005955	2.05	Uncharacterized ORF	7.70E-28
CAL0005556	2.05	Uncharacterized ORF	9.43E-25
CAL0001252	2.05	Protein part of the CCR4-NOT transcription regulatory complex	9.66E-129
CAF0006960	2.04	Ortholog(s) have structural constituent of ribosome activity	9.86E-60
CAL0003480	2.04	Serine/threonine protein phosphatase catalytic subunit	1.00E-40
CAL0005343	2.04	Involved in ubiquitin-mediated protein degradation	2.91E-93
CAL0001018	2.04	Putative serine/threonine protein kinase; Hog1p-induced	1.19E-35
CAL0004213	2.04	Uncharacterized ORF	0.0135147
CAL0002501	2.04	Dubious ORF; Late-stage biofilm-induced gene	0.144972
CAL0003745	2.04	Ortholog(s) have chromatin binding activity	2.60E-61
CAL0000365	2.03	Putative cell wall assembly regulatory protein;	2.15E-20
CAL0001016	2.03	Uncharacterized ORF; Ortholog(s) have protein anchor activity	2.26E-83
CAL0001175	2.03	Putative mitochondrial exonuclease	6.43E-14

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0004029	2.03	Uncharacterized ORF	3.13E-39
CAL0001427	2.03	Putative Arf3p GTPase activating protein; Hap43p-repressed gene	1.89E-31
CAL0001302	2.03	Putative GPI-anchored adhesin-like protein	4.17E-24
CAL0002855	2.03	Putative S-adenosyl-L-homocysteine hydrolase	1.00E-300
CAL0004269	2.03	Putative GTPase-activating protein; involved in cell signaling pathways that control cell polarity	5.78E-31
CAL0004406	2.03	Uncharacterized ORF	2.08E-65
CAL0002762	2.03	Putative component of the SBF transcription complex involved in G1/S cell-cycle progression	1.45E-57
CAL0002547	2.03	Putative protein disulfide-isomerase	1.00E-300
CAL0001722	2.02	Uncharacterized ORF	2.03E-11
CAL0000127	2.02	Uncharacterized ORF	2.15E-25
CAL0006318	2.02	Protein with similarity to fatty acid desaturase (stearoyl-CoA desaturase)	2.26E-33
CAL0005717	2.02	Ortholog(s) have Rho GTPase activator activity	7.35E-81
CAL0002765	2.02	Protein with a predicted role in docking and fusion of post-Golgi vesicles with the plasma membrane	6.67E-62
CAL0003877	2.02	Ortholog(s) have role in pseudohyphal growth	6.58E-13
CAL0005023	2.02	Uncharacterized ORF	1.92E-24
CAL0004481	2.02	Subtilisin-like protease (proprotein convertase)	1.68E-69
CAL0001621	2.02	Putative serine palmitoyltransferase component	7.37E-86
CAL0003712	2.02	Uncharacterized ORF; Hap43p-repressed gene	8.14E-101
CAL0005225	2.02	Uncharacterized ORF	6.84E-30
CAL0006108	2.02	Uncharacterized ORF; Hap43p-repressed gene	5.78E-231
CAL0001280	2.02	Transcription factor; regulates hyphal gene expression	1.23E-52
CAL0000071	2.01	Alpha-1,2-mannosyl transferase	1.00E-300
CAL0005672	2.01	Ortholog(s) have trans-hexaprenyltranstransferase activity	6.47E-113
CAL0120555	2.01	Uncharacterized ORF	7.04E-13
CAL0003666	2.01	Putative role in nonsense-mediated mRNA decay	2.93E-98
CAL0006369	2.01	Putative cytochrome c oxidase assembly protein	2.38E-30
CAL0003146	2.01	Ortholog(s) have FAD transmembrane transporter activity	1.68E-07
CAL0001700	2.01	Ortholog(s) have DNA helicase activity	5.70E-56
CAL0001915	2.01	Putative transcription factor, regulator of purine biosynthetic genes	2.74E-39
CAL0000132	2.01	Phosphorylated protein	2.23E-34
CAL0004392	2.01	Putative phenylalanine-tRNA ligase	1.91E-92
CAL0002780	2.01	Putative thiol-specific monooxygenase	3.24E-15
CAL0004359	2.01	Putative DNA-dependent ATPase	2.87E-19
CAL0006187	2.01	Ortholog(s) have structural constituent of ribosome activity	1.01E-156
CAL0001654	2.01	Uncharacterized ORF; Has domain(s) with predicted binding activity	3.35E-16
CAL0004881	2.01	Uncharacterized ORF	2.13E-31
CAL0003506	2.01	Uncharacterized ORF; Putative acyl-CoA thioesterase	5.45E-09
CAL0005220	2.01	Putative high-affinity, high-capacity xanthine-uric acid/H ⁺ symporter	2.45E-08
CAL0000068	2.01	Ortholog(s) have structural constituent of ribosome activity	2.27E-156
CAL0004274	2.01	Uncharacterized ORF	1.93E-07

Table A-5 continued

Gene ID	Fold up-regulated	Function	P-value
CAL0000743	2.01	Alpha-1,2-mannosyltransferase	5.09E-75
CAL0000027	2.01	Transcriptional activator that likely regulates genes involved in phosphatidylcholine and phosphatidylinositol biosynthesis	8.50E-06
CAL0002272	2.01	Uncharacterized ORF; Ortholog(s) have role in translational termination and nucleus localization	5.45E-138
CAL0002739	2.00	Required asymmetric inheritance of oxidatively damaged proteins	1.18E-07
CAL0003059	2.00	Probable transcription factor required for filamentous growth	7.61E-21
CAL0004311	2.00	Uncharacterized ORF	6.27E-16
CAL0002493	2.00	Predicted methyltransferase activity and role in RNA capping	3.28E-23
CAL0001948	2.00	Uncharacterized ORF	4.62E-17
CAL0006370	2.00	Putative transcription factor with zinc cluster DNA-binding motif	1.42E-09
CAL0001272	2.00	MAP kinase kinase kinase (MAPKKK) that regulates Hog1p activation and signaling	4.85E-74

Table A-6: List of all the genes down-regulated in the *psd1*Δ/*psd2*Δ mutant compared to the wild-type strain

Gene ID	Fold-downregulated	CGD Function	P-value
CAL0004548	132.07	Alternative oxidase	1.00E-300
CAL0000931	115.14	Uncharacterized ORF	1.00E-300
CAL0000982	73.36	Formate dehydrogenase	1.00E-300
CAL0001883	50.68	Uncharacterized ORF	1.00E-300
CAF0006908	36.29	Uncharacterized ORF; Hap43p-repressed gene; hypoxia downregulated	1.00E-300
CAL0001258	19.28	Uncharacterized ORF; Hap43p-induced gene	1.00E-300
CAL0005695	18.42	Uncharacterized ORF	3.21E-58
CAL0120803	17.58	Uncharacterized ORF	1.69E-195
CAL0000550	14.28	Glycerophosphoinositol permease	1.00E-300
CAL0002678	13.70	Similar to heat-shock protease protein	1.08E-143
CAL0002987	10.18	Putative oxidoreductase, iron utilization; regulated by Sfu1p, Sef1p, Hap43p	1.64E-55
CAL0004080	9.95	Uncharacterized ORF; Hap43p-repressed gene	1.00E-300
CAL0000590	9.51	Uncharacterized ORF	1.00E-300
CAL0002189	8.56	Uncharacterized ORF	1.00E-300
CAL0005929	7.97	Putative oxidoreductase	1.00E-300
CAL0000501	7.75	Putative potassium transporter	3.63E-224
CAL0004550	7.70	Alternative oxidase	1.80E-66
CAL0003857	7.53	Uncharacterized ORF; Hap43p-repressed gene	3.23E-140
CAL0001071	7.21	Nitric oxide dioxygenase	1.26E-200
CAL0003313	7.07	Late-stage biofilm-induced gene; induced by nitric oxide; Hap43p-repressed	1.00E-300
CAL0000448	6.66	UDP-glucose 4-epimerase	1.00E-300
CAL0004676	6.29	Protein similar to Pcl5p and other cyclins for Pho85p kinase; Gcn4p-induced	3.85E-247
CAL0006389	6.21	Phosphoenolpyruvate carboxykinase	1.00E-300
CAL0002175	6.15	Uncharacterized ORF	2.32E-135
CAL0000451	5.98	Putative galactose-1-phosphphate uridyl transferase; downregulated by hypoxia	1.00E-300
CAL0003136	5.70	Putative high-affinity maltose transporter	1.00E-300
CAL0005139	5.66	Uncharacterized ORF; Hap43p-repressed gene	1.00E-300
CAL0001847	5.66	Protein of unknown function; Plc1p-regulated	3.94E-55
CAL0000195	5.57	Uncharacterized ORF; Hap43p-induced gene	3.36E-86
CAL0003582	5.54	Putative transcription factor with zinc finger DNA-binding motif	1.00E-300
CAL0003856	5.32	Dubious ORF	5.08E-96
CAL0002350	5.29	Response regulator of a two-component system involved in stress, morphogenesis, virulence	1.00E-300
CAL0004997	5.26	Uncharacterized ORF	2.49E-168
CAL0001097	5.24	Uncharacterized ORF; Similar to cell wall proteins	8.61E-155
CAL0005709	4.92	Putative phosphatidyl synthase	1.00E-300
CAL0003246	4.90	TEA/ATTS transcription factor involved in pheromone response in white cells	1.00E-300

Table A-6 continued

Gene ID	Fold-downregulated	CGD Function	P-value
CAL0004456	4.90	Copper- and zinc-containing superoxide dismutase	1.00E-300
CAL0003028	4.88	Uncharacterized ORF	1.00E-300
CAL0002696	4.77	High-affinity glucose transporter	1.00E-300
CAL0005548	4.61	Plasma membrane-localized protein	4.29E-20
CAL0004872	4.39	Uncharacterized ORF	1.00E-300
CAL0000706	4.39	Uncharacterized ORF	1.00E-300
CAL0003529	4.35	Uncharacterized ORF	4.59E-284
CAL0001013	4.13	Uncharacterized ORF	8.02E-133
CAL0002216	4.08	ADP-ribosylation factor, probable GTPase involved in intracellular transport	4.88E-19
CAL0003860	4.07	Predicted type 2C protein phosphatase	1.00E-300
CAL0004432	3.94	Possible role in polyamine transport; MFS-MDR family	3.72E-291
CAL0003510	3.88	Uncharacterized ORF	1.00E-300
CAL0120801	3.87	Uncharacterized ORF	1.00E-300
CAF0006934	3.80	Protein involved in ATP biosynthesis	1.00E-300
CAL0000843	3.70	Uncharacterized ORF; Hap43p-repressed gene	8.16E-146
CAL0004487	3.70	Major cell-surface ferric reductase under low-iron conditions	1.00E-300
CAL0003807	3.59	Putative protein phosphatase inhibitor; Hap43p-repressed	1.00E-300
CAL0120807	3.57	Putative adhesin-like protein	1.41E-293
CAL0005504	3.52	Protein similar to <i>S. cerevisiae</i> Ecm21p ; alkaline upregulated by Rim101p	8.71E-36
CAL0006378	3.46	Uncharacterized ORF; Hap43p-repressed gene; biofilm-induced	0.000130068
CAL0005271	3.35	Copper- and zinc-containing superoxide dismutase	8.01E-254
CAL0003653	3.34	Putative alcohol dehydrogenase	1.58E-207
CAL0004170	3.32	Predicted flavin adenine dinucleotide binding, oxidoreductase activity and role in oxidation-reduction process	3.24E-128
CAL0120629	3.26	Uncharacterized ORF	1.00E-300
CAL0004199	3.25	Uncharacterized ORF; Hap43p-repressed gene	1.26E-41
CAL0003785	3.16	Uncharacterized ORF; Beta-mannosyltransferase	0.00445478
CAL0003833	3.14	Uncharacterized ORF	4.87E-147
CAL0001177	3.09	Uncharacterized ORF	5.25E-29
CAL0120835	2.98	Uncharacterized ORF	4.26E-06
CAL0001581	2.95	Uncharacterized ORF; Protein similar to pirin	1.10E-26
CAL0004585	2.92	Uncharacterized ORF	1.00E-300
CAL0005860	2.92	Uncharacterized ORF	3.46E-79
CAL0120981	2.92	Uncharacterized ORF	1.24E-25
CAL0120705	2.92	Uncharacterized ORF	5.01E-25
CAL0000450	2.91	Putative UDP-glucose-4-epimerase	1.00E-300
CAL0002961	2.89	Uncharacterized ORF; Hap43p-repressed gene	6.97E-14
CAL0003891	2.85	Uncharacterized ORF	9.13E-102
CAL0001598	2.85	Uncharacterized ORF	1.47E-42
CAL0004943	2.85	Uncharacterized ORF	8.23E-49
CAL0004792	2.85	Putative fumarate hydratase	1.00E-300

Table A-6 continued

Gene ID	Fold-downregulated	CGD Function	P-value
CAL0000972	2.84	Putative methyltransferase	1.42E-181
CAL0004974	2.83	Uncharacterized ORF	1.00E-300
CAL0002816	2.82	Uncharacterized ORF	2.16E-229
CAL0120493	2.80	Pseudogene	7.08E-30
CAL0001619	2.78	Uncharacterized ORF; ATP sulfurlyase of sulfate assimilation	3.17E-08
CAL0004502	2.77	Protein that affects filamentous growth; late-stage biofilm-induced gene	1.20E-25
CAL0004289	2.74	Putative subunit of the F1F0-ATPase complex	1.96E-15
CAL0000682	2.72	Uncharacterized ORF; Similar to glutathione peroxidase	9.16E-96
CAL0006076	2.71	Putative transmembrane protein	1.00E-300
CAL0005475	2.70	Putative NAD-specific glutamate dehydrogenase	1.00E-300
CAL0005645	2.70	Putative glucose transporter of the major facilitator superfamily	1.06E-08
CAL0002676	2.69	Putative glycerophosphoinositol permease	1.00E-300
CAL0004471	2.67	Putative protein with a predicted role in mitochondrial iron metabolism; Hap43p-repressed gene	3.00E-137
CAL0000649	2.66	Ortholog(s) have oxidoreductase activity	3.09E-257
CAL0002133	2.65	Uncharacterized ORF	7.41E-284
CAL0005376	2.64	Ortholog(s) have oxidoreductase activity	7.60E-121
CAL0002044	2.63	Putative glutamate decarboxylase	1.00E-300
CAL0004247	2.60	GPI-anchored cell wall protein	6.82E-106
CAF0007037	2.59	Protein localized to the mitochondrial membrane	1.00E-300
CAL0001649	2.57	Uncharacterized ORF; Hap43p-induced gene	1.00E-300
CAL0003690	2.56	Putative adenylsulfate kinase; predicted role in sulfur metabolism	1.00E-300
CAL0004054	2.56	Uncharacterized ORF	1.51E-10
CAL0003826	2.55	Uncharacterized ORF	6.54E-61
CAF0006952	2.54	Uncharacterized ORF	5.91E-18
CAF0007053	2.54	Uncharacterized ORF	1.34E-11
CAL0004298	2.51	Transcription factor with zinc cluster DNA-binding motif involved in glycolysis	1.00E-300
CAL0006005	2.51	Uncharacterized ORF	1.00E-300
CAL0005909	2.50	Protein with Gal4p-like DNA-binding domain	1.00E-300
CAL0006377	2.50	Putative 3-dehydroquinate dehydratase	8.38E-253
CAL0005485	2.49	Putative endoplasmic reticulum protein; possibly adherence-induced	8.86E-53
CAL0001146	2.49	Putative constitutive acid phosphatase; negatively regulated by Rim101p	1.00E-300
CAL0002956	2.46	Putative phosphate permease	1.77E-273
CAL0002899	2.46	Putative GPI-anchored protein; Hap43p-repressed gene	8.00E-33
CAL0120690	2.45	Uncharacterized ORF	1.48E-62
CAL0120739	2.45	Uncharacterized ORF	8.64E-06
CAL0000788	2.44	Putative glucoamylase	1.23E-221
CAL0120789	2.44	Uncharacterized ORF	4.63E-06
CAL0004657	2.43	Cytochrome c oxidase	1.36E-72
CAL0000439	2.42	Putative glucose transporter of the major facilitator superfamily	2.50E-104

Table A-6 continued

Gene ID	Fold-downregulated	CGD Function	P-value
CAL0001572	2.41	Uncharacterized ORF	1.00E-300
CAL0005980	2.39	Putative F0-ATP synthase subunit 4	6.46E-30
CAF0007126	2.38	Mitochondrial ATPase complex subunit	3.60E-46
CAL0120954	2.35	Uncharacterized ORF	1.00E-300
CAL0001467	2.35	Glycerol permease involved in glycerol uptake	1.00E-300
CAF0007051	2.34	Putative mitochondrial F1F0 ATP synthase subunit	1.03E-64
CAL0002512	2.33	Protein similar to <i>S. cerevisiae</i> Sds24p; transcription regulated by Mig1p and Tup1p	2.30E-89
CAL0000917	2.33	Nucleoside diphosphate kinase	1.00E-300
CAL0120750	2.32	Uncharacterized ORF	1.00E-300
CAL0003972	2.29	Uncharacterized ORF	1.00E-300
CAL0002995	2.29	Transcriptional repressor; regulates chlamydospore development	9.44E-250
CAL0005201	2.28	Transcription factor that binds upstream of ribosomal protein genes	1.87E-51
CAL0000320	2.27	Putative proline oxidase; alkaline upregulated by Rim101p	1.00E-300
CAL0000857	2.27	Uncharacterized ORF; Hap43p-repressed gene	1.00E-300
CAL0003212	2.26	Uncharacterized ORF; Hap43p-repressed gene	1.10E-223
CAL0001980	2.26	Uncharacterized ORF	1.83E-17
CAL0000358	2.26	Uncharacterized ORF	1.53E-92
CAL0004380	2.25	Uncharacterized ORF	1.03E-44
CAL0003363	2.24	Putative alcohol dehydrogenase	2.39E-58
CAL0003390	2.23	Putative constitutive acid phosphatase	3.73E-48
CAL0001669	2.23	Uncharacterized ORF	1.00E-300
CAL0003361	2.23	Uncharacterized ORF	3.96E-96
CAL0000446	2.23	Galactokinase	7.24E-43
CAL0000301	2.22	Uncharacterized ORF; Hap43p-repressed gene; repressed by nitric oxide	2.33E-68
CAL0006052	2.22	Uncharacterized ORF; Hap43p-repressed gene; repressed by nitric oxide	1.00E-300
CAF0007033	2.21	Putative cytochrome c oxidase	1.00E-300
CAL0003939	2.20	Uncharacterized ORF	1.00E-300
CAL0003242	2.20	Imidazoleglycerol-phosphate dehydratase, enzyme of histidine biosynthesis	1.00E-300
CAL0120612	2.19	Uncharacterized ORF	1.00E-300
CAL0004762	2.19	Putative NADH dehydrogenase that could act alternatively to complex I in respiration	3.92E-136
CAL0000684	2.19	Uncharacterized ORF; Putative thiol peroxidase	0.0109287
CAL0004848	2.17	Putative DNA-binding transcription factor	1.00E-300
CAL0005196	2.17	Uncharacterized ORF	1.03E-14
CAL0003019	2.16	Uncharacterized ORF	2.82E-67
CAL0001782	2.15	Putative inducible acid phosphatase	0.00392063
CAL0003397	2.14	Uncharacterized ORF	6.48E-14
CAL0005651	2.13	Uncharacterized ORF; Ortholog(s) have role in iron-sulfur cluster assembly	1.61E-08
CAF0007449	2.13	Uncharacterized ORF	2.92E-131
CAL0004062	2.12	Uncharacterized ORF; Hap43p-repressed gene	2.93E-102

Table A-6 continued

Gene ID	Fold-downregulated	CGD Function	P-value
CAL0005907	2.12	Uncharacterized ORF	2.22E-131
CAL0003434	2.11	Plasma membrane-localized protein; repressed by nitric oxide	6.59E-11
CAL0002597	2.11	Ortholog(s) have omega peptidase activity, role in glutathione catabolic process	1.00E-300
CAL0001093	2.11	Protein related to flavohemoglobins	1.00E-300
CAL0000792	2.08	Glutathione-dependent formaldehyde dehydrogenase involved in glycine catabolism	6.32E-88
CAL0004747	2.07	Uncharacterized ORF; regulated by Sef1p-, Sfu1p-, and Hap43p	1.44E-87
CAL0003892	2.06	Protein involved in proteolytic activation of Rim101p, which regulates pH response	1.00E-300
CAL0002622	2.06	Putative homocitrate synthase; repressed by nitric oxide and by hypoxia	3.19E-47
CAL0003655	2.06	Mitochondrial ADP/ATP carrier protein involved in ATP biosynthesis	1.44E-64
CAL0120862	2.05	Uncharacterized ORF	7.09E-209
CAL0005662	2.05	Zinc cluster transcription factor	1.00E-300
CAL0001727	2.04	Uncharacterized ORF	1.00E-300
CAL0120825	2.04	Uncharacterized ORF	6.11E-141
CAL0001540	2.04	Uncharacterized ORF	6.20E-09
CAL0004908	2.02	Uncharacterized ORF	1.00E-300
CAL0005780	2.02	Putative NADH-cytochrome-b5 reductase; soluble protein in hyphae;	2.61E-09
CAL0120936	2.02	Uncharacterized ORF	1.00E-300
CAL0004024	2.01	Putative cation conductance protein	1.00E-300
CAF0007034	2.01	Putative subunit VIIa of cytochrome c oxidase	2.46E-49
CAL0000596	2.01	Putative ubiquinol-cytochrome-c reductase, subunit 7; Hap43p-repressed gene	2.42E-107
CAL0001616	2.01	Ortholog(s) have inorganic cation transmembrane transporter activity	1.70E-273
CAL0003729	2.01	Ortholog(s) have phosphoprotein phosphatase activity	1.00E-300
CAL0003252	2.00	F1-ATP synthase complex subunit	5.08E-171

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Vita

Anthony E. Montedonico received his B.S. in Microbiology (honors) from the University of Tennessee, Knoxville. He performed undergraduate research in the laboratory of Dr. Todd Reynolds and completed his senior honors thesis studying *S. cerevisiae* biofilm formation. He stayed in Knoxville and enrolled in graduate school in the Department of Microbiology at the University of Tennessee in 2008 to continue his research and to pursue his passion for fungal biology. He has received several travel awards to present his research at internationally-renowned conferences. He graduated with a Doctor of Philosophy in December 2012. He has an interest in drug discovery, cell wall dynamics and the roles of lipids, especially phospholipids, in biological processes of the cell. He hopes to continue his research and teaching interests and explore novel biological problems.