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Signal Transduction and Transcriptional Regulation Pathways Essential for Azole Resistance in *Candida albicans*

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Signal Transduction and Transcriptional Regulation Pathways Essential for Azole Resistance in *Candida albicans*

Abstract

Candida albicans is the most prevalent human fungal pathogen, found as a commensal organism in the mucosa, gastrointestinal, and urogenital tracts of humans. This pathogenic fungus causes a wide spectrum of diseases, including the mucosal infection oropharyngeal candidiasis (OPC) which frequently affects patients with human immunodeficiency virus (HIV). The azole antifungals (such as fluconazole) are the most widely used and important ergosterol biosynthesis inhibitors (EBIs) for the treatment of *Candida* infections, including OPC. However, the azoles are fungistatic against *C. albicans* and therefore have limited efficacy against this organism, especially for immunocompromised patients. In *C. albicans*, the transcription factor Upc2 is central to the regulation of ergosterol biosynthesis. UPC2 activating mutations contribute to azole resistance, whereas disruption increases azole susceptibility. We further investigated the relationship of UPC2 to fluconazole susceptibility, particularly in azole-resistant strains. In addition to the reduced fluconazole minimum inhibitory concentration (MIC) previously observed with UPC2 disruption, we observed a reduced minimum fungicidal concentration (MFC) in a *upc2Δ/Δ* mutant relative to its azole-susceptible parent SC5314. Moreover, *upc2Δ/Δ* was unable to grow on solid media containing 10 µg/mL fluconazole and exhibited increased susceptibility and a clear zone of inhibition when subjected to Etest. Time-kill analysis showed increased azole activity against *upc2Δ/Δ* compared to SC5314. UPC2 disruption in strains carrying specific resistance mutations also resulted in reduced MICs and MFCs. UPC2 disruption in a highly azole-resistant clinical isolate containing multiple resistance mechanisms likewise resulted in a reduced MIC and MFC. This mutant was unable to grow on solid media containing 10 µg/mL fluconazole and exhibited increased susceptibility and a clear zone of inhibition when subjected to Etest. Time-kill analysis showed increased azole activity against the *upc2Δ/Δ* mutant in the resistant background. Microarray analysis showed attenuated fluconazole induction of genes involved in sterol biosynthesis as well as iron transport and homeostasis in the absence of UPC2. Taken together, our results demonstrate that the UPC2 transcriptional network is universally essential for azole resistance in *C. albicans* and represents an attractive target for enhancing azole antifungal activity. Fungal survival in the presence of the azoles is permitted by specific signal transduction and transcriptional activation programs. In an effort to identify additional transcriptional pathways involved in fluconazole susceptibility, we sought to identify transcription factors (TFs) essential for this process. From a collection of *C. albicans* strains disrupted for genes encoding TFs (Homann et al., PLoS Genet. 2009;5:e1000783), 4 exhibited marked reduction in MFC in both RPMI and YPD media independent of any noted growth defect in medium alone as compared to the parent strain. In addition to UPC2, one gene of interest (GOI) (CAS5) was unable to recover from fluconazole exposure at concentrations as low as 2 µg/mL after 72 hours in YPD medium, showed reduced susceptibility and a clear zone of inhibition by Etest, was unable to grow on solid media containing 10 µg/mL fluconazole, and exhibited increased susceptibility by time-kill analysis. CAS5 disruption in highly azole resistant clinical isolates containing multiple resistance mechanisms did not alter susceptibility. However, CAS5 disruption in strains containing specific resistance mutations resulted in a moderate reduction in MIC and MFC. Genome-wide transcriptional analysis was performed in the presence of fluconazole and was consistent with the suggested role of CAS5 in cell wall organization while also suggesting a role in iron transport and homeostasis. These findings suggest that Cas5 regulates a transcriptional network that influences susceptibility of *C. albicans* to fluconazole. Further delineation of this transcriptional network may identify targets for potential cotherapeutic strategies to enhance the activity to the azole class of antifungals. Genome-wide expression profiling identified that ergosterol biosynthesis genes depend on Upc2 for their fluconazole-inducibility, and cell wall maintenance genes depend on Cas5. In the presence of fluconazole, the expression of iron ion transport and homeostasis genes were also identified to be dependent on Upc2 as well as Cas5. The identification of these target genes validated the known and

putative roles for Upc2 and Cas5, and also provided insight into novel roles for these transcription factors in azole susceptibility.

Document Type

Dissertation

Degree Name

Doctor of Philosophy (PhD)

Program

Biomedical Sciences

Research Advisor

P. David Rogers, Pharm.D, Ph.D.

Keywords

Antifungal, azole, *Candida albicans*, Rrsistance, transcriptional regulation

Subject Categories

Bacterial Infections and Mycoses | Diseases | Fungi | Medicine and Health Sciences | Organisms

**Signal Transduction and Transcriptional Regulation Pathways Essential for Azole
Resistance in *Candida albicans***

A Dissertation
Presented for
The Graduate Studies Council
The University of Tennessee
Health Science Center

In Partial Fulfillment
Of the Requirements for the Degree
Doctor of Philosophy
From The University of Tennessee

By
Erin M. Vasicek
December 2013

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DEDICATION

To my family and friends for their love, patience, and encouragement. No amount of homemade cookies can express my gratitude.

ACKNOWLEDGEMENTS

First I would like to express my utmost gratitude to my advisor, Dave Rogers, who challenged me throughout my research and provided opportunities to interact with other researchers while experiencing the country, and the world. I would also like to thank the members of my committee, Jim Dale, Ramin Homayouni, Richard Lee, and Nathan Wiederhold for their suggestions, guidance, and critical reviews in order to improve my research.

My gratitude also extends to the current and former members of the Rogers lab, Kathy Barker, Kelly Caudle, Chris Hoehamer, Teresa Liu, Stephanie Flowers, Sarah Whaley, Beth Berkow, Qing Zhang, Andy Nishimoto, and Gabaergic Kayihura for their friendship and support. I am also extremely grateful to Todd Reynolds at University of Tennessee in Knoxville, Tennessee for allowing me to spend a month working in his lab.

Last but not least, I would like to thank my entire family for believing in me throughout this adventure. In addition, I would like to thank all my friends, near and far, for keeping me balanced.

ABSTRACT

Candida albicans is the most prevalent human fungal pathogen, found as a commensal organism in the mucosa, gastrointestinal, and urogenital tracts of humans. This pathogenic fungus causes a wide spectrum of diseases, including the mucosal infection oropharyngeal candidiasis (OPC) which frequently affects patients with human immunodeficiency virus (HIV). The azole antifungals (such as fluconazole) are the most widely used and important ergosterol biosynthesis inhibitors (EBIs) for the treatment of *Candida* infections, including OPC. However, the azoles are fungistatic against *C. albicans* and therefore have limited efficacy against this organism, especially for immunocompromised patients.

In *C. albicans*, the transcription factor Upc2 is central to the regulation of ergosterol biosynthesis. *UPC2* activating mutations contribute to azole resistance, whereas disruption increases azole susceptibility. We further investigated the relationship of *UPC2* to fluconazole susceptibility, particularly in azole-resistant strains. In addition to the reduced fluconazole minimum inhibitory concentration (MIC) previously observed with *UPC2* disruption, we observed a reduced minimum fungicidal concentration (MFC) in a *upc2Δ/Δ* mutant relative to its azole-susceptible parent SC5314. Moreover, *upc2Δ/Δ* was unable to grow on solid media containing 10 µg/mL fluconazole and exhibited increased susceptibility and a clear zone of inhibition when subjected to Etest. Time-kill analysis showed increased azole activity against *upc2Δ/Δ* compared to SC5314. *UPC2* disruption in strains carrying specific resistance mutations also resulted in reduced MICs and MFCs. *UPC2* disruption in a highly azole-resistant clinical isolate containing multiple resistance mechanisms likewise resulted in a reduced MIC and MFC. This mutant was unable to grow on solid media containing 10 µg/mL fluconazole and exhibited increased susceptibility and a clear zone of inhibition when subjected to Etest. Time-kill analysis showed increased azole activity against the *upc2Δ/Δ* mutant in the resistant background. Microarray analysis showed attenuated fluconazole induction of genes involved in sterol biosynthesis as well as iron transport and homeostasis in the absence of *UPC2*. Taken together, our results demonstrate that the *UPC2* transcriptional network is universally essential for azole resistance in *C. albicans* and represents an attractive target for enhancing azole antifungal activity.

Fungal survival in the presence of the azoles is permitted by specific signal transduction and transcriptional activation programs. In an effort to identify additional transcriptional pathways involved in fluconazole susceptibility, we sought to identify transcription factors (TFs) essential for this process. From a collection of *C. albicans* strains disrupted for genes encoding TFs (Homann et al., PLoS Genet. 2009;5:e1000783), 4 exhibited marked reduction in MFC in both RPMI and YPD media independent of any noted growth defect in medium alone as compared to the parent strain. In addition to *UPC2*, one gene of interest (GOI) (*CAS5*) was unable to recover from fluconazole exposure at concentrations as low as 2 µg/mL after 72 hours in YPD medium, showed reduced susceptibility and a clear zone of inhibition by Etest, was unable to grow on solid media containing 10 µg/mL fluconazole, and exhibited increased susceptibility by time-

kill analysis. *CAS5* disruption in highly azole resistant clinical isolates containing multiple resistance mechanisms did not alter susceptibility. However, *CAS5* disruption in strains containing specific resistance mutations resulted in a moderate reduction in MIC and MFC. Genome-wide transcriptional analysis was performed in the presence of fluconazole and was consistent with the suggested role of *CAS5* in cell wall organization while also suggesting a role in iron transport and homeostasis. These findings suggest that Cas5 regulates a transcriptional network that influences susceptibility of *C. albicans* to fluconazole. Further delineation of this transcriptional network may identify targets for potential co-therapeutic strategies to enhance the activity to the azole class of antifungals.

Genome-wide expression profiling identified that ergosterol biosynthesis genes depend on Upc2 for their fluconazole-induceability, and cell wall maintenance genes depend on Cas5. In the presence of fluconazole, the expression of iron ion transport and homeostasis genes were also identified to be dependent on Upc2 as well as Cas5. The identification of these target genes validated the known and putative roles for Upc2 and Cas5, and also provided insight into novel roles for these transcription factors in azole susceptibility.

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LIST OF ABBREVIATIONS

ABC	ATP binding cassette
AIDS	Acquired immune deficiency syndrome
AmB	Amphotericin B
AP-1	Activator protein-1
APC	Antigen presenting cell
ARE	AP-1 recognition element
bHLH	Basic helix-loop-helix
bZip	Basic leucine zipper
c-CRD	carboxyl terminus cysteine rich domain
cDNA	Complementary deoxyribonucleic acid
CDR	<i>Candida</i> drug resistance
CFU	Colony forming units
CLSI	Clinical and Laboratory Standards Institute
CRD	Cysteine rich domain
cRNA	Complementary ribonucleic acid
CWI	Cell wall integrity
DBD	DNA binding domain
DNA	Deoxyribonucleic acid
DRE	Drug response element
Etest	Epsilometer test
FLC	Fluconazole
FRT	<i>FLP</i> recognition target
GEO	Gene Expression Omnibus
GI	Gastrointestinal track
GO	Gene Ontology
GOF	Gain-of-function
GOI	Gene of interest
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HOG	High osmolarity glycerol
ICU	Intensive care unit
IV	Intravenous
MAPK	Mitogen activated protein kinase
MDR	Multidrug resistance
MFC	Minimum fungicidal concentration
MFS	Major facilitator superfamily
MIC	Minimum inhibitory concentration
n-CRD	Amino terminus cysteine rich domain
Nou	Nourseothricin
Nou ^R	Nourseothricin-resistant
Nou ^S	Nourseothricin-susceptible
OD	Optical density
OPC	Oropharyngeal candidiasis

ORF	Open reading frame
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PKA	Protein Kinase-A
PRR	Pattern recognition receptor
qRT-PCR	Quantitative real time-polymerase chain reaction
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
SBI	Sterol biosynthesis inhibitors
SD	Sabouraud dextrose
SRE	Sterol response element
TF	Transcription factor
TLR	Toll-like receptor
UV	Ultra Violet
VVC	Vulvovaginal candidiasis
YPD	Yeast peptone dextrose
YRE	yAP-1 response element
5-FOA	5-Fluoroorotic acid

CHAPTER 1. INTRODUCTION

CANDIDA ALBICANS

Physiology

Candida albicans is an opportunistic fungal pathogen commonly found as a commensal organisms in the oropharyngeal cavity, gastrointestinal track, and vagina of up to 75% of humans (1, 2). It is a dimorphic fungus and exists as a blastospore (or yeast cell) at temperatures below 35°C with glucose as a carbon source and in a filamentous cell when above 35°C and in the presence of serum (3). Blastospores are spherical, single cells that undergo mitosis by budding. After the completion of mitosis, a septum forms between the two cells and separation results in two asymmetrical mother and daughter cells similar to *Saccharomyces cerevisiae* (4). Under filamentous conditions, *C. albicans* can form hyphae or pseudohyphae. The formation of hyphae occurs when a blastospore induces the elongation site and subsequently extends a germ tube and forms a septum without constrictions after mitosis, but the cells do not separate (5). Instead, the hypha continues to elongate. Pseudohyphae are formed when a blastospore buds but the cells remain attached to each other after the formation of the septum, and the daughter cell subsequently elongates into a shape resembling a hypha. As the cells undergo mitosis, this process repeats with each terminal cell resulting in a chain of elongated cells (4-6).

The ability of *C. albicans* to change its morphology between these forms is considered to be a virulence factor, promoting disseminated infection and forming biofilms, and is regulated by various environmental signals (7-10). Although it typically colonizes the mucocutaneous surfaces, the hyphal phase is able to invade into deeper tissues and cause damage, particularly when the host defenses are compromised (11). *C. albicans* switches back to yeast cells once in the bloodstream in order for dissemination (12). These morphologies also play a role in the formation of biofilms, which typically occur on the surfaces of indwelling devices such as catheters and dentures. Biofilms begin forming when blastospores adhere to these devices and then form a thick hyphal layer, which subsequently disperses more fungal cells (13). Biofilms have been shown to be resistance to antifungal drugs and is due to prevention of drug penetration by the extracellular matrix, drug efflux, and persister cells within the biofilms (14-18).

The yeast to hyphal transition, which occurs during fungal invasion, also significantly alters the composition of the cell wall. The outermost layer of the *Candida* cell wall is composed of mannans and mannoproteins, while the inner layer is composed of β -1,3-glucan and chitin. Although the outer layer of mannan masks underlying cell wall components such as β -1,3-glucan, they become exposed during hyphal transition at the bud scars and also by removal of this outer layer by heat inactivation, antifungal drugs, or mutations in the mannan layer (19-25). These cell wall components are the main source of pathogen-associated molecular patterns (PAMPs) recognized by the

pattern recognition receptors (PRRs) of the cells of the immune system, and their interaction is critical for establishing an anti-*Candida* immune response (26).

Genetics

C. albicans is a diploid yeast with no haploid stage during its cell cycle. Sequencing of its genome shows that it possesses 6,354 putative genes contained in 8 chromosomes (27). It lacks a conventional sexual cycle and instead its reproduction is mostly clonal. There does exist a mating type locus on chromosome 5, but the occurrence of meiotic recombination is infrequent (28).

Due to its lack of a traditional sexual cycle or a haploid stage, the diploid state of *C. albicans* makes it difficult to isolate homozygous mutants carrying recessive mutations. In the past, mild mutagenesis only resulted in the generation of auxotrophs (29, 30). Combining mild mutagenesis with ultraviolet light increases the types of mutations, but also generates unwanted genomic alterations and therefore genetically undefined mutants. Reliable methods for gene disruption have been developed which utilize electroporation transformation by homologous recombination, which occurs with a plasmid-resident gene, and results in chromosomal integration and ensures stable inheritance.

Auxotrophic strains were developed for use in transformation experiments, and the most common marker used is *URA3* because of the ease of selection for the correct transformants. The URA-blaster method of gene disruption uses this marker flanked by direct repeats of bacterial *hisG* sequences. The cassette is constructed to contain the 5' and 3' regions of the targeted gene on either side of the *hisG* sequences. Upon transformation into a *ura⁻* strain, one allele is replaced with the cassette by homologous recombination. Positive selection for the presence of the disruption cassette in the first allele occurs by growth on media lacking uridine. Counterselection then occurs by plating on media containing 5-fluorooronic acid (5-FOA), which only allows for the growth of *ura⁻* strains which arise by homologous recombination between the *hisG* sequences. The presence of the *URA3* gene, encoding orotidine 5'-phosphate decarboxylase, results in the conversion of 5-FOA to 5-fluorouracil, which is toxic to the cell. The counterselected heterozygous cells then undergo a second round of transformation and selection for disruption of the second allele. However, auxotrophic strains exhibit decreased virulence and limit the background from which genes may be deleted. Additionally, repeated use of this method leaves behind *hisG* sequences, providing homology sites for unwanted integration events (31).

Transformation methods using a dominant resistance marker suitable for routine transformation of clinical isolates would be ideal. Two such methods have been developed independent of auxotrophy. These methods utilize dominant resistance markers conferring resistance to either mycophenolic acid or nourseothricin which are fused to a site-specific *FLP* recombinase, modified from *S. cerevisiae*, controlled by a *SAP2* or *MAL2* promoter respectively. The markers are flanked by direct repeats of the

FLP recognition target (FRT) and cassettes are constructed to contain the 5' and 3' regions of the targeted gene on either side of the FRT sites. After integration into the first allele, the promoter is induced and the *FLP* recombinase removes the dominant marker and *FLP* sequences. A second round generates a mutant which differs from its background strain only by the lack of a gene and the presence of FRT sequences (32-34).

Epidemiology of Infection

In recent decades, fungi have emerged as a major cause of disease particularly for individuals who are immunocompromised or hospitalized with a serious underlying disease (35-39). This increased incidence of infections caused by fungi correlates to the growth of the immunocompromised and immunosuppressed patient population. Immune defects caused by hematopoietic stem cell transplantation, solid organ transplantation, major surgery, HIV/AIDS, neoplastic disease, immunosuppressive therapy, advanced age, or premature birth are all high risk factors for fungal infections (37, 38, 40-44).

The most important opportunistic fungal pathogen is the *Candida* spp. and are the fourth leading cause of nosocomial blood stream infections in the United States (45). As stated previously, *Candida* spp. are common commensal organisms in the oropharyngeal cavity, gastrointestinal track, and vagina of humans. This organism is capable of opportunistic infection upon disruption of the normal flora by broad spectrum antimicrobial treatment, a breach in the mucocutaneous barrier due to surgery or the presence of catheters, or defects in the host immune system caused by neutropenia, HIV/AIDS, chemotherapy, and organ or bone marrow transplantation (2).

The most common fungal infections that occur are invasive and mucosal infections. Invasive candidiasis can occur in the bloodstream or in one or more organs and candidemia is the most common form of invasive candidiasis (45), typically occurring in hospitalized patients with serious underlying conditions. The incidence of nosocomial candidiasis has steadily increased over the past three decades along with the widespread use of central venous catheters, broad spectrum antimicrobial agents, corticosteroids and cytotoxic chemotherapeutic agents. Such immunosuppression impairs the ability to fight opportunistic infections. Patients with candidemia are twice as likely to die during hospitalization as compared to noncandidal blood stream infections (46), with 10-49% mortality (47, 48).

Mucosal candidiasis mainly presents as vulvovaginal candidiasis (VVC) or oropharyngeal candidiasis (OPC). VVC typically occurs in women of childbearing age, but also occurs due to antibiotic use, immunosuppressive therapy, hormone replacement therapy, and diabetes mellitus (49). OPC can occur in elderly patients with dentures, providing a surface on which *Candida* can adhere, which is resolved by removal of the dentures and proper dental hygiene (50, 51). In AIDS patients, OPC is most the commonly encountered opportunistic infection (52-54). Such an infection occurs on the soft and hard palates, pharynx, tongue, and buccal mucosa, and is typically the first

clinical sign of an underlying HIV infection (55). The occurrence of OPC increases as CD4⁺ cells decrease below 200 cells/mm³ (56).

The increasing susceptibility of HIV/AIDS patients to opportunistic fungal infections occurring alongside the loss in CD4⁺ cells implicates that the cytokines produced by these cells are important for immunity to *Candida* (57). Indeed, functional cell-mediated immune responses are important for the host's defense against *Candida* spp. This is made evident by the engagement of PRRs on antigen presenting cells (APCs) by *Candida*, resulting in the secretion of specific cytokines which in turn promote the differentiation of activated CD4⁺ T cells into Th17 cells (58). These cells express the cytokine IL-17 and signaling through the IL-17 receptor (IL-17R) is crucial for an effective anti-*Candida* immune response (59).

Due to their enhanced susceptibility, up to 90% of all AIDS patients contract OPC and many die as a direct consequence (56). A significant decrease in HIV-related opportunistic infections, including OPC, occurred upon the advent of highly active antiretroviral therapy (HARRT). However, significant morbidity associated with *Candida* continues for the HIV/AIDS patient population due to high cost, insufficient monitoring, and poor patient compliance (55, 60).

Treatment of *Candida* Infections

Despite the wide spectrum of disease caused by *C. albicans*, there are few fundamentally different classes of clinical antifungals available for treatment due to limited unique and important fungal-specific targets which are not found in the host (61). Currently available antifungal drugs act primarily on the fungal cell wall and ergosterol found in the cell membrane. The fungal cell wall displays an electron-dense fibrillar outer layer composed of mannoproteins and an inner layer of glucans (such as β -1,3-glucan) and chitin which confer elasticity and tensile strength to the cell wall (62, 63). Ergosterol is found in the fungal cell membrane and regulates membrane fluidity and asymmetry, and ultimately membrane integrity and transport across the membrane (64). Perturbation of either of these components impacts the fungal cell's survival. Both components are exploitable drug targets as the cell wall is essential for survival, and human cells lack both cell walls and ergosterol. The currently available antifungals which are primarily used for treatment include the polyenes, the azoles and the echinocandins (65).

Polyenes, such as amphotericin B (AmB), directly bind to membrane ergosterol and as a result cause impairment of yeast cell physiology (66-71). Channel formation and subsequent membrane permeabilization is a secondary mechanism that enhances the antifungals activity (72). It has a broad spectrum of action including most major fungal pathogens and was the sole drug available to control fungal infections for 30 years (73). For oral infection its use is usually limited to topical application as it is not readily absorbed from the gut (74). It is primarily given intravenously (IV) for systemic infections but has been associated with significant nephrotoxicity. The development of

lipid formulations allowed for higher doses with less toxicity but they are more costly (75-77). Despite its fungicidal activity, cases of resistance have occasionally been reported (78).

The azoles represented a major advancement in ability to safely and effectively treat local and systemic fungal infections, and their high safety profile led to extensive use (65, 79). Fluconazole (FLC) inhibits ergosterol production essential in cell membrane formation (80) by targeting the product of *ERG11*, a critical cytochrome P450 enzyme of the ergosterol biosynthesis pathway known as lanosterol 14 α -demethylase which demethylates lanosterol (81). Inhibition of this enzyme results in depletion of ergosterol and accumulation of toxic sterol intermediates including 14 α -methylated sterols which alters membrane structure and function, and ultimately disrupts its integrity (79, 82). The azoles are available in both IV and oral formulations, and are currently the only oral option. Oral administration of fluconazole undergoes relatively little metabolism, is absorbed rapidly, is less toxic than AmB and is relatively inexpensive (77, 80, 83, 84). One drawback is its fungistatic activity instead of being fungicidal (61, 85) and as a result facilitates the development of resistance (86-88).

The echinocandins, such as caspofungin, are the most recent class of antifungal drugs. They act by inhibiting (1,3)- β -D-glucan synthase which is required for glucan synthesis, an important structure in the fungal cell wall (77). This class of antifungal represents significant advancement in antifungal therapy attributed to their fungicidal activity, proven clinical efficacy, and limited toxicity and drug-drug interactions. However, they are only available IV and are associated with moderate cost which somewhat limits their utility.

FUNGISTATIC AZOLE ACTIVITY AND AZOLE RESISTANCE

Clinical Impact of Fungistatic Azole Activity

As mentioned above, the fungistatic nature of azoles limits their efficacy (61, 85). The fungal cells are able to grow despite the inhibitory effects of the antifungal agent (61). Therefore, the inability to kill fungal cells may facilitate the development of resistance creating problems for treatment, especially for immunocompromised patients with OPC (89, 90). OPC is typically treated with low doses of fluconazole (100 mg/day) over a long period of time. Upon the occurrence of a relapse, the dosage is increased (up to 800 mg/day).

The incidence of OPC has decreased with the use of HARTT. However despite this advancement, resistance has been shown to still occur and many resistance mechanisms are maintained even after the antifungal agent is no longer present (61). This suggests that for the treatment of fungal infection it is important that the antifungal agent have enhanced abilities (91-93). As a result the development of novel co-

therapeutic agents imparting enhanced activity upon the azoles would represent a favorable strategy by which to advance treatment (90, 94-96).

Response Mechanisms That Allow Fungistatic Azole Activity

Azole antifungal inhibition of ergosterol biosynthesis pathway is not lethal in *C. albicans*, suggesting that coping mechanisms exist such as specific stress responses which may circumvent azole activity and permit survival of the fungal cells in the presence of the azoles. Indeed, *C. albicans* activates specific pathways in response to external stresses. Response mechanisms implicated in the fungistatic effect of the azoles include: increases in drug efflux pump and ergosterol biosynthesis gene expression (97), the Ras-cAMP-PKA pathway (98), the calcineurin pathway (99-101), the Ssk1p/Chk1p pathway (102), and the cell wall integrity (CWI) pathway (103-106).

Upon ketoconazole exposure, expression of genes involved in drug efflux pump (*CDR1*, *CDR2*, *MDR1*) and ergosterol biosynthesis (*NCP1*, *MCR1*, *CYB5*, *ERG2*, *ERG3*, *POT14*, *ERG25*, *ERG251*) were induced (97). These efflux pumps are typically expressed as low or undetectable levels and become upregulated in the presence of certain chemicals. Increased expression of these drug efflux pumps reduces the intracellular concentration of drug and therefore decreases its effect. Also, many of the ergosterol biosynthesis genes identified to be induced upon ketoconazole exposure exist downstream from Erg11p, the azole target, and respond to ergosterol depletion by facilitating the production of alternate sterols including lanosterol, eburicol, obtusifoliol, 14 α -methyl fecosterol, and 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol to be inserted into the cell membrane (107). Integration of these sterols into the plasma membrane disrupts its integrity resulting in its altered structure and function (79, 82, 108). Under these conditions, growth is inhibited but the organism remains viable (109).

Hsp90 is a molecular chaperone that stabilizes the regulators of many eukaryotic cellular signaling pathways, and its inhibition disrupts many cellular processes (110, 111). Interestingly, compromise of its function blocks the development of azole resistance and renders drug-resistant isolates susceptible (112), such as a resistant *erg3 Δ /* Δ mutant (113). It has also been shown to improve the efficacy of treatment in a murine model of disseminated infection (114). Of the many pathways in which Hsp90 interacts, it has been shown to play roles in some of the pathways implicated in the response to azoles.

Ras1 is a small GTPase that regulates the cAMP-protein kinase A (PKA) pathway controlling morphogenesis in response to nutrient limitation, pH, CO₂, and serum (12). In response to these stimuli, the guanine exchange factor Cdc25 activates Ras1 to its GTP-bound state which continues on to stimulate Cdc35 and cAMP-associated protein (Cap1/Srv2) for the production of cAMP (115, 116). This increase in intracellular cAMP activates the catalytic subunits of PKA which activates the transcriptional regulator of morphogenesis, Efg1 (117, 118). Also playing a role in the transition from yeast to hyphal growth is the Hsp90 molecular chaperone. Pharmacological disruption of Hsp90

function induces hyphal growth even in the absence of external cues, and therefore is a negative regulator of the Ras-cAMP-PKA pathway (9). Disruption of the genes encoding adenyl cyclase (*Cdc35*) or *Cap1/Srv2* renders the cells hypersusceptible to the azoles, with a fungicidal effect. This has been correlated to the fungal cell's inability to up-regulate a multidrug transporter gene *CDR1* (98), known to pump fluconazole out of the cell.

Calcineurin is a protein phosphatase consisting of a catalytic A (*Cna1*) and a regulatory B (*Cnb1*) subunit which are highly conserved between yeasts and mammals (119). The molecular chaperone Hsp90 has been shown to interact with the *Cna1* subunit, stabilizing the protein for activation in response to stress (99, 120, 121). In response to stress, calcineurin dephosphorylates downstream transcriptional regulators, including the transcription factor *Crz1* which regulates genes involved in cell wall biosynthesis and ion homeostasis (122-125). Calcineurin is implicated as being required for the fungistatic activity of the azoles (100, 126). Hsp90 or calcineurin compromise imparts fungicidal activity upon the azole antifungals (114, 122, 124). Calcineurin pathway mutants disrupted for the calcineurin subunit genes *CNA1* and *CNB1* treated in combination with an azole and cyclosporine A (which inhibits calcineurin) also render the azoles fungicidal. This effect does not involve changes in the expression of the transporter genes (99-101).

The high osmolarity glycerol (HOG) pathway is mediated by the Hog1 mitogen activated protein kinase (MAPK) in response to osmotic and oxidative stress (127-129). It has also been implicated in morphogenesis and cell wall formation. Hog1 is phosphorylated by the Pbs2 MAP kinase downstream from the *Ssk1p/Chk1p* pathway (102). Environmental signals cause the *Chk1* histidine kinase to autophosphorylate which then dephosphorylates *Ssk1* and stimulates the Pbs2-Hog1 MAPK cascade (130). Disruption of either genes encoding the response regulator (*Ssk1p*) or histidine kinase (*Chk1p*) caused the fungal cells to become hypersusceptible to the azoles due to the inability to pump out the drug resulting in fluconazole accumulation within the cells (102). Additionally, when *CHK1* is disrupted, the properties of the cell surface are altered along with lowered glucan content (131-133). On the other hand, disruption of *HOG1* did not result in altered susceptibility to fluconazole, but did result in resistance nikkomycin Z and Congo red (127).

In *S. cerevisiae*, the cell wall integrity MAP kinase (CWI MAPK) pathway is the major contributor to cell wall assembly and maintenance (85). Upon sensing stress at the surface of the fungal plasma membrane, the linear MAPK cascade amplifies the initial signal for transmission to downstream transcriptional regulators such as *Rlm1p*. This regulator is responsible for the majority of the transcriptional output produced by this pathway (63, 103). This pathway is conserved in *C. albicans* and has been previously suggested to be critical for the fungistatic activity of sterol biosynthesis inhibitors (104). *CaCAS5* encodes a transcriptional regulator of the cell wall damage response and is postulated to be the functional equivalent of *ScRlm1p* (134). When the cell wall integrity pathway is disrupted, specifically by disrupting the kinase genes *PKC1* and *MKC1*, the cells exhibit increased fluconazole susceptibility (103, 106). The upstream protein kinase

C, Pkc1p, phosphorylates the MAPK Mkc1p in response to fluconazole, but is interrupted by its absence. Hsp90 depletion was also shown to destabilize Mkc1p in the same study, therefore blocking *PKC1*-signaling. Additionally, deletion of *PKC1* in a resistant *erg3Δ/Δ* mutant resulted in enhanced susceptibility (106).

The transcription factors and their target genes have yet to be identified for many of the pathways involved in the response to azole activity and there may be other yet to be identified pathways involved. Target genes of these pathways would be useful as co-therapeutic drug targets to improve the activity of azole against *C. albicans*.

Emergence of Clinical Azole Resistance

When an infection progresses or persists despite treatment with antifungals, this is known as clinical resistance. There are two forms of resistance. Organisms that are resistant prior to exposure to an antifungal agent have primary or intrinsic resistance while secondary resistance arises after initiation of antifungal treatment (77). Due to their fungistatic activity and widespread use in the treatment of fungal infections, antifungal drug resistance most commonly occurs for the azoles. Their fungistatic nature makes it difficult to completely clear all of the fungal cells, especially in an immunocompromised individual, resulting in reoccurring infection.

The emergence of fluconazole resistance is attributed to many factors. First, *Candida* infections are not usually due to newly acquired resistant strains, but rather commensal strains that either become more virulent or that no longer have to compete with other normal flora (135). The use of low doses of fluconazole over extended periods of time also creates an ideal environment for the selection of resistant strains. Transmission between individuals also can occur, although typically among immunocompromised individuals (136, 137).

In order to characterize fluconazole susceptibility in the clinical setting, the Clinical and Laboratory Standards Institute (CLSI) Subcommittee for Antifungal Susceptibility Testing established standard breakpoints: sensitive ≤ 2 $\mu\text{g/ml}$, susceptible-dose dependent (S-DD) = 4 $\mu\text{g/ml}$, resistant ≥ 8 $\mu\text{g/ml}$ (138). Appropriate measurement of resistance in vitro is often correlated with treatment failure (139). However, there are many others factors that can impact the likelihood of therapeutic success such as clinical status of the patient, presence of foreign material, and location of infection (79, 140).

Due to the rise in the immunosuppressed patient population the usage of antifungal agents has also increased, coincidentally along with a rise in resistance. The azoles are the most important antifungal agent because they are relatively safe, can be orally delivered, and are relatively inexpensive. Fluconazole is the most widely used azole for treating OPC, which is the most common AIDS-associated opportunistic infection (141, 142). Repetition and lengthy duration of therapy for OPC in AIDS patients has led to an increased incidence of treatment failures secondary to the emergence of azole resistance (77, 89, 143, 144). Recurrent OPC most often occurs

when CD4⁺ T cells drop below 50 cells/mm³. In fact, one study identified 33% of late-stage AIDS patients with drug-resistant *Candida* present in their oral cavities (145).

Other patient populations are increasingly exposed to azoles for treatment and prophylaxis including those undergoing aggressive cancer therapy, surgical intensive care unit (ICU) patients, nonneutropenic patients, and women with VVC (146-150). However, the increased use of the azole antifungals in the immunocompromised patient population has the largest effect on the frequency, morbidity, and overall response to therapy of fungal infections.

It is logical for resistance to antifungal agents to develop as a result of their use for treatment. An additional complication of fluconazole resistance is the potential to develop cross-resistance to other azoles and the polyenes, as the activities of both antifungals are associated with ergosterol. As a result, the ability to limit the emergence of resistance to these agents and the development of more potent drugs is clinically important. Strategies to avoid and suppress resistance have yet to be defined, but one suggested method is to utilize combination therapy in order to broaden efficacy and preserve the utility of the azole class of antifungals (151).

Molecular Mechanisms of Resistance to Azoles

Several mechanisms of azole resistance have been documented in *C. albicans*. One method involves the export of the azole antifungal out of the cell by constitutive overexpression of efflux pumps, preventing the intracellular accumulation of effective concentrations. At least two efflux systems exist in *C. albicans* and have been found to be overexpressed in resistant isolates: the ATP-binding cassette superfamily (ABC) and the major facilitator superfamily (MFS) (152-156).

ABC transporters rely on the binding and hydrolysis of ATP for energy and have a broad specificity including many of the azoles (157). Two ABC transporters, encoded by *CDR1* and *CDR2*, have been implicated in azole resistance. In clinical matched isolates, increased expression of *CDR1* and *CDR2* is correlated with decreased azole susceptibility (155, 156, 158). This was further demonstrated by experimentally controlling overexpression of *CDR1* which conferred resistance to fluconazole (159). Deletion of *CDR1* from a clinical isolate with resistance to several azoles resulted in a large reduction in resistance, while deletion of *CDR2* had a lesser effect (160).

Unlike ABC transporters, MFS transporters rely on the energy of the proton motive force generated by the proton gradient across the cell membrane and are relatively specific for fluconazole and voriconazole (161). The main MFS transporter identified to contribute to fluconazole resistance is Mdr1. Increased expression of *MDR1* has been detected in resistant isolates when compared to their susceptible matched pairs (152, 158). Experimental overexpression of *MDR1* was unable to confer fluconazole resistance, because higher levels of *MDR1* expression than what were achieved are necessary to confer resistance to fluconazole (162). The role of *MDR1* in fluconazole

resistance was confirmed when deletion from an *MDR1*-overexpressing resistant isolate resulted in decreased fluconazole resistance (33).

Other mechanisms by which azole resistance develops involve changes in lanosterol demethylase or other components of the ergosterol biosynthesis pathway. Inhibition of lanosterol demethylase by the azoles results in accumulation of the methylated toxic sterol 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol which inhibits fungal cell growth (108). In order to compensate for this loss-of-function inhibition due to azole activity, there exists a bypass pathway through the inactivation of *ERG3* product, $\Delta^{5,6}$ desaturase. This results in the accumulation of alternate, nontoxic sterols (such as fecosterol) in place of the toxic sterols, allowing the organism to circumvent inhibited cell growth, and results in azole resistance (108, 163).

Drug target alterations also lead to resistance by decreased drug affinity, while still maintaining an active target. White et al. analyzed the *ERG11* sequences of 17 sequential isolates which develop resistance and identified a point mutation resulting in a substitution between two residues known to be involved in interacting with the heme in the active site (156, 164, 165). Such point mutations in *ERG11* may result in conformational changes that prevent effective binding between azoles and their target without affecting function of the enzyme in ergosterol biosynthesis (165, 166). Several point mutations have been identified in resistant isolates and lead to decreased azole susceptibilities (165-167).

Overexpression of *ERG11* is another mechanism of resistance and results in overproduction of the target enzyme, lanosterol demethylase. This increased production overwhelms the intracellular azole concentration such that the drug does not inhibit the reaction completely (152, 156, 167). *ERG11* upregulation is found to be present along with one of the efflux pump resistance mechanisms in at least 25% of clinical resistant isolates (167).

All of these mechanisms impact reduced azole susceptibility, but high-level azole resistance appears to accumulate gradually over time with the acquisition of multiple mechanisms (167, 168). Sanglard, et al. (169) studied set of 16 sequential *C. albicans* isolated from five AIDS patients, selected based on their increasing fluconazole resistance following prolonged treatment. The observed decreased in fluconazole accumulation was shown to be associated with a 10-fold increase in mRNA levels of *MDR1* (normal levels for *CDR1*). Additionally, White, et al. examined 17 sequential isolates from an HIV patient and found increases in *ERG11*, *MDR1* and *CDR1*, further suggesting that high-level azole resistance results from contributions of several mechanisms (156).

TRANSCRIPTIONAL REGULATORS

Transcription Factor Classes

Two major classes of transcription factors are involved in drug resistance, the first being the basic leucine zipper (bZip) proteins. The bZip class is found only in eukaryotic cells and is characterized by a highly conserved 60-80 amino acid long basic leucine zipper motif shaped in a bipartite α -helix which binds the DNA major groove (170). The DNA binding domain (DBD) is located in the N-terminal portion of the bZip motif and consists of two basic amino acid clusters which are responsible for binding to DNA. The leucine zipper is located in the C-terminal portion and has leucine residues every 7 amino acids (170). It is through the leucine zipper that the bZip class proteins dimerize and bind DNA as either homodimers or heterodimers resulting in gene transcription activation or repression (170, 171).

Within the bZip class, there exist 13 different families of proteins. Two families, the Jun and the Fos, dimerize and form the activator protein-1 (AP-1) transcription factor family implicated in drug resistance (171). These proteins bind DNA as heterodimers with one Jun protein and one Fos protein or homodimers with two Jun proteins. The dimers bind DNA at a specific AP-1 recognition element (ARE) motif which is found in the promoters and enhancers of genes involved in several cellular processes.

Fungal AP-1 transcription factors are referred to as yeast AP-1 (yAP-1) and bind to the yAP-1 response element (YRE) (172, 173). Yeast bZip transcription factors differ by being the only bZips to contain two cysteine rich domains (CRD) located in the amino terminus (n-CRD) and carboxy terminus (c-CRD) of the 3' bZip domain (174). These CRDs act in the localization and activation of yAP-1 transcription factors.

The largest class of eukaryotic transcriptional regulators is the zinc finger proteins (175), which are defined by a zinc finger motif, structured as one α -helix and a pair of antiparallel β strands, containing cysteines and histidines which bind zinc atoms for structure, function and stabilization. Within this class exist three families of zinc finger proteins: classical zinc fingers (Cys_2His_2) bind DNA as monomers, Cys_4 zinc fingers bind as either homodimers or heterodimers, and zinc cluster proteins (Cys_6) bind as monomers, homodimers and heterodimers. The zinc cluster transcription factors have six cysteines bound by two zinc atoms and are exclusive to fungi (175-177).

The zinc cluster transcription factors are typically found in the nucleus and many are constitutively bound to their promoters (175). These transcription factors are comprised of a DBD, regulatory domain, and acidic domain. Their DBD contains zinc finger motif, linker, and dimerization domains. The zinc finger motif binds as dimers in the DNA major groove, the linker designates DNA binding specificity, and the dimerization domain resembles the leucine zipper in bZip proteins (175, 178). Their regulatory domain is specific to zinc cluster transcription factors and upon its deletion,

renders them constitutively active (179, 180). Phosphorylation or dephosphorylation of the acidic domain acts as the activation domain (175).

Transcription Factors and Fungistatic Azole Activity

Some of the transcriptional regulators of the stress responses which circumvent azole activity and permit survival in the presence of the azoles have been identified, but the direct downstream targets of many remain unclear.

EFG1 encodes a transcription factor of the basic helix-loop-helix (bHLH) class and is a member of the APSES family fungal-specific regulators (181, 182). The bHLH domain promotes dimerization and DNA binding to the Efg1 recognition sequence (EGR or E-box) box, which is found in many genes involved in morphogenesis. Indeed, *EFG1* plays a role in the induction and repression of cell wall genes in yeast and hyphal forms (183). This agrees with its function downstream of the Ras-cAMP-PKA pathway. Upon disruption of *EFG1*, an increase in membrane fluidity was observed concurrent with a decrease in *ERG11* expression, rendering the cell hypersusceptible to fluconazole (184).

One of the downstream regulators of the calcineurin pathway is Crz1 (100). It is a classical Cys₂His₂ zinc finger and has been found to control the expression of several Ca²⁺/calcineurin-responsive genes through CDRE sequences in their promoters (122, 185). It has also been documented that disruption of *CRZ1* results in hypersusceptibility to fluconazole (100, 122). Interestingly, disruption of the catalytic α -subunit of protein kinase CK2, *CKA2*, results in fluconazole resistance through overexpression of *CDR1* and *CDR2*, but subsequent *CRZ1* disruption suppresses this phenotype (186).

In the *S. cerevisiae* CWI pathway, the transcription factor ScRlm1 plays a major role. This pathway is conserved in *C. albicans* and the functional homolog to ScRlm1 has been identified as Cas5, a classical Cys₂His₂ zinc finger protein (134, 187). Microarray analysis in the presence of caspofungin identified the requirement of Cas5 for the expression of many caspofungin-responsive genes, including those cell wall protein and cell wall maintenance genes. Motifs were also identified that may represent the Cas5-binding sites in the promoter regions of 10 out of 13 Cas5-dependent genes examined. Additionally, disruption of *CAS5* resulted in hypersusceptibility to caspofungin, Congo red, and sodium dodecyl sulfate (134). Due to its role in the CWI pathway, it is possible that it is also hypersusceptible to the azoles, similarly seen for other components of this pathway.

Transcription Factors and Azole Resistance

Constitutive overexpression of the efflux pumps involved in azole-resistance is due to constitutively active transcription factors, as a result of gain-of-function (GOF) mutations. A few of these transcription factors have been identified.

The *C. albicans* bZip yAP-1 transcription factor, Cap1, is closely related to the *S. cerevisiae* Yap1 and has been identified to be involved in multidrug resistance (188). Cap1p is primarily involved in protecting the cell against oxidative stress (189-191). Genome-wide location and expression analysis was undertaken in order to determine which genes Cap1 directly binds and regulates. Targeted groups included genes involved in osmotic stress response, response to drugs (including *MDR1*), phospholipid transport, and nitrogen utilization (192). Cells disrupted for *CAP1* were unable to upregulate *MDR1* in response to fluconazole and the formation of a hyperactive Cap1, by disruption of the c-CRD, resulted in fluconazole resistance and increased *MDR1* expression (189). Cap1 binds to the Yap1 response element (YRE) motif located in the *MDR1* promoter, but the absence of this motif in a fluconazole-resistant clinical isolate did not alter *MDR1* expression, suggesting there is another regulator controlling its expression during resistance (193, 194).

Constitutive *MDR1* overexpression resulting in multidrug resistance is due to GOF mutations in the zinc cluster transcription factor Mrr1. *MRR1* was identified as one of the genes upregulated in three *MDR1*-overexpressing fluconazole-resistant isolates when compared to their fluconazole-susceptible matched isolates. These isolates were identified as being homozygous for mutated *MRR1* GOF alleles (195). Inactivation of this *MRR1* allele resulted in the loss of *MDR1* overexpression and increased fluconazole susceptibility. Deletion of *MRR1* reduced fluconazole resistance more than *MDR1*, suggesting that additional Mrr1p-controlled mechanisms are involved. Meanwhile, introduction of the GOF allele into a fluconazole-susceptible background resulted in constitutive overexpression of *MDR1* and multidrug resistance. Other transcription factors have been implicated in the upregulation of *MDR1*, but Mrr1 is the central regulator of *MDR1* expression in fluconazole resistance.

Tac1p is a zinc cluster transcription factor is another transcription factor involved in regulating clinical azole-resistance (196). It binds the drug response element (DRE) consensus sequence located in the promoters of *CDR1* and *CDR2* (196, 197). Disruption of *TAC1* abolishes *CDR1* and *CDR2* expression and is hypersusceptible to fluconazole. Azole resistance occurs when the isolates have become homozygous for a GOF *TAC1* allele, likely because zinc cluster transcription factors act as dimers. Such a *TAC1* allele was isolated from an azole-resistant strain which overexpressed *CDR1* and *CDR2*, and when placed in the background of a susceptible strain was still able to confer resistance and *CDR1* and *CDR2* overexpression. However, this resistance depends on the presence of *CDR1* and *CDR2* (198).

Upc2p is a zinc cluster transcription that controls inducible ergosterol gene expression in the presence of the azoles. When *UPC2* is disrupted in a susceptible background, it becomes hypersusceptible to sterol biosynthesis inhibitors (SBIs), including fluconazole in addition to abolished *ERG* gene transcription and reduced ergosterol levels (199, 200). It binds to a sterol response element (SRE) motif found in the promoter region of *ERG* genes and also *UPC2* itself. Genome-wide location profiling identified the Upc2p gene groups targets involved in ergosterol biosynthesis (including *ERG11*), drug transport (*MDR1*), and transcription factors (*UPC2*) (201). The first GOF

mutation in Upc2 was identified in matched isolates with no *CDR1*, *CDR1*, or *MDR1* overexpression by Northern blot (152). Microarray analysis revealed that *UPC2*, *ERG11* and *MDR1* were overexpressed in the resistant isolate, containing a G648D GOF mutation (202). When the mutated GOF allele was introduced into a fluconazole-susceptible background, both *ERG11* and *MDR1* were upregulated. Other *UPC2* GOF mutations have also been identified to confer fluconazole-resistance and increased *ERG11* expression (203-205).

CENTRAL HYPOTHESIS AND RESEARCH OBJECTIVES

C. albicans responds to antifungal stress through specific signal transduction and transcriptional activation pathways, which circumvent azole activity and permit survival of the fungal cells in the presence of the azoles. Several response mechanisms have been identified to mediate fluconazole susceptibility. Increased drug efflux pump expression results in decreased intracellular concentrations of the azoles. Increased *ERG11* expression results in increased intracellular concentrations of lanosterol demethylase and therefore the amount of enzyme increases that is not bound by the azoles. Additionally, increased expression of other genes involved in the ergosterol biosynthesis pathway in response to ergosterol depletion leads to accumulation of alternate sterols which results in inhibited growth but the cell remains viable. Disruption of components of the Ras-cAMP-PKA pathway results in decreased inducible *CDR1* gene expression resulting in fungicidal azole activity, while disruption of the transcriptional regulator, Efg1, results in decreased *ERG11* expression and hypersusceptibility to fluconazole. Compromise of the calcineurin pathway renders the azole fungicidal and disruption of one possible downstream regulator, Crz1, results in fluconazole hypersusceptibility. Disruption of either Ssk1 or Chk1 upstream of the HOG pathway resulted in intracellular fluconazole accumulation and hypersusceptibility. Disruption of Mkc1 and Pkc1 of the CWI pathway results in increased susceptibility to the azoles. The downstream transcriptional regulators and their target genes have yet to be identified for many of these pathways and there may also be novel pathways which have yet to be identified.

I hypothesized that pathways centrally involved in the response mechanisms to azole activity, permitting survival in their presence, require key transcriptional regulators. With the completion of the *C. albicans* genome sequencing project many genes were identified to code for transcription factors, and microarray technology can be used to identify their downstream targets. By studying mutants deleted for specific transcription factors, their impact on fluconazole susceptibility can be elucidated and their fluconazole-inducible target genes can be identified. Among the transcription factors identified, we found both previously described as well as novel roles for these transcriptional regulators in fluconazole susceptibility.

Upc2, a zinc cluster transcription factor, has been previously identified as the transcriptional regulator of the ergosterol biosynthesis pathway. The disruption of this transcription factor has also been shown to enhance susceptibility to fluconazole, while GOF mutations result in azole resistance. My first research objective (Chapter 2) was to

examine susceptible and resistant strains disrupted for *UPC2* by various susceptibility tests and gene expression analysis in an effort to elucidate the extent of its involvement in fluconazole susceptibility. I hypothesized that Upc2 is essential for fluconazole susceptibility in both susceptible and resistant backgrounds. Our studies showed that disruption of *UPC2* enhanced susceptibility and overcame resistance by downregulation of *ERG* genes, specifically *ERG11*. Additionally, we identified a novel role for Upc2 in regulating fluconazole-inducible iron homeostasis.

In research objective 2 (Chapter 3), we examined a library of mutants disrupted for genes encoding specific transcription factors in an effort to identify those which had enhanced susceptibility to fluconazole. I hypothesized that transcriptional regulators of known and possibly novel pathways would be essential for responding to azole activity. Our studies characterized the zinc finger transcription factor Cas5 in regulating fluconazole susceptibility, through its role as the transcriptional regulator of the CWI pathway. Gene expression profiling identified genes involved in cell wall maintenance whose fluconazole-induced expression was dependent on *CAS5*. Additionally, we identified a novel role for Cas5 in iron homeostasis, similar to Upc2.

CHAPTER 2. *UPC2* IS UNIVERSALLY ESSENTIAL FOR AZOLE RESISTANCE IN *CANDIDA ALBICANS*

INTRODUCTION

The increased frequency of invasive fungal infections in recent decades is directly related to an expansion in the immunocompromised patient population including those with HIV/AIDS, cancer chemotherapy patients, neutropenic patients, recipients of transplants with indwelling catheters, and patients receiving antibiotics (65, 206, 207). *Candida* species collectively are the fourth-leading cause of nosocomial infections in the United States and such infections are associated with unacceptably high rates of mortality (65, 208, 209). *Candida albicans* is the most prevalent opportunistic human fungal pathogen and causes a wide variety of infections spanning from superficial, mucosal infections to invasive disseminated disease despite the availability of effective antifungal treatment. Moreover, the most common opportunistic infection among AIDS patients is oropharyngeal candidiasis (OPC), and chronic cases continue despite the availability of highly active anti-retroviral therapy (HAART) due to poor compliance, lack of access, and treatment failure of HAART (52, 53, 210-212).

The azoles, particularly fluconazole, are the most widely used class of antifungals for the treatment of *Candida* infections (65, 79). Fluconazole acts by inhibiting the protein product of *ERG11*, lanosterol 14 α -demethylase, which leads to ergosterol depletion and accumulation of toxic methylated sterol precursors resulting in inhibition of growth (213). In an immunocompromised host, the fungistatic nature of fluconazole limits its efficacy against this organism (61, 85). *C. albicans* exhibits inhibited growth in the presence of this fungistatic drug and it is generally believed that such fungistatic activity facilitates the development of resistance creating problems for treatment, especially in immunocompromised patients with OPC (89, 90). Currently known mechanisms of resistance to the azoles include alterations in the expression of drug efflux pumps (*CDR1*, *CDR2* and *MDR1*) and ergosterol biosynthesis genes *ERG*, and mutations in *ERG11* (77). These mechanisms are frequently combined resulting in a stepwise development of resistance over time.

In *C. albicans*, *UPC2* is a zinc-cluster transcription factor that regulates the expression of genes involved in ergosterol biosynthesis, including *ERG11* (199, 214). Disruption of *ERG11* or pharmacologic inhibition of the enzyme it encodes leads to reduced ergosterol production and the accumulation of alternate sterols including lanosterol, eburicol, obtusifolliol, 14 α -methyl fecosterol, and 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol (107). Biosynthesis of these alternate sterols does not require *ERG11*, but does require other genes in the ergosterol biosynthesis pathway, some of which appear to be regulated by Upc2. We reasoned that since Upc2 is required for the transcriptional activation of other genes of the sterol biosynthesis pathway, the disruption of *UPC2* might result in enhanced activity of the azole antifungals in azole-resistant as well as -susceptible isolates. In the present study, we further examined the role of *UPC2* in azole antifungal activity against both azole-susceptible and azole -resistant strains of

C. albicans, in particular an azole-resistant clinical isolate and mutant strains that represent four of the major azole resistance mechanisms. Taken together, our results indicate that the *UPC2* transcriptional network is universally essential for fluconazole resistance in *C. albicans*.

MATERIALS AND METHODS

Strains and Growth Conditions

All *C. albicans* strains (**Table 2-1**) were stored as frozen stock in 40% glycerol at -80°C. YPD (1% yeast extract, 2% peptone, and 1% dextrose) agar plates and YPD liquid medium were used for routine growth of strains at 30°C. For CFU counts during time-kill analysis, PDA (0.4% potato starch, 2% dextrose, and 1.5% agar) plates were used, and cultures grown on PDA were incubated at 35°C.

Drug Susceptibility Testing

The minimum inhibitory concentrations (MICs) of fluconazole (FLC) were determined by using broth microdilution as described by the Clinical and Laboratory Standards Institute M27-A2, modified by using YPD media, and were read both visually and spectrophotometrically at 24, 48, and 72 hours. Minimum fungicidal concentrations (MFCs) were measured by removing 2 µl from each well of the MIC plate and plating onto YPD agar. Also, serial dilutions from a 0.1 OD₆₀₀ suspension were diluted 4-fold and 2 µl of each dilution plated onto YPD agar plates with and without 10 µg/ml fluconazole and then incubated at 30°C for 24 and 48 hours. Fluconazole activity was also assessed by Epsilon test strips (Etest strips) (Biomérieux) according to manufacturer's instructions with the following modifications. A standardized cell suspension (0.5 McFarland) was used to create a confluent lawn across YPD agar plates prior to Etest strip placement and then incubated at 30°C for 24 and 48 hours. Time-kill analyses were performed with a 0.5 McFarland cell suspension, which was 10-fold diluted into YPD media with and without 10 µg/ml fluconazole and incubated at 35°C. Aliquots were removed at 0, 6, 12, and 24 hours, 10-fold serially diluted and plated onto PDA. CFUs were counted in duplicate after 48 hours at 35°C and plotted on a log-scale curve versus time (215).

Construction of *ERG11* Strains with Mutant Alleles

CaERG11 coding sequences were amplified by PCR (*Pfu* DNA polymerase; Stratagene) from *C. albicans* genomic DNA using the primers *ERG11*-A and *ERG11*-E. Products were cloned into pCR-BLUNTII-TOPO using a Zero Blunt TOPO PCR Cloning Kit (Invitrogen) and transferred into *Escherichia coli* TOP10 cells with selection on LB agar plates containing 50 µg/ml kanamycin. Plasmid DNA was purified (QIAprep;

Table 2-1. *C. albicans* strains used in this study

Strain	Designation	Strain Background	Relevant Characteristics or Genotype	Source
SC5314	SC5314	N/A	<i>UPC2-1/UPC2-2</i>	ATCC
Clinical isolate 2-79	2-79	N/A	Susceptible isolate	Redding, 1994
Clinical isolate 12-99	12-99	N/A	Resistant isolate	Redding, 1994
UPC2M4A	<i>upc2Δ/Δ</i>	SC5314	<i>upc2-1Δ::FRT/upc2-2Δ::FRT</i>	Dunkel, 2008
UPC2M2K21A	<i>upc2Δ/Δ+UPC2</i>	UPC2M2A	<i>upc2-1Δ::FRT/UPC2^{S1-1}-caSAT1</i>	Dunkel, 2008
12-99UPC2A5C1A	12-99 <i>upc2Δ/Δ</i>	12-99	<i>upc2Δ::FRT/upc2Δ::FRT</i>	This study
10C1B1M1	<i>ERG11^{K143R}</i>	SC5314	<i>ERG11^{K143R}::FRT/ERG11^{K143R}::FRT</i>	This study
10C1B1M1UPC2C9H	<i>ERG11upc2Δ/Δ</i>	10C1B1M1	<i>UPC2-1/UPC2-2</i> <i>ERG11^{K143R}::FRT/ERG11^{K143R}::FRT</i>	This study
SCMRR1R34A	<i>MRR1^{P683S}</i>	SC5314	<i>upc2-1Δ::FRT/upc2-2Δ::FRT</i> <i>MRR1^{P683S}::FRT/MRR1^{P683S}::FRT</i>	Schubert, 2011
<i>Δupc2</i> MRR1R34A	<i>MRR1upc2Δ/Δ</i>	SCMRR1R34A	<i>UPC2-1/UPC2-2</i> <i>MRR1^{P683S}::FRT/MRR1^{P683S}::FRT</i>	Schubert, 2011
SCTAC1R34A	<i>TAC1^{G980E}</i>	SC5314	<i>upc2-1Δ::FRT/upc2-2Δ::FRT</i> <i>TAC1^{G980E}::FRT/TAC1^{G980E}::FRT</i>	Sasse, 2011
SCupc2TAC1R34A1A14A	<i>TAC1upc2Δ/Δ</i>	SCTAC1R34A	<i>UPC2-1/UPC2-2</i> <i>TAC1^{G980E}::FRT/TAC1^{G980E}::FRT</i> <i>upc2-1Δ::FRT/upc2-2Δ::FRT</i>	This study

Qiagen; Germantown, MD) and sequenced on an ABI Model 3130XL Genetic Analyzer using the *ERG11* sequencing primers (**Table 2-2**), resulting in full-length sequence from both strands of the *CaERG11* gene. The sequencing was performed using six sets of clones derived from three independent PCRs for each strain/isolate sequenced.

Sequenced plasmids of the *ERG11* ORF whose predicted translation indicated an amino acid substitution were digested with restriction enzymes *ApaI* and *XhoI*, which excised the full-length ORF from the plasmid, and the *ERG11* alleles were cloned upstream of the *SAT1*-flipper cassette into the *ApaI* and *XhoI* sites of plasmid pSFS2 (34). The *ERG11* downstream segments were amplified with ExTaq (Takara) using primers *ERG11-C* and *ERG11-D* and cloned downstream of the *SAT1*-flipper cassette in pSFS2 using the *NotI* and *SacII* sites.

Construction of *UPC2* Deletion Strains

Plasmid pSFS2 contains the entire *SAT1*-flipper cassette (34). The *SAT1*-flipper cassette consists of the *SAT1* selectable marker which confers resistance to nourseothricin and the *FLP* flipper recombinase gene both flanked by *FRT* sites (flipper recombinase target sequences). The *UPC2* deletion cassette (pUPC2M2) was developed by Dunkel et al, where the 5' upstream sequence from positions -373 to +15 was cloned downstream of the *SAT1*-flipper cassette while the 3' sequence from positions +2097 to +2437 was cloned upstream of the *SAT1*-flipper cassette (**Table 2-2**) (216). Upon transformation with the gel-purified *SacI-ApaI* fragment from pUPC2M2 into the parent strain the *SAT1*-flipper was inserted into the coding region of one allele from positions +16 to +2096, and such positive transformants (Nou^R) were selected on YPD-nourseothricin agar plates containing 200µg/ml of nourseothricin. Induction of the *FLP* gene occurred by growing the transformants in YPD medium for 24 hours without selective pressure. Positive cells (Nou^S) were selected by replica plating onto YPD plates with and without 200 µg/ml of nourseothricin. Upon induction of the *FLP* gene, the cassette was excised such that only one copy of the *FRT* site remains in the locus. Another round was required to disrupt the second allele. Appropriate gene disruption and complementation for two independent strains was confirmed by Southern hybridization (34).

Isolation of Genomic DNA and Southern Hybridization

Genomic DNA was isolated as described previously (217). Four µg of DNA were digested with an appropriate restriction endonuclease, separated on a 1% agarose gel, and after staining with ethidium bromide, were transferred by vacuum blotting onto a nylon membrane and fixed by UV cross-linking. Southern hybridization with enhanced chemiluminescence-labeled probes was performed with the Amersham ECL Direct nucleic acid labeling and detection system according to the instructions of the manufacturer.

Table 2-2. Primers used in this study

Primer	Sequence
qRT-PCR	
<i>ACT1</i> -F	5'-ACGGTGAAGAAGTTGCTGCTTTAGTT-3'
<i>ACT1</i> -R	5'-CGTCGTCACCGGCAAAA-3'
<i>BMR1</i> -F	5'-ACATAAATACTTTGCCCATCCAGAA-3'
<i>BMR1</i> -R	5'-AAGAGTTGGTTTGTAAATCGGCTAAA-3'
<i>CDR1</i> -F	5'-ATTCTAAGATGTCGTCGCAAGATG-3'
<i>CDR1</i> -R	5'-AGTTCTGGCTAAATTCGTAATGTTTTTC-3'
<i>CDR2</i> -F	5'-TAGTCCATTCAACGGCAACATT-3'
<i>CDR2</i> -R	5'-CACCCAGTATTTGGCATTGAAA-3'
<i>CFL4</i> -F	5'-GCAATGGTTGACAGGTTGGAA-3'
<i>CFL4</i> -R	5'-GCAATGTGACGATGATAAGTGACAA-3'
<i>ERG11</i> -F	5'-CCCCTATTAATTTGTTTTCCCTAATTTAC-3'
<i>ERG11</i> -R	5'-CACGTTCTCTTCTCAGTTTAATTTCTTTC-3'
<i>FET3</i> -F	5'-GCCGGTGTCTTAGGTTTAGCC-3'
<i>FET3</i> -R	5'-CTAGCAACTCTTTCTTCAACATCGG-3'
<i>FRP1</i> -F	5'-CTTCCAATACCATCCATTACGAT-3'
<i>FRP1</i> -R	5'-ATCTCCCCACTTTCAGCAAGAC-3'
<i>FTR1</i> -F	5'-ATTGTTGTTTCAGTGCTTTTGGC-3'
<i>FTR1</i> -R	5'-GGTCGGAACCTACCACCCATAGA-3'
<i>ERG11</i> cloning	
<i>ERG11</i> -F	5'-GGGCCCTTTGAGAACAGCCACACGACAAC-3'
<i>ERG11</i> -R	5'- <u>CTCGAGG</u> ACAAAAACCATCAACCGATTCCG-3'
<i>ERG11</i> sequencing	
<i>ERG11</i> seq B	5'-TATTTTCACTGCTTCAAGATCT-3'
<i>ERG11</i> seq C	5'-CCAAAAGGTCATTATGTTTTAG-3'
<i>ERG11</i> seq E	5'-AATGAGGTTTTTCACCTAAATG-3'
<i>ERG11</i> seq F	5'-CCCTTTACCGAAAACCTGGAGTA-3'
T7	5'-TAATACGACTCACTATAGGG-3'
M13R	5'-CAGGAAACAGCTATGACC-3'
<i>UPC2</i> mutant construction	
<i>UPC2</i> -A	5'-GGGCCC <u>G</u> AGATCTTGATGTCATTAG-3'
<i>UPC2</i> -B	5'- <u>CTCGAG</u> CTATATCTTCAATGAACTG-3'
<i>UPC2</i> -C	5'-CCGCGGACAGGTCAATACCGCGTAG-3'
<i>UPC2</i> -D	5'- <u>GAGCTC</u> GTTCCTCTAGTATCACTCTT-3'

Underlined sequence reflects the introduction of a restriction site sequence.

RNA Isolation for qRT-PCR

RNA was isolated using a small-scale hot phenol method of RNA isolation described by Schmitt et al (218). Briefly, overnight cultures were diluted to an OD₆₀₀ of 0.2 in 20 ml YPD and then incubated at 30°C with shaking for 3 hours. Cells were collected by centrifugation, and stored at -80°C. Cell pellets were resuspended in 950 µl of AE buffer and then transferred to a 2-ml RNase-free microcentrifuge tube containing 950 µl acid phenol (pH 4.3) with 1% SDS. Cells were incubated at 65°C for 10 minutes then lysates were clarified by centrifugation. The supernatant was then divided into two new 2-ml microcentrifuge tubes containing 950 µl of chloroform and mixed. The sample was then subjected to centrifugation again, and the top aqueous layer was transferred to a new tube containing 1 ml of isopropanol and 100 µl 2M sodium acetate. The RNA pellet was subsequently washed with 500 µl of 70% ethanol and collected by centrifugation. The RNA pellet was resuspended in DNase/RNase-free H₂O. Quantity and purity were determined spectrophotometrically at absorbances of A₂₆₀ and A₂₈₀.

Quantitative RT-PCR

First-strand cDNAs were synthesized from 1 µg of total RNA using SuperScript first-strand synthesis system for RT-PCR (Invitrogen). Gene-specific primers (**Table 2-2**) were designed using Primer Express software (Applied Biosystems) and synthesized by Integrated DNA Technologies (Coralville, IA). Quantitative PCRs were performed in triplicate using the 7000 sequence detection system (Applied Biosystems), independently amplifying *ACT1* (normalizing gene) and the genes of interest (GOI) as described previously (219).

RNA Isolation for Microarray

RNA was isolated using a large-scale version of the hot phenol method of RNA isolation described by Schmitt et al. (218). Briefly, overnight cultures were diluted to an OD₆₀₀ of 0.005 in 100 ml YPD and then incubated at 30°C with shaking for an additional 8 hours to an OD₆₀₀ of 1.0. Cultures were diluted again to an OD₆₀₀ of 0.025 in 100 ml fresh YPD, allowed to incubate at 30°C with shaking for one doubling, inoculated with or without 10 µg/ml FLC, and then incubated at 30°C with shaking for 6 hours. Cells were collected by centrifugation, and stored at -80°C. Cell pellets were resuspended in 12 ml of AE buffer and then transferred to 50-ml Oak Ridge tubes treated with RNase Away (Molecular BioProducts) containing 12 ml acid phenol (pH 4.3) with 1% SDS. Cells were incubated at 65°C for 10 minutes then lysates were clarified by centrifugation. The supernatant was then transferred to a new tube containing 15 ml of chloroform and mixed. The sample was then subjected to centrifugation again, and the top aqueous layer was transferred to a new tube containing one volume of isopropanol and 0.1 volume 2M sodium acetate. The RNA pellet was subsequently washed with 10 ml of 70% ethanol and collected by centrifugation. The RNA pellet was resuspended in DNase/RNase-free

H₂O. Quantity and purity were determined spectrophotometrically at absorbances of A₂₆₀ and A₂₈₀.

Transcriptional Profiling

Gene expression profiles were obtained by hybridizing labeled cRNAs generated from *C. albicans* total RNA onto Affymetrix *C. albicans* custom expression arrays (CAN07; 49-5241) (216), which have been described previously (201). Microarray hybridization and analysis were performed as described previously (201). Genes were considered to be differentially expressed in response to drug if their expression changed by ≥ 1.5 -fold in two independent experiments. Genes induced by FLC were considered to be *UPC2*-dependent if the induction was abrogated in the deletion mutant was ≥ 2.0 -fold (50%) less than the wild-type. All microarray data is available for download from the NCBI at *GEO* (Accession number: pending).

RESULTS

Disruption of *UPC2* Results in Enhanced Fluconazole Activity

In order to further investigate the requirement of *UPC2* for susceptibility to fluconazole, we subjected the *upc2* Δ/Δ mutant derived from azole-susceptible isolate SC5314 to various azole susceptibility tests examining both MIC and MFC using nutrient rich YPD media in order to detect strong phenotypes despite existing in an environment that promotes growth. Consistent with previous observations, disruption of *UPC2* resulted in a marked reduction in fluconazole MIC by broth microdilution (**Table 2-3**), Etest (**Figure 2-1A**), 72 hr regrowth (**Figure 2-1B**), and spot assay (**Figure 2-1C**). Interestingly at 24 hr in YPD, the fluconazole MFC for SC5314 was >64 $\mu\text{g/ml}$ whereas for the *upc2* Δ/Δ mutant it was 0.25 $\mu\text{g/ml}$ (**Table 2-3**). At 48 hr the MIC, as measured by Etest, for SC5314 was 1.0 $\mu\text{g/ml}$ and a halo of reduced growth (but not a clear zone of inhibition) was observed up to the Etest strip consistent with the fungistatic nature of fluconazole. The 48 hr MIC by Etest for the *upc2* Δ/Δ mutant was 0.032 $\mu\text{g/ml}$ and a clear zone of inhibition around the Etest strip was observed (**Figure 2-1A**). We also used a 72 hr endpoint for a broth microdilution assay in YPD as a way to assess the ability of the organism to resume growth in the presence of fluconazole. SC5314 was able to resume growth in all concentrations of fluconazole tested whereas the *upc2* Δ/Δ mutant was not (**Table 2-3**, **Figure 2-1B**). When plated on YPD agar plates containing 10 $\mu\text{g/ml}$ fluconazole, growth was reduced in the presence of fluconazole for the *upc2* Δ/Δ mutant as compared to SC5314 (**Figure 2-1C**). Time-kill analysis showed increased fungistatic activity of 10 $\mu\text{g/ml}$ fluconazole against the *upc2* Δ/Δ mutant as compared to its parent strain (**Figure 2-1D**). All phenotypes were reverted by reintegration of one allele of the disrupted gene.

Table 2-3. MICs and MFCs in YPD in SC5314 background

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>UPC2-1/UPC2-2</i>	0.5	0.5	>64	>64	>64	>64
<i>upc2Δ/Δ</i>	<i>upc2-1Δ::FRT/upc2-2Δ::FRT</i>	≤0.125	0.25	0.25	0.25	0.25	0.25
<i>upc2Δ/Δ+UPC2</i>	<i>upc2-1Δ::FRT/UPC2^{SI-1}-caSAT1</i>	0.5	0.5	>64	>64	>64	>64

Figure 2-1. *UPC2* impacts fluconazole susceptibility in SC5314 background

(A) Effect of *UPC2* on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) MIC heat map of SC5314, *UPC2* mutant, and complimented derivative. Susceptibility was determined by broth microdilution in YPD at 72 hours. Growth was quantified spectrophotometrically and assigned to a colorimetric scale. (C) Effect on *UPC2* on the ability to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 μ l aliquots were spotted onto YPD agar +/- 10 μ g/mL FLC and incubated for 48 hours. (D) Effect of fluconazole on *UPC2* by time-kill assay. SC5314 or *upc2* Δ/Δ cells were diluted in YPD medium containing fluconazole (10 μ g/mL) or the solvent, DMSO. After 0, 6, 12, and 24 hours, samples from each were diluted and plated for colony forming units.

Disruption of *UPC2* in Strains Containing Resistance Mutations in *MRR1*, *TAC1*, and *ERG11* Also Enhances Fluconazole Activity

In order to investigate the requirement for *UPC2* in the setting of specific mechanisms of azole resistance, independent mutants were constructed in strains containing two copies of a gene conferring reduced susceptibility to fluconazole: *MRR1*^{P683S}, *TAC1*^{G980E}, or *ERG11*^{K143R}. The *MRR1*^{P683S} and *TAC1*^{G980E} alleles contain gain-of-function mutations which render the transcription factors they encode constitutively active, resulting in upregulation of either *MDR1* or *CDR1* and *CDR2*, respectively, and decreased fluconazole susceptibility (195, 196, 220, 221). The *ERG11*^{K143R} allele contains a point mutation postulated to be located near the azole access channel, interfering with entry of fluconazole, resulting in decreased fluconazole susceptibility (222). Again the disruption of *UPC2* in each background resulted in a marked reduction in MIC and MFC by all methods and the trend in susceptibility seen for *MRR1upc2Δ/Δ* was consistent with what we have previously observed (214). The MFCs at 24 hr in YPD for *ERG11upc2Δ/Δ*, *MRR1upc2Δ/Δ* and *TAC1upc2Δ/Δ* were all reduced from 8 μg/ml, >64 μg/ml, and >64 μg/ml in their background strains to 1 μg/ml, 4 μg/ml, and 2 μg/ml respectively (**Table 2-4**). At 48 hr the MICs by Etest for *ERG11upc2Δ/Δ*, *MRR1upc2Δ/Δ* and *TAC1upc2Δ/Δ* were all reduced from 1.5 μg/ml, 4 μg/ml, and 8 μg/ml in their background strains to 0.19 μg/ml, 0.5 μg/ml, and 0.5 μg/ml respectively (**Figure 2-2A**). Growth was also reduced when plated on YPD agar plates containing fluconazole 10 μg/ml for the *MRR1upc2Δ/Δ* and *TAC1upc2Δ/Δ* strains as compared to their background strain (**Figure 2-2B**). Consistent with their fluconazole MICs, both the *ERG11*^{K143R} strain and its *upc2Δ/Δ* derivative were unable to grow at this concentration of fluconazole.

Disruption of *Upc2* Overrides Clinical Drug Resistance

Since disruption of *UPC2* in strains containing one resistance mechanism resulted in enhanced fluconazole activity, we wanted to further examine the extent to which disruption of *UPC2* influences high-level azole resistance in the presence of multiple resistance mechanisms. We constructed *upc2Δ/Δ* mutant strains in the background of an azole-resistant clinical isolate (12-99) known to carry four of the most common mechanisms of azole resistance: overexpression of *CDR1* and *CDR2*, overexpression of *MDR1*, overexpression of *ERG11*, and mutation in *ERG11* (223). As was observed for isolate SC5314 and the isogenic resistant strains, the disruption of *UPC2* resulted in a marked reduction in MIC and MFC by all methods. The MFC at 24 hr in YPD for 12-99 was >64 μg/ml whereas for 12-99*upc2Δ/Δ* was 16 μg/ml (**Table 2-5**). The fluconazole MIC by Etest at 48 hr was >256 μg/ml and confluent growth was observed for 12-99, whereas an MIC of 1.5 μg/ml and a clear zone of inhibition was observed for 12-99*upc2Δ/Δ* (**Figure 2-3A**). Likewise, in broth microdilution assays after 72 hr the parent strain was able to grow in all concentrations of fluconazole tested whereas 12-99*upc2Δ/Δ* grew less well (**Figure 2-3B**). Growth was also reduced when plated on YPD agar plates containing fluconazole 10 μg/ml for 12-99*upc2Δ/Δ* as compared to its parent strain (**Figure 2-3C**). As was observed in the SC5314 background, time-kill analysis also

Table 2-4. MICs and MFCs in YPD in background of strains expressing resistance mechanisms

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>UPC2-1/UPC2-2</i>	0.5	0.5	>64	>64	>64	>64
<i>ERG11</i> ^{K143R}	<i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT	4	8	8	8	8	32
<i>ERG11upc2Δ/Δ</i>	<i>UPC2-1/UPC2-2</i> <i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT	0.5	1	1	1	1	1
<i>MRR1</i> ^{P683S}	<i>upc2-1Δ</i> ::FRT/ <i>upc2-2Δ</i> ::FRT <i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT	16	>64	>64	>64	>64	>64
<i>MRR1upc2Δ/Δ</i>	<i>UPC2-1/UPC2-2</i> <i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT	2	2	4	4	4	4
<i>TAC1</i> ^{G980E}	<i>upc2-1Δ</i> ::FRT/ <i>upc2-2Δ</i> ::FRT <i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT	16	>64	>64	>64	>64	>64
<i>TAC1upc2Δ/Δ</i>	<i>UPC2-1/UPC2-2</i> <i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT <i>upc2-1Δ</i> ::FRT/ <i>upc2-2Δ</i> ::FRT	2	2	2	2	2	2

Figure 2-2. *UPC2* impacts fluconazole susceptibility in background of strains expressing resistance mechanisms

(A) Effect of *UPC2* in resistant backgrounds on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) Effect of *UPC2* in resistance backgrounds on the ability to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 μ L aliquots were spotted onto YPD agar +/- 10 μ g/mL FLC and incubated for 48 hours.

Table 2-5. MICs and MFCs in YPD of strains in 12-99 background

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>UPC2-1/UPC2-2</i>	0.5	0.5	>64	>64	>64	>64
2-79	Susceptible isolate	1	1	>64	>64	>64	>64
12-99	Resistant isolate	>64	>64	>64	>64	>64	>64
12-99 <i>upc2</i> Δ/Δ	<i>upc2</i> Δ::FRT/ <i>upc2</i> Δ::FRT	4	4	8	16	16	16

Figure 2-3. UPC2 impacts fluconazole susceptibility in 12-99 background

(A) Effect of *UPC2* in 12-99 on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) MIC heat map of 2-79, 12-99, and *UPC2* mutant.

Susceptibility was determined by broth microdilution in YPD at 72 hours. Growth was quantified spectrophotometrically and assigned to a colorimetric scale. (C) Effect of *UPC2* in 12-99 on the ability to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 µl aliquots were spotted onto YPD agar +/- 10 µg/mL FLC and incubated for 48 hours. (D) Effect of fluconazole on *UPC2* in 12-99 by time-kill assay. 12-99 or 12-99*upc2*Δ/Δ cells were diluted in YPD medium containing fluconazole (10 µg/mL) or the solvent, DMSO. After 0, 6, 12, and 24 hours, samples from each were diluted and plated for colony forming units.

revealed an increased fungistatic effect for fluconazole at 10 µg/ml against 12-99*upc2*Δ/Δ (**Figure 2-3D**).

Expression of *ERG11*, *CDR1*, *CDR2*, and *MDR1* When *UPC2* Is Disrupted in Resistant Backgrounds

In order to determine if the enhanced fluconazole activity was due to decreased expression *ERG11* or genes encoding efflux pumps, we measured *ERG11*, *CDR1*, *CDR2*, and *MDR1* mRNA abundance by qRT-PCR in the strains containing a single resistance mechanism, isolate 12-99 and their respective *upc2*Δ/Δ mutants (**Figure 2-4**). As expected, the *upc2*Δ/Δ mutant constructed in the SC5314 background showed a reduction in baseline *ERG11* expression relative to its parent strain. This was also the case for the *upc2*Δ/Δ mutants constructed in the *ERG11*^{K143R} and clinical isolate 12-99 backgrounds. However, both the *TAC1*^{G980E} and *MRR1*^{P683S} strains exhibited reduced *ERG11* expression relative to SC5314 with no appreciable additional reduction in expression when *UPC2* was disrupted. Disruption of *UPC2* did not result in decreased expression of *CDR1*, *CDR2*, or *MDR1* in any background. Interestingly, disruption of *UPC2* in *ERG11upc2*Δ/Δ resulted in an increase in expression of these transporter genes, the significance of which is unclear. These data suggest that the enhanced activity of fluconazole observed in resistant strains lacking *UPC2* is not due to changes in transporter gene expression levels, but may be associated with a reduction in *ERG* gene expression, particularly *ERG11*.

Comparison of the Gene Expression Profiles of Wild-Type Strain SC5314 and *upc2*Δ/Δ Exposed to Fluconazole

In order to identify genes whose expression in response to fluconazole is influenced by Upc2, we compared the transcriptional profiles of SC5314 and its *upc2*Δ/Δ derivative after treatment with or without 10 µg/ml fluconazole for 6 hours. Genes were considered to be differentially expressed in response to fluconazole if their expression changed by ≥1.5-fold in two independent experiments (**Table A-1** and **Table A-2**). Fluconazole inducible genes were also considered to be *UPC2*-dependent if their induction was abrogated in the deletion mutant and was ≥2.0-fold (50%) less than compared to SC5314. Using these criteria, there were 127 genes upregulated by fluconazole whose induction was abrogated in the absence of *UPC2* (**Table 2-6**). The most common biological processes represented by these genes included the lipid metabolic process, iron ion transport and iron homeostasis, transport, response to stress and chemical stimulus, and the oxidation-reduction process.

Validation of Microarray Data by Real Time RT-PCR

In order to validate the differential expression of genes identified by microarray, we examined the mRNA abundance for 5 genes of interest using the same RNA isolated

Figure 2-4. Expression of *ERG11*, *CDR1*, *CDR2*, and *MDR1* as measured by qRT-PCR

Expression levels for *ERG11*, *CDR1*, *CDR2*, and *MDR1* were measured in triplicate by qRT-PCR and compared to the expression levels in SC5314 (A) and 2-79 (B) in YPD. Error bars represent the standard error of the mean.

Table 2-6. Genes upregulated by at least 1.5-fold by fluconazole which are dependent upon Upc2

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Lipid metabolic process	orf19.1598	<i>ERG24</i>	2.0	1.6	0.9	0.7	0.5	0.4
	orf19.1631	<i>ERG6</i>	1.8	1.7	0.4	0.5	0.2	0.3
	orf19.2670		1.6	1.8	0.4	0.7	0.2	0.4
	orf19.3240	<i>ERG27</i>	2.8	2.4	1.4	1.1	0.5	0.4
	orf19.4982		2.1	2.3	0.7	0.8	0.3	0.4
	orf19.7585	<i>INO1</i>	9.2	20.5	0.9	0.8	0.1	0.0
	orf19.922	<i>ERG11</i>	1.6	1.5	0.8	0.7	0.5	0.4
Iron ion transport	orf19.1415	<i>FRE10</i>	2.0	4.2	0.2	0.1	0.1	0.0
	orf19.1932	<i>CFL4</i>	35.6	359	8.0	22	0.2	0.1
	orf19.4211	<i>FET3</i>	2.8	6.2	0.7	0.2	0.3	0.0
	orf19.4215	<i>FET34</i>	2.1	5.2	0.0	0.1	0.0	0.0
	orf19.5634	<i>FRP1</i>	8.9	8.2	0.1	0.2	0.0	0.0
	orf19.7219	<i>FTR1</i>	3.2	7.8	0.1	0.0	0.0	0.0
Iron ion homeostasis	orf19.1264	<i>CFL2</i>	2.0	6.0	0.2	0.8	0.1	0.1
	orf19.1715	<i>IRO1</i>	4.2	8.1	2.2	1.4	0.5	0.2
	orf19.5636	<i>RBT5</i>	1.7	1.6	0.2	0.2	0.1	0.2
	orf19.7114	<i>CSA1</i>	1.7	3.2	0.7	0.6	0.4	0.2
Transport	orf19.1352	<i>TIM22</i>	3.6	4.4	1.3	2.0	0.4	0.4
	orf19.2023	<i>HGT7</i>	20.8	21.6	9.4	7.8	0.5	0.4

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.2292	<i>OPT4</i>	5.2	3.7	0.0	0.0	0.0	0.0
	orf19.2350		2.5	4.1	0.9	0.6	0.4	0.1
	orf19.2785	<i>ATP7</i>	3.1	3.1	1.3	1.5	0.4	0.5
	orf19.3026	<i>MAS1</i>	1.7	1.6	0.9	0.8	0.5	0.5
	orf19.3195	<i>HIP1</i>	1.5	2.6	0.7	0.8	0.5	0.3
	orf19.3232		24.6	5.3	1.6	1.6	0.1	0.3
	orf19.3668	<i>HGT2</i>	48.3	35.5	20.1	12.3	0.4	0.3
	orf19.3746	<i>IFC1</i>	2.3	2.7	0.2	0.0	0.1	0.0
	orf19.4335	<i>TNA1</i>	194.9	29.8	0.4	0.2	0.0	0.0
	orf19.4384	<i>HXT5</i>	70.6	75.5	16.9	9.7	0.2	0.1
	orf19.4682	<i>HGT17</i>	45.0	24.7	4.2	4.5	0.1	0.2
	orf19.4690		16.3	20.2	1.0	1.4	0.1	0.1
	orf19.5307	<i>JEN2</i>	10.3	2.7	0.4	1.3	0.0	0.5
	orf19.5753	<i>HGT10</i>	20.6	1.6	2.6	0.8	0.1	0.5
	orf19.6148		4.4	47.3	2.0	13.8	0.4	0.3
	orf19.6249	<i>HAK1</i>	5.4	5.8	1.4	1.7	0.3	0.3
	orf19.6993	<i>GAP2</i>	30.6	10.6	6.0	3.0	0.2	0.3
	orf19.7093	<i>HGT13</i>	40.9	18.8	9.8	2.6	0.2	0.1
Response to stress	orf19.1434		1.7	2.7	0.8	0.9	0.5	0.3
	orf19.3239	<i>CTF18</i>	2.7	2.4	1.4	0.8	0.5	0.4
	orf19.3672	<i>GAL10</i>	6.0	7.3	2.7	3.8	0.5	0.5

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.4082	<i>DDR48</i>	5.8	5.7	1.9	1.3	0.3	0.2
	orf19.4093	<i>PES1</i>	2.7	3.5	0.6	1.3	0.2	0.4
	orf19.4317	<i>GRE3</i>	1.5	1.5	0.3	0.7	0.2	0.5
	orf19.496		2.6	2.3	1.1	0.6	0.4	0.3
	orf19.5902	<i>RAS2</i>	7.6	6.2	2.3	1.5	0.3	0.2
	orf19.7221	<i>SET3</i>	4.6	2.9	2.1	1.4	0.5	0.5
	orf19.921	<i>HMS1</i>	2.6	2.8	0.8	1.3	0.3	0.5
Response to chemical stimulus	orf19.4645	<i>BEMI</i>	1.5	1.9	0.4	0.6	0.3	0.3
	orf19.5591	<i>ADO1</i>	2.4	2.9	0.7	0.8	0.3	0.3
	orf19.6102	<i>RCA1</i>	2.5	3.4	1.1	1.4	0.5	0.4
	orf19.7374	<i>CTA4</i>	1.7	2.2	0.8	1.0	0.5	0.5
Oxidation- reduction process	orf19.1411		2.7	4.8	1.4	1.1	0.5	0.2
	orf19.1710	<i>ALH1</i>	2.0	1.9	0.7	0.8	0.4	0.4
	orf19.1940		1.7	1.5	0.4	0.7	0.2	0.5
	orf19.2091		2.2	2.3	0.8	0.9	0.4	0.4
	orf19.2108	<i>SOD6</i>	7.9	10.6	1.1	1.6	0.1	0.1
	orf19.4274	<i>PUT1</i>	6.8	8.8	2.5	0.6	0.4	0.1
	orf19.4747	<i>HEM14</i>	1.7	2.0	0.1	0.2	0.0	0.1
Filamentous	orf19.1300		2.1	4.4	0.3	0.6	0.1	0.4

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
growth	orf19.2809	<i>CTN3</i>	41.1	36.9	12.6	11.5	0.3	0.3
	orf19.3969	<i>SFL2</i>	1.5	2.4	0.7	0.6	0.5	0.2
	orf19.5741	<i>ALS1</i>	66.8	79.0	4.8	3.1	0.1	0.0
Organelle organization	orf19.109	<i>MST1</i>	1.5	1.6	0.5	0.5	0.4	0.3
	orf19.2387		1.7	1.5	0.7	0.7	0.4	0.4
	orf19.2984		1.9	2.0	0.7	1.0	0.4	0.5
	orf19.3782.2	<i>HTS1</i>	1.5	1.8	0.6	0.5	0.4	0.3
	orf19.4051		1.8	1.7	0.9	0.8	0.5	0.5
	orf19.415		2.2	2.3	1.2	1.0	0.5	0.5
	orf19.4273		5.1	7.6	1.4	2.0	0.3	0.3
	orf19.4478		1.6	1.8	0.5	0.8	0.3	0.5
	orf19.4657	<i>NAM2</i>	2.8	2.2	1.4	0.6	0.5	0.3
	orf19.5705		1.6	2.0	0.7	0.7	0.4	0.3
	orf19.6047		<i>TUF1</i>	1.8	2.8	0.3	0.3	0.2
DNA or RNA metabolic process	orf19.1956	<i>NOP1</i>	1.5	1.8	0.8	1.0	0.5	0.5
	orf19.3138		1.5	2.4	0.7	0.7	0.5	0.3
	orf19.7569		<i>SIK1</i>	1.5	2.2	0.6	0.9	0.4
Translation	orf19.828		1.8	1.5	0.2	0.4	0.1	0.3

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/		<i>upc2Δ</i> /Δ+FLC/		Ratio (B/A)	
			SC5314 (A)		<i>upc2Δ</i> /Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Biological process	orf19.4054	<i>CTA24</i>	1.8	2.5	0.5	1.0	0.3	0.4
	orf19.6003		2.4	2.1	0.9	0.7	0.4	0.4
Precursor metabolites and energy	orf19.1770	<i>CYCI</i>	1.9	2.2	0.6	0.7	0.3	0.3
	orf19.2439.1		1.6	2.6	0.8	1.2	0.5	0.5
	orf19.3290	<i>RIP1</i>	1.8	2.0	1.0	1.1	0.5	0.5
	orf19.5893		1.6	2.2	0.7	0.8	0.5	0.4
Vitamin metabolic process	orf19.2590		1.8	2.8	0.0	0.2	0.0	0.1
	orf19.410.3		5.6	5.7	1.0	0.8	0.2	0.1
	orf19.6057		2.5	1.8	0.2	0.2	0.1	0.1
Other	orf19.2836	<i>COQ4</i> <i>GCA1</i>	3.4	5.9	1.6	1.5	0.5	0.3
	orf19.3008		2.5	1.8	0.4	0.8	0.2	0.5
	orf19.4899		4.4	5.2	1.2	1.9	0.3	0.4
	orf19.6348		3.0	2.4	0.6	1.2	0.2	0.5
	orf19.7263		5.3	6.0	2.6	2.2	0.5	0.4
Unknown	orf19.1070		8.6	8.0	2.4	1.6	0.3	0.2
	orf19.1277		6.6	9.3	3.4	2.7	0.5	0.3
	orf19.1371		1.5	1.6	0.8	0.6	0.5	0.4
	orf19.1440		2.1	2.6	0.8	0.9	0.4	0.3

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		<i>upc2Δ/Δ</i> +FLC/ <i>upc2Δ/Δ</i> (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.1562	<i>CIRT4B</i>	3.7	4.5	1.7	1.3	0.5	0.3
	orf19.2068		1.6	1.5	0.6	0.6	0.4	0.4
	orf19.2414		3.1	3.3	0.7	1.7	0.2	0.5
	orf19.2691		1.5	1.5	0.4	0.7	0.3	0.5
	orf19.2839		1.6	2.6	0.7	0.6	0.4	0.2
	orf19.287		2.8	2.9	1.4	1.5	0.5	0.5
	orf19.3154		2.1	2.3	0.6	0.6	0.3	0.2
	orf19.3499		79.5	20.5	10.6	10.1	0.1	0.5
	orf19.3881		1.9	2.0	0.5	0.5	0.2	0.2
	orf19.4013		2.6	2.2	0.3	0.4	0.1	0.2
	orf19.4070		5.0	8.9	1.8	1.9	0.4	0.2
	orf19.4144		2.4	3.2	0.9	0.9	0.4	0.3
	orf19.4391		3.2	1.5	1.0	0.7	0.3	0.5
	orf19.4658		1.6	1.8	0.5	0.8	0.3	0.5
	orf19.467		32.3	7.1	2.8	0.8	0.1	0.1
	orf19.5019		3.5	3.7	0.9	1.9	0.2	0.5
	orf19.5205	<i>PGA7</i>	3.9	4.9	1.9	2.2	0.5	0.4
	orf19.5547		2.8	2.4	0.8	1.1	0.3	0.4
	orf19.5635		4.9	4.1	0.5	0.4	0.1	0.1
	orf19.6186		12.1	13.3	2.8	2.7	0.2	0.2
	orf19.6187		1.7	1.7	0.9	0.7	0.5	0.4
	orf19.6194		1.5	1.6	0.6	0.9	0.4	0.5

Table 2-6. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.6690		2.4	2.3	0.6	0.7	0.3	0.3
	orf19.670.2		47.4	152.7	18.3	14.1	0.4	0.1
	orf19.6713		2.2	3.7	0.9	0.3	0.4	0.1
	orf19.6905		2.4	3.3	1.2	1.4	0.5	0.4
	orf19.6970		4.5	4.4	2.0	1.8	0.4	0.4
	orf19.6983		2.1	9.1	0.4	0.0	0.2	0.0
	orf19.7380		3.0	3.2	1.6	1.3	0.5	0.4
	orf19.7567		1.9	2.1	0.2	0.2	0.1	0.1
	orf19.7675		1.7	1.9	0.8	0.8	0.5	0.4
	orf19.951		2.3	2.4	0.8	1.2	0.3	0.5

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).

for the microarray experiments. In addition to *ERG11*, four others were chosen based on their involvement in iron transport and homeostasis. The correlation between the microarray data and that obtained by real time RT-PCR was good (**Figure 2-5**). The expressions of *CFL4*, *FET3*, *FRP1*, and *FTR1* were upregulated in the wild type SC5314 when treated with fluconazole, but could not respond to the same extent when *UPC2* was disrupted. As expected, *ERG11* was also shown to respond to fluconazole in a *UPC2*-dependent fashion.

These data suggest that the enhanced activity of fluconazole observed in both susceptible and resistant strains lacking *UPC2* may be due to dysregulation of iron homeostasis, in addition to the inability to upregulate genes involved in the ergosterol biosynthesis pathway.

DISCUSSION

Identifying novel drug targets that improve the efficacy of fluconazole is important in order to develop new therapeutic strategies to preserve the azole class of antifungals and overcome azole resistance. *UPC2* has been well characterized with regard to its impact on fluconazole susceptibility and its role regulating genes of the ergosterol biosynthesis pathway (199-201, 224). Silver et al. and MacPherson et al. identified Upc2p as the key regulator of ergosterol metabolism in *C. albicans*, showing that azole-inducible expression of *ERG2*, *ERG7*, *ERG11*, and *ERG25* is diminished in the absence of *UPC2* (199, 200). We and others have established that in some azole-resistant isolates specific mutations render *UPC2* constitutively active, resulting in increased *ERG* gene expression (including *ERG11*), cellular ergosterol levels, and increased fluconazole resistance (156, 168, 201, 203, 204, 216, 219). This suggests that *UPC2* influences azole susceptibility through the regulation of this pathway.

In the present study, we observed that not only did the disruption of *UPC2* result in enhanced fluconazole susceptibility as measured by MIC, but also resulted in a substantial reduction in fluconazole MFC at 24, 48, and 72 hrs. Indeed disruption of *UPC2* in an azole-susceptible strain prevented its regrowth in YPD medium in the presence of higher fluconazole concentrations after 72 hours, resulted in a clear zone of inhibition around a fluconazole Etest strip, and prevented growth on solid media containing a therapeutically relevant concentration of fluconazole (10 µg/ml). Time-kill analysis also demonstrated an enhanced effect for fluconazole 10 µg/ml against the *upc2Δ/Δ* mutant as compared to its parent strain. Taken together, these data underscore the contribution of the Upc2 transcriptional activation pathway to azole susceptibility.

We then wished to determine if disruption of *UPC2* might have similar effects in fluconazole resistant isolates. For this purpose we chose isogenic strains containing resistance mutations in *ERG11*, *MRR1*, or *TAC1*. The strain containing the *ERG11*^{K143R} mutation had an MIC of 8 µg/ml at 48 and 72 hours in YPD. Consistent with this, it was unable to grow in the presence of 10 µg/ml fluconazole. Although this background was not as highly resistant as others, its respective *UPC2* deletion mutant had a marked drop

Figure 2-5. Validation of fluconazole-inducible and Upc2-dependent iron gene expression by qRT-PCR

Expression levels for *CFL4*, *FET3*, *FRP1*, *FTR1*, and *ERG11* were measured in triplicate by qRT-PCR and compared to the expression levels in SC5314 in YPD. Shown are the relative n-fold changes in gene expression in the in SC5314 and *upc2*Δ/Δ treated with fluconazole (FLC). Error bars represent the standard error of the mean.

in both fluconazole MIC and MFC. The *MRR1*^{P683S} and *TAC1*^{G980E} resistant strains both had an MIC and MFC of >64 µg/ml at 48 and 72 hours in YPD medium which was reduced markedly in the respective *upc2Δ/Δ* mutants at all time points. This trend in susceptibility is consistent with what has been previously observed for this *MRR1*^{P683S} *upc2Δ/Δ* mutant (214). However, unlike the halo of reduced confluent growth observed around the Etest strip with clinical isolate SC5314 (and generally observed with other *C. albicans* isolates), such growth was not observed with these resistant strains despite higher MICs and MFCs of >64 µg/ml. It has recently been shown that the *MRR1*^{P683S} and *TAC1*^{G980E} constructed mutants exhibit a fitness defect associated with introduction of these specific resistance mutations, whereas clinical isolates carrying such mutations appear to have regained fitness (225). Such clinical isolates have likely evolved compensatory mutations which mitigate these fitness defects. This may explain the unusual growth pattern of these mutants when subjected to susceptibility testing by Etest. Despite the absence of reduced confluent growth, the *upc2Δ/Δ* mutants in each background showed increased susceptibility to fluconazole by Etest and an inability to grow in the presence of fluconazole at 10 µg/ml. Therefore, the loss of *UPC2* in strains containing a single mechanism of resistance also resulted in enhanced susceptibility to fluconazole and to a greater extent than that observed for their susceptible parent strain SC5314.

The same phenotype was also observed upon disruption of *UPC2* in a highly resistant clinical isolate, 12-99, which carries all four of the most common mechanisms of resistance. 12-99*upc2Δ/Δ* exhibited a substantial reduction in fluconazole resistance as compared to isolate 12-99. Although 12-99*upc2Δ/Δ* was able to resume growth in lower concentrations of fluconazole at 72 hours in broth microdilution plates, it was not able to grow to the extent of 12-99 or its susceptible matched isolate, 2-79, both of which were able to grow even in the highest concentration of fluconazole. Similarly to what was observed for *upc2Δ/Δ* in the SC5314 background, fluconazole exhibited a greater fungistatic effect against 12-99*upc2Δ/Δ* as compared to its parent azole-resistant isolate. Indeed the loss of *UPC2* allowed fluconazole to overcome all four mechanisms of fluconazole resistance operative in isolate 12-99, and resulted in greater fluconazole susceptibility than was seen with its susceptible matched isolate, 2-79. Moreover, this resulted in a more pronounced effect by MFC, 72 hour regrowth, and time kill analysis than was observed for the susceptible wild type strain, SC5314.

One hypothesis for how the disruption of *UPC2* overcomes drug resistance is that it influences the expression of the efflux pump genes *CDR1*, *CDR2*, and *MDR1* in these strains. Indeed, it has been shown that in *C. albicans* Upc2 binds to the promoters of *CDR1* and *MDR1* and can regulate their expression (201). However, the expression of these genes was not affected in the absence of *UPC2* in both the constructed mutant strains or resistant clinical isolate 12-99. The disruption of *UPC2* had such an effect on susceptibility that even with constitutive upregulation of these efflux pumps, the strains were no longer resistant to fluconazole. *CDR1* and *CDR2* remained upregulated in *TAC1*^{G980E} and 12-99 as did *MDR1* in *MRR1*^{P683S} and 12-99.

Another possible explanation for the increased susceptibility to fluconazole observed in the *upc2Δ/Δ* mutants is a reduction in baseline or inducible expression of genes involved in sterol biosynthesis. In the presence of fluconazole, the *ERG11* gene product, lanosterol demethylase, is inhibited leading to the accumulation of toxic sterol precursors and the production of alternate sterols such as lanosterol, eburicol, obtusifoliol, 14 α -methyl fecosterol and 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol (107). Integration of these sterols into the plasma membrane disrupts its integrity resulting in its altered structure and function (79, 82, 226). Under these conditions, growth is inhibited but the organism remains viable (109). Fluconazole treatment also induces the expression of *ERG11* and other genes of the ergosterol biosynthesis pathway leading to increased production of not only lanosterol demethylase, but enzymes involved in alternate sterol production (227). However when *UPC2* is disrupted, *ERG11* expression would be reduced which would diminish the amount of drug target expressed in the cell. Expression of genes encoding enzymes needed for the biosynthesis of alternative sterols would also be reduced, further compromising the organism in the face of fluconazole exposure. Indeed expression of *ERG11* was reduced in *ERG11upc2Δ/Δ*, *MRR1upc2Δ/Δ* and *TAC1upc2Δ/Δ* as well as 12-99*upc2Δ/Δ* as measured by real-time RT-PCR, and inducible expression of *ERG6*, *ERG24*, and *ERG27* (as well as *ERG11*) was reduced in the *upc2Δ/Δ* mutant as compared to its parent strain SC5314 upon exposure to fluconazole as measured by microarray analysis. *ERG6* and *ERG27* both encode enzymes believed to be involved in the production of alternate sterols (Figure 2-6).

Of particular interest, another core set of fluconazole-induced genes we found to be dependent upon *UPC2* included those in the gene ontology process categories of iron transport (*CFL4*, *FET3*, *FET34*, *FRE10*, *FRP1*, *FTR1*) and iron homeostasis (*CFL2*, *CSA1*, *IRO1*, *RBT5*). Iron starvation has been shown to enhance the susceptibility of *C. albicans* to fluconazole (228-231). Prasad et al. observed that an *ftr1Δ/Δ* mutant, which is defective in high-affinity iron uptake, also exhibited enhanced susceptibility to fluconazole (228). Upon examination of *ERG11* mRNA abundance and membrane ergosterol levels of the *ftr1Δ/Δ* mutant, a reduction in *ERG11* expression and ergosterol content was observed compared to wild-type cells which led to increased membrane fluidity and consequently increased passive diffusion of fluconazole. In contrast *ERG3*, which acts in the biosynthesis of 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol (232), was upregulated. This phenotype reverted upon the addition of iron to the medium. A similar observation has been made in *Cryptococcus neoformans* where disruption of the genes encoding the ferroxidase (*CFO1*) and an iron permease (*CFT1*) of the high-affinity reductive iron uptake system, resulted in increased susceptibility to azole antifungals (233).

As many enzymes in the ergosterol biosynthesis pathway require iron, it is tempting to speculate that decreased iron uptake, as suggested by the diminished expression of iron transport and homeostasis genes exhibited by the *upc2Δ/Δ* mutant relative to its parent strain in the presence of fluconazole, would have a significant impact on the activity of these enzymes. This, combined with decreased expression of the genes encoding enzymes involved in ergosterol biosynthesis, (*ERG11* in particular), may

Figure 2-6. Sterol biosynthesis pathway in *C. albicans*

Genes in boldface were found to be responsive to fluconazole in a Upc2-dependent manner. Dotted lines denote multiple enzymatic steps (232, 234, 235).

account for the substantial effect on fluconazole susceptibility observed in these strains upon disruption of *UPC2*. While further investigation is needed to determine which Upc2 targets influence susceptibility to the azole antifungals, our findings suggest that the Upc2 and the transcriptional activation pathway it regulates represents a potential target for overcoming azole antifungal resistance in *C. albicans*.

CHAPTER 3. CAS5 IMPACTS THE ACTIVITY OF FLUCONAZOLE AGAINST *CANDIDA ALBICANS*

INTRODUCTION

Candida albicans is the most prevalent opportunistic human fungal pathogen, causing mucosal, cutaneous and systemic infections including oropharyngeal candidiasis (OPC), which is the most common opportunistic infection among AIDS patients (65). *Candida* species collectively are also the fourth-leading cause of nosocomial infections and are associated with high mortality rates (208, 209). The azole antifungals, particularly fluconazole (FLC), are the most widely used for treatment of *Candida* infections (65). However, *C. albicans* exhibits inhibited growth in the presence of azole antifungals, thus these agents are fungistatic against this organism (206). Identifying strategies to impart increased susceptibility to the azoles could improve their efficacy against *C. albicans* and may also diminish the development of resistance.

In *C. albicans* inhibition of the ergosterol biosynthesis pathway is not lethal suggesting that specific stress responses may circumvent or counter the activity of the azoles. Such an adaptive response would permit fungal survival in the presence of these antifungal agents. Indeed, in response to antifungal stress, specific signal transduction and transcriptional activation programs are affected (97). Drug efflux pump and ergosterol biosynthesis gene expression (236), the Ras-cAMP-PKA pathway (98), the calcineurin pathway (99-101), the Ssk1p/Chk1p pathway (102), and the cell wall integrity pathway (105, 237) have all been implicated as stress responses upon azole exposure yet the transcriptional activation pathways involved in these processes have yet to be identified. Moreover, it is likely that additional biological processes influence susceptibility to the azole antifungals.

Recently a library of *C. albicans* TF mutants was created and examined in 55 different growth conditions. Phenotypic profiling in response to various stress agents, including fluconazole, identified the possible biological roles of many previously uncharacterized TFs (187). While initial screens identified several TFs that affect fluconazole susceptibility, we undertook a more comprehensive analysis of this library in order to identify transcriptional activation programs that influence fluconazole activity and would therefore be implicated in the mechanisms by which *C. albicans* survives in the presence of the azoles. We identified one TF in particular, Cas5, whose disruption results enhanced azole activity indicating a role for the Cas5 transcriptional network in the susceptibility of *C. albicans* to the azole antifungal agents.

MATERIALS AND METHODS

Strains and Growth Conditions

All *C. albicans* strains (**Table 3-1**) were stored as frozen stock in 40% glycerol at -80°C. YPD (1% yeast extract, 2% peptone, and 1% dextrose) agar plates and YPD liquid medium were used for routine growth of strains at 30°C. For colony forming unit (CFU) counts during time-kill analysis, PDA (0.4% potato starch, 2% dextrose, and 1.5% agar) plates were used, and cultures grown on PDA were incubated at 35°C.

Drug Susceptibility Testing

The minimum inhibitory concentrations (MICs) of fluconazole were determined by using broth microdilution as described by the Clinical and Laboratory Standards Institute M27-A2, modified by using both RPMI and YPD media, and were read both visually and spectrophotometrically at 24, 48, and 72 hours. Minimum fungicidal concentrations (MFCs) were measured by removing 2 µl from each well of the MIC plate and plating onto SD or YPD agar, respectively. Also, serial dilutions from a 0.1 OD₆₀₀ suspension were diluted 4-fold and 2 µl of each dilution plated onto YPD agar plates with and without 10 µg/ml fluconazole and then incubated at 30°C for 24 and 48 hours. Fluconazole activity was also assessed by Epsilon test strips (Etest strips) (Biomerieux) according to manufacturer's instructions with the following modifications. A standardized cell suspension (0.5 McFarland) was used to create a confluent lawn across YPD agar plates prior to Etest strip placement and then incubated at 30°C for 24 and 48 hours. Time-kill analyses were performed with a 0.5 McFarland cell suspension, which was 10-fold diluted into YPD media with and without 10 µg/ml fluconazole and incubated at 35°C. Aliquots were removed at 0, 6, 12, and 24 hours, 10-fold serially diluted and plated onto PDA. CFUs were counted in duplicate after 48 hours at 35°C and plotted on a log-scale curve versus time (238).

Construction of Gene Deletion Strains

Plasmid pBSS2 contains the entire *SAT1*-flipper disruption cassette from pSFS2 in a pBluescript vector backbone. The *SAT1*-flipper cassette consists of the *SAT1* selectable marker which confers resistance to nourseothricin and the *FLP* flipper recombinase gene both flanked by *FRT* sites (flipper recombinase target sequences). The target gene's 5' flanking sequence was cloned upstream of the *SAT1*-flipper cassette while the 3' flanking sequence was cloned downstream of the *SAT1*-flipper cassette (**Table 3-2**). Upon transformation into the SC5314 strain the *SAT1*-flipper was inserted into the coding region of one allele, and such positive transformants (Nou^R) were selected on YPD-nourseothricin agar plates containing 200 µg/ml of nourseothricin. Induction of the *FLP* gene occurred by growing the transformants in YPD medium for 24 hours without selective pressure. Positive cells (Nou^S) were selected by replica plating onto

Table 3-1. *C.albicans* strains used in this study

Strain	Designation	Strain Background	Relevant Characteristics or Genotype	Source
SC5314	SC5314	N/A	<i>CAS5/CAS5 CZF1/CZF1 MKC1/MKC1 RPN4/RPN4</i>	ATCC
SCCAS5A5A66C	<i>cas5Δ/Δ</i>	SC5314	<i>cas5Δ::FRT/cas5Δ::FRT</i>	This study
SCCAS5A5A66C1B	<i>cas5Δ/Δ+CAS5</i>	SCCAS5A5A66C	<i>cas5Δ::FRT/CAS5-caSAT1</i>	This study
SCRPN4A1A21C	<i>rpn4Δ/Δ</i>	SC5314	<i>rpn4Δ::FRT/rpn4Δ::FRT</i>	This study
SCRPN4A1A21C6A	<i>rpn4Δ/Δ+RPN4</i>	SCRPN4A1A21C	<i>rpn4Δ::FRT/RPN4-caSAT1</i>	This study
SCCZF1G1D12	<i>czf1Δ/Δ</i>	SC5314	<i>czf1Δ::FRT/czf1Δ::FRT</i>	This study
SCMKC1B5A4A	<i>mkc1Δ/Δ</i>	SC5314	<i>mkc1Δ::FRT/mkc1Δ::FRT</i>	This study
SCMKC1B5A4A9A	<i>mkc1Δ/Δ+MKC1</i>	SCMKC1B5A4A	<i>mkc1Δ::FRT/MKC1-caSAT1</i>	This study
Clinical isolate2-79	2-79	N/A	Susceptible isolate	Redding, 1994
Clinical isolate12-99	12-99	N/A	Resistant isolate	Redding, 1994
12-99CAS5A1A72	12-99 <i>cas5Δ/Δ</i>	12-99	<i>cas5Δ::FRT/cas5Δ::FRT</i>	This study
G2	G2	G1	Susceptible isolate	Franz, 1998
G5	G5	G2	Resistant isolate	Franz, 1998
G5CAS5B16D3D	G5 <i>cas5Δ/Δ</i>	G5	<i>cas5Δ::FRT/cas5Δ::FRT</i>	This study

Table 3-1. (Continued)

Strain	Designation	Strain Background	Relevant Characteristics or Genotype	Source
Gu2	Gu2	Gu1	Susceptible isolate	Franz, 1999
Gu5	Gu5	Gu2	Resistant isolate	Franz, 1999
Gu5CAS5A23A4A	Gu5 <i>cas5</i> Δ/Δ	Gu5	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study
S1	S1	N/A	Susceptible isolate	Franz, 1999
S2	S2	S1	Resistant isolate	Franz, 1999
S2CAS5B6C5A	S2 <i>cas5</i> Δ/Δ	S2	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study
SCUPC2R14A	<i>UPC2</i> ^{G648D}	SC5314	<i>UPC2</i> ^{G648D} ::FRT/ <i>UPC2</i> ^{G648D} ::FRT <i>CAS5/CAS5</i>	Heilmann, 2010
SCUPC2R14ACAS5A5A21Y	<i>UPC2cas5</i> Δ/Δ	SCUPC2R14A	<i>UPC2</i> ^{G648D} ::FRT/ <i>UPC2</i> ^{G648D} ::FRT <i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study
10C1B1M1	<i>ERG11</i> ^{K143R}	SC5314	<i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT <i>CAS5/CAS5</i>	Chapter 2
10C1B1M1CAS5-1A51B	<i>ERG11cas5</i> Δ/Δ	10C1B1M1	<i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT <i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study
SCMRR1R34A	<i>MRR1</i> ^{P683S}	SC5314	<i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT <i>CAS5/CAS5</i>	Schubert, 2011
SCMRR1R34ACAS5-2A3	<i>MRR1cas5</i> Δ/Δ	SCMRR1R34A	<i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT <i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study
SCTAC1R34A	<i>TAC1</i> ^{G980E}	SC5314	<i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT <i>CAS5/CAS5</i>	Sasse, 2011
SCTAC1R34ACAS5-2A1	<i>TAC1cas5</i> Δ/Δ	SCTAC1R34A	<i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT <i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	This study

Table 3-2. Primers used in this study

Method	Primer	Sequence
qRT-PCR	<i>ACT1</i> -F	5'-ACGGTGAAGAAGTTGCTGCTTTAGTT-3'
	<i>ACT1</i> -R	5'-CGTCGTCACCGGCAAAA-3'
	<i>BMR1</i> -F	5'-ACATAAATACTTTGCCCATCCAGAA-3'
	<i>BMR1</i> -R	5'-AAGAGTTGGTTTGTAATCGGCTAAA-3'
	<i>CDR1</i> -F	5'-ATTCTAAGATGTCGTCGCAAGATG-3'
	<i>CDR1</i> -R	5'-AGTTCTGGCTAAATTCGTAATGTTTTTC-3'
	<i>CDR2</i> -F	5'-TAGTCCATTCAACGGCAACATT-3'
	<i>CDR2</i> -R	5'-CACCCAGTATTTGGCATTGAAA-3'
	<i>CFL4</i> -F	5'-GCAATGGTTGACAGGTTGGAA-3'
	<i>CFL4</i> -R	5'-GCAATGTGACGATGATAAGTGACAA-3'
	<i>ERG11</i> -F	5'-CCCCTATTAATTTTGTTCCTAATTTAC-3'
	<i>ERG11</i> -R	5'-CACGTTCTCTTCTCAGTTTAATTTCTTTC-3'
	<i>ERG26</i> -F	5'-ATTAGAGTGTGCGATGCCATT-3'
	<i>ERG26</i> -R	5'-CGTCGTCGTAGTCGTCAC-3'
	<i>FTR1</i> -F	5'-ATTGTTGTTTCAGTGCTTTTGGC-3'
	<i>FTR1</i> -R	5'-GGTCGGAACACCACCCATAGA-3'
	<i>PGA13</i> -F	5'-CTCCGAAGAAGAAGGAAGCGT-3'
	<i>PGA13</i> -R	5'-GGTGAAATCAGTGACGGTGACTT-3'
	<i>PGA31</i> -F	5'-CTTTCCTTTGATGGTTCCGACT-3'
	<i>PGA31</i> -R	5'-GGGTCATTGATGTTCTTGGA-3'
Mutant construction	<i>CAS5</i> -A	5'-TTCAACTTCAAGGTACCTGTTGATATTCTA-3'
	<i>CAS5</i> -B	5'-CTAACGGTCTCGAGAAATTTGTGATAGTT-3'
	<i>CAS5</i> -C	5'-ATTGTGGTAACCGCGGTAATCGTAAAGATA-3'
	<i>CAS5</i> -D	5'-GCATATTTAGGAGCTCCTAGTGGTGATTTA-3'
	<i>CAS5</i> -E	5'-GCATACTGCATATTTAGCTCGAGCTAGTGG-3'
	<i>RPN4</i> -A	5'-AGATCAATATAGGGCCCTAAAGGACATCTT-3'
	<i>RPN4</i> -B	5'-GTGTATTATTGGCTCGAGTAAAAGTGTTGT-3'
	<i>RPN4</i> -C	5'-TAGATTTAATCCGCGGAGCTAAAGAGAATG-3'
	<i>RPN4</i> -D	5'-CTGGTGAAGAGCTCTAAAGTGATATTATTG-3'
	<i>RPN4</i> -E	5'-CTGGTGAAGAACTCGAGAGTGATATTATTG-3'
	<i>CZF1</i> -A	5'-GCAAAATTCAAAGGGCCCGACCTAATTC-3'
	<i>CZF1</i> -B	5'-GTGGTTGTGCTCGAGGAGAGGTATTGG-3'
	<i>CZF1</i> -C	5'-CAATTGAAGCGGCCGCGTTTGGTAGAATC-3'
	<i>CZF1</i> -D	5'-GTCAACTTTTGCCGCGGCACACACGTAAA-3'
	<i>MKC1</i> -A	5'-TTTGATAATAAAAAGGGGCCCTTGAGAA-3'
	<i>MKC1</i> -B	5'-GATAAATTCCTGGCTCGAGACCTTATTTAC-3'
	<i>MKC1</i> -C	5'-AAGAGTATATAAAGCCCGCGGAAGAGCTTG-3'
	<i>MKC1</i> -D	5'-AACAAGACTGAGCTCAACAAAAGAAATGTA-3'
	<i>MKC1</i> -E	5'-AACAAGACTGAACTCGAGAAAAGAAATGTA-3'

Underlined sequence reflects the introduction of a restriction site sequence.

YPD plates with and without 200 µg/ml of nourseothricin. Upon induction of the *FLP* gene, the cassette was excised such that only one copy of the *FRT* site remains in the locus. Another round was required to disrupt the second allele (34).

For gene complementation, the target gene's open reading frame (ORF) $\pm$ ~1kb was cloned in place of the target gene's 5' flanking sequence in the aforementioned disruption cassette. Upon transformation into the SC5314 strain, one allele of the gene's coding region was inserted into the original locus in conjunction with the *SAT1*-flipper, and such positive transformants (Nou^R) were selected on YPD-nourseothricin agar plates containing 200 µg/ml of nourseothricin. Induction of the *FLP* gene occurred by growing the transformants in YPD medium for 24 hours without selective pressure. Positive cells (Nou^S) were selected on by replica plated onto YPD plates with and without 200 µg/ml of nourseothricin. Upon induction of the *FLP* gene, the cassette is excised such that only the gene's allele with a downstream *FRT* was left in the locus. Appropriate gene disruption and complementation was confirmed by Southern hybridization (34).

Isolation of Genomic DNA and Southern Hybridization

Genomic DNA was isolated as described previously (217). Four µg of DNA were digested with an appropriate restriction endonuclease, separated on a 1% agarose gel, and after staining with ethidium bromide, were transferred by vacuum blotting onto a nylon membrane and fixed by UV cross-linking. Southern hybridization with enhanced chemiluminescence-labeled probes was performed with the Amersham ECL Direct nucleic acid labeling and detection system according to the instructions of the manufacturer.

RNA Isolation for qRT-PCR

RNA was isolated using a small-scale hot phenol method of RNA isolation described by Schmitt et al (218). Briefly, overnight cultures were diluted to an OD₆₀₀ of 0.2 in 20 ml YPD and then incubated at 30°C with shaking for 3 hours. Cells were collected by centrifugation, and stored at -80°C. Cell pellets were resuspended in 950 µl of AE buffer and then transferred to a 2-ml RNase-free microcentrifuge tube containing 950 µl acid phenol (pH 4.3) with 1% SDS. Cells were incubated at 65°C for 10 minutes then lysates were clarified by centrifugation. The supernatant was then divided into two new 2-ml microcentrifuge tubes containing 950 µl of chloroform and mixed. The sample was then subjected to centrifugation again, and the top aqueous layer was transferred to a new tube containing 1 ml of isopropanol and 100 µl 2M sodium acetate. The RNA pellet was subsequently washed with 500 µl of 70% ethanol and collected by centrifugation. The RNA pellet was resuspended in DNase/RNase-free H₂O. Quantity and purity were determined spectrophotometrically at absorbances of A₂₆₀ and A₂₈₀.

Quantitative RT-PCR

First-strand cDNAs were synthesized from 1 µg of total RNA using SuperScript first-strand synthesis system for qRT-PCR (Invitrogen). Gene-specific primers (**Table 3-2**) were designed using Primer Express software (Applied Biosystems) and synthesized by Integrated DNA Technologies (Coralville, IA). Quantitative PCRs were performed in triplicate using the 7000 sequence detection system (Applied Biosystems), independently amplifying *ACT1* (normalizing gene) and the genes of interest (GOI) as described previously (219).

RNA Isolation for Microarray

RNA was isolated using a large-scale version of the hot phenol method of RNA isolation described by Schmitt et al. (218). Briefly, overnight cultures were diluted to an OD₆₀₀ of 0.005 in 100 ml YPD and then incubated at 30°C with shaking for an additional 8 hours to an OD₆₀₀ of 1.0. Cultures were diluted again to an OD₆₀₀ of 0.025 in 100 ml fresh YPD, allowed to incubate at 30°C with shaking for one doubling, inoculated with or without 10 µg/ml fluconazole, and then incubated at 30°C with shaking for 6 hours. Cells were collected by centrifugation, and stored at -80°C. Cell pellets were resuspended in 12 ml of AE buffer and then transferred to a 50-ml Oak Ridge tubes treated with RNase Away (Molecular BioProducts) containing 12 ml acid phenol (pH 4.3) with 1% SDS. Cells were incubated at 65°C for 10 minutes then lysates were clarified by centrifugation. The supernatant was then transferred to a new tube containing 15 ml of chloroform and mixed. The sample was then subjected to centrifugation again, and the top aqueous layer was transferred to a new tube containing one volume of isopropanol and 0.1 volume 2M sodium acetate. The RNA pellet was subsequently washed with 10 ml of 70% ethanol and collected by centrifugation. The RNA pellet was resuspended in DNase/RNase-free H₂O. Quantity and purity were determined spectrophotometrically at absorbances of A₂₆₀ and A₂₈₀.

Transcriptional Profiling

Gene expression profiles were obtained by hybridizing labeled cRNAs generated from *C. albicans* total RNA onto Affymetrix *C. albicans* custom expression arrays (CAN07; 49-5241) (216), which have been described previously (201). Microarray hybridization and analysis were performed as described previously (201). Genes were considered to be differentially expressed in response to drug if their expression changed by ≥ 1.5 -fold in two independent experiments. Genes induced by fluconazole were considered to be *CAS5*-dependent if the induction was abrogated in the deletion mutant was ≥ 2.0 -fold (50%) less than the wild-type. All microarray data is available for download from the NCBI at *GEO* (Accession number: pending).

RESULTS

Disruption of *Cas5* Enhances Fluconazole Activity against *C. albicans*

In order to identify transcriptional activation programs that influence azole susceptibility of *C. albicans* to the azole antifungals, we first screened a collection of TF deletion mutants for those that displayed a increase in fluconazole susceptibility as measured by broth microdilution in RPMI medium (187). In order to detect marked reductions in MIC, a lower range of fluconazole concentrations from 0.0071825 to 4 µg/ml were used, and 19 TFs were identified from the library with reductions in fluconazole MIC in RPMI medium. These hits were then rescreened for those that also displayed a reduction in fluconazole MFC in both RPMI and YPD media (**Table B-1**). We identified four TF mutants that met the criteria: *CAS5*, *RPN4*, *UPC2* and *CZF1* (**Table 3-3**). The observation that disruption of *UPC2* increased susceptibility to fluconazole was not surprising as this has been reported previously (199, 200), validating the results of our screen. The remaining three TF deletion mutants which exhibited a reduction in MFC in both RPMI and YPD were prioritized as being of greatest interest and independent mutants were generated in the SC5314 background (**Table 3-4**). In order to determine the extent to which disruption of these TF genes impacts fluconazole susceptibility, the deletion mutants were subjected to various susceptibility tests using the nutrient rich YPD media in order to detect strong phenotypes despite existing in an environment that promotes growth.

CAS5 and *RPN4* disruption had a moderate impact on fluconazole MIC, and a marked impact on MFC by broth microdilution (**Table 3-4**) and Etest (**Figure 3-1A**), while *CZF1* had no impact by all methods. The fluconazole MFC at 24 hours in YPD for SC5314 was >64 µg/ml whereas the *cas5*Δ/Δ, *rpn4*Δ/Δ, and *czf1*Δ/Δ mutants were 1, 16, and >64 µg/ml respectively (**Table 3-4**), indicating that *CZF1* could not be independently confirmed as influencing susceptibility to fluconazole and therefore did not proceed through any further examination. For SC5314, the 48 hour MIC by Etest was 1.0 µg/ml and a halo of reduced growth was observed up to the Etest strip consistent with the fungistatic nature of fluconazole. At 48 hours, the MICs by Etest for the *cas5*Δ/Δ and *rpn4*Δ/Δ mutant were 0.25 µg/ml and 1.5 µg/ml respectively. A large clear zone of inhibition was observed for *cas5*Δ/Δ but was smaller for *rpn4*Δ/Δ (**Figure 3-1A**). Using a 72 hour endpoint for a broth microdilution assay in YPD as a way to assess the ability of the organism to resume growth in the presence of fluconazole we found that SC5314 and *rpn4*Δ/Δ were able to resume growth in all concentrations of fluconazole tested whereas the *cas5*Δ/Δ mutant only grew at the lowest concentrations (**Figure 3-1B**). When plated on YPD agar plates containing 10 µg/ml of fluconazole, growth was reduced for the *cas5*Δ/Δ mutant, but only slightly for *rpn4*Δ/Δ, as compared to SC5314 (**Figure 3-1C**). Time-kill analysis showed increased fungistatic activity for 10 µg/ml fluconazole against the *cas5*Δ/Δ mutant, but not *rpn4*Δ/Δ, as compared to their parent strain (**Figure 3-1D**). *RPN4* was not pursued in further analysis due to its minimal impact at the therapeutically relevant concentration of fluconazole, 10 µg/ml. All phenotypes were reverted by reintegration of one allele of the disrupted gene.

Table 3-3. Library hits with reduced MICs and MFCs in both RPMI and YPD

Clone ID	CGD	orf19 Designation	MIC (µg/ml)		MFC (µg/ml)	
			RPMI	YPD	RPMI	YPD
WT	--	--	0.25	0.5	>4	>64
TF3	<i>RPN4</i>	orf19.1069	0.0625	0.25	0.125	32
TF33	<i>CAS5</i>	orf19.4670	0.125	0.25	4	2
TF77	<i>UPC2</i>	orf19.391	0.03125	0.125	1	1
TF104	<i>CZF1</i>	orf19.3127	0.0625	0.5	0.25	8

Table 3-4. MICs and MFCs in YPD in SC5314 background

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>CAS5/CAS5 CZF1/CZF1</i> <i>MKC1/MKC1 RPN4/RPN4</i>	0.5	0.5	>64	>64	>64	>64
<i>cas5Δ/Δ</i>	<i>cas5Δ::FRT/cas5Δ::FRT</i>	0.5	1	1	1	1	1
<i>cas5Δ/Δ+CAS5</i>	<i>cas5Δ::FRT/CAS5-caSAT1</i>	0.5	0.5	>64	>64	>64	>64
<i>rpn4Δ/Δ</i>	<i>rpn4Δ::FRT/rpn4Δ::FRT</i>	0.5	1	>64	8	8	8
<i>rpn4Δ/Δ+RPN4</i>	<i>rpn4Δ::FRT/RPN4-caSAT1</i>	0.5	0.5	>64	>64	>64	>64
<i>czf1Δ/Δ</i>	<i>czf1Δ::FRT/czf1Δ::FRT</i>	0.5	0.5	>64	>64	>64	>64
<i>mkc1Δ/Δ</i>	<i>mkc1Δ::FRT/mkc1Δ::FRT</i>	0.5	0.5	1	1	1	1
<i>mkc1Δ/Δ+MKC1</i>	<i>mkc1Δ::FRT/MKC1-caSAT1</i>	0.5	0.5	>64	>64	>64	>64

Figure 3-1. Effects of *CAS5* and *RPN4* on fluconazole susceptibility in SC5314 background

(A) Effect on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) MIC heat map of SC5314, mutants, and complimented derivative. Susceptibility was determined by broth microdilution in YPD at 72 hours. Growth was quantified spectrophotometrically and assigned to a colorimetric scale. (C) Effect on the ability to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 µl aliquots were spotted onto YPD agar +/- 10 µg/mL FLC and incubated for 48 hours. (D) Effect of fluconazole by time-kill assay. SC5314, *cas5*Δ/Δ, or *rpn4*Δ/Δ cells were diluted in YPD medium containing FLC (10 µg/mL) or the solvent, DMSO. After 0, 6, 12, and 24 hours, samples from each were diluted and plated for colony forming units.

MKC1* Disruption Does Not Fully Phenocopy *CAS5

The cell wall integrity (CWI) pathway has been previously shown to be critical for the activity of sterol biosynthesis inhibitors and is specific to fungal cells (103-105). This pathway is conserved in *C. albicans* from *Saccharomyces cerevisiae* and in *C. albicans* the transcription factor Cas5p is postulated to be the functional equivalent of the *S. cerevisiae* CWI transcriptional regulator Rlm1 (134, 239). Looking upstream of Rlm1 in the CWI pathway is Slt2, a stress-activated kinase phosphorylated by Pkc1p. The functional equivalent of this protein in *C. albicans* is Mkc1 and is required for the maintenance of cell wall integrity as well as the azole stress response in *C. albicans* (103, 240, 241). In order to determine if deletion of *MKC1* in SC5314 phenocopies deletion of *CAS5*, we similarly tested an *mkc1*Δ/Δ mutant for susceptibility to fluconazole.

Disruption of *MKC1* had no change in fluconazole MIC at 24 or 48 hours, but a marked impact on MFC by broth microdilution (**Table 3-4**), and MIC by Etest (**Figure 3-2A**). The fluconazole MFC at 24 hours in YPD for SC5314 was >64 μg/ml whereas the *mkc1*Δ/Δ mutant was 1 μg/ml (**Table 3-4**). At 48 hours, the MIC by Etest for SC5314 was 0.5 μg/ml and a halo of reduced growth was observed up to the Etest strip consistent with the fungistatic nature of fluconazole. Meanwhile, the *mkc1*Δ/Δ mutant had an MIC by Etest of 0.25 μg/ml and a large clear zone of inhibition was observed (**Figure 3-2A**). We also used a 72 hour endpoint for a broth microdilution assay as a way to assess the ability of the organism to regrow in the presence of fluconazole. SC5314 and *mkc1*Δ/Δ+*MKC1* were able to resume growth in all concentrations of fluconazole tested whereas the *mkc1*Δ/Δ mutant only grew at the lowest concentrations (**Figure 3-2B**). When plated on YPD agar plates containing 10 μg/ml fluconazole, growth was reduced in the presence of fluconazole for the *mkc1*Δ/Δ mutant as compared to SC5314 (**Figure 3-2C**). Time-kill analysis showed slightly increased activity of 10 μg/ml fluconazole against the *mkc1*Δ/Δ mutant as compared to the parent strain, but some regrowth was observed after 24 hours (**Figure 3-2D**). All phenotypes were reverted by reintegration of one allele of the disrupted gene.

Disruption of *Cas5* Does Not Override Combinations of Clinical Drug Resistance Mechanisms

In order to examine the extent at which Cas5p is involved, we constructed *cas5*Δ/Δ mutant strains in the background of an azole-resistant clinical isolate (12-99) known to carry four of the most common mechanisms of azole resistance: overexpression of *CDR1* and *CDR2*, overexpression of *MDR1*, overexpression of *ERG11*, and a mutation in *ERG11* (242). The disruption of *CAS5* in isolate 12-99 did not result in a reduction in MIC or MFC, and therefore was not able to overcome the combined mechanisms of resistance operative in this isolate (**Table 3-5**). At 48 hours, the MIC by Etest for 12-99 was >256 μg/ml and confluent growth was observed up to the Etest strip consistent with the resistance of 12-99 to fluconazole, and the same was observed for 12-99*cas5*Δ/Δ, although this strain exhibited slower growth (data not shown). Time-kill analysis

Figure 3-2. *MKC1* effects fluconazole susceptibility in SC5314 background
(A) Effect of *MKC1* on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) MIC heat map of SC5314, mutant, and complimented derivative. Susceptibility was determined by broth microdilution in YPD at 72 hours. Growth was quantified spectrophotometrically and assigned to a colorimetric scale. (C) Effect of *MKC1* on the ability to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 μ l aliquots were spotted onto YPD agar +/- 10 μ g/mL FLC and incubated for 48 hours. (D) Effect of fluconazole on *MKC1* in time-kill assay. SC5314 or *mkc1* Δ/Δ cells were diluted in YPD medium containing FLC (10 μ g/mL) or the solvent, DMSO. After 0, 6, 12, and 24 hours, samples from each were diluted and plated for colony forming units.

Table 3-5. MICs and MFCs in YPD in background of clinical resistant strains

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>CAS5/CAS5</i>	0.5	0.5	>64	>64	>64	>64
2-79	Susceptible isolate	1	1	>64	>64	>64	>64
12-99	Resistant isolate	>64	>64	>64	>64	>64	>64
12-99 <i>cas5</i> Δ/Δ	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	>64	>64	>64	>64	>64	>64
G2	Susceptible isolate	1	>64	>64	>64	>64	>64
G5	Resistant isolate	>64	>64	>64	>64	>64	>64
G5 <i>cas5</i> Δ/Δ	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	32	32	32	>64	>64	>64
Gu2	Susceptible isolate	0.5	1	>64	>64	>64	>64
Gu5	Resistant isolate	>64	>64	>64	>64	>64	>64
Gu5 <i>cas5</i> Δ/Δ	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	>64	>64	>64	>64	>64	>64
S1	Susceptible isolate	2	>64	>64	>64	>64	>64
S2	Resistant isolate	>64	>64	>64	>64	>64	>64
S2 <i>cas5</i> Δ/Δ	<i>cas5</i> Δ::FRT/ <i>cas5</i> Δ::FRT	64	>64	>64	>64	>64	>64

revealed an overall growth defect but this did not alter the effect of fluconazole against 12-99*cas5*Δ/Δ at 10 μg/ml (**Figure 3-3**).

Isolate 12-99 simultaneously overexpresses *CDR1* and *CDR2* as well as *MDR1* (156). Since previous studies have shown that azole resistant isolates usually do not simultaneously overexpress these transporter genes (154, 167, 243), *CAS5* was also disrupted in the clinical isolates S2, G5, and Gu5. Isolate S2 has been previously shown to have a G464S amino acid substitution in Upc2p which renders it constitutively active, resulting in upregulation of *ERG11* and increased fluconazole resistance (195, 202). Isolate G5 has a G997V amino acid substitution in Mrr1p, resulting in increased *MDR1* expression and is also homozygous for an *ERG11* mutation (152, 195). Isolate Gu5 displays increased expression of *CDR1* and *CDR2* (153). As with isolate 12-99, the disruption of *CAS5* in these three clinical isolates did not markedly alter the MIC or MFC in these resistant backgrounds when examined by broth microdilution (**Table 3-5**). The only change observed was a 24 hour MIC of 32 μg/ml for G5*cas5*Δ/Δ reduced from >64 μg/ml by broth microdilution, and a 48 hour Etest MIC of 24 μg/ml reduced from >256 μg/ml (data not shown).

Disruption of *CAS5* in Strains Containing Resistance Mutations in *MRR1*, *TAC1*, and *ERG11* Impacts Fluconazole Susceptibility

Since the deletion of *CAS5* was unable to override the common resistance mechanisms acting together we sought to investigate the requirement for *CAS5* for specific mechanisms of resistance. Independent mutants were constructed in background strains containing two copies of a gene conferring reduced susceptibility to fluconazole: *MRR1*^{P683S}, *TAC1*^{G980E}, *UPC2*^{G648D} or *ERG11*^{K143R}. The *MRR1*^{P683S}, *TAC1*^{G980E} and *UPC2*^{G648D} alleles contain gain-of-function mutations which render the transcription factors they encode constitutively active, resulting in upregulation of *MDR1*, *CDR1* and *CDR2*, and *ERG11* respectively, and decreased fluconazole susceptibility (195, 196, 202, 220, 221). The *ERG11*^{K143R} allele contains a point mutation postulated to be located near the azole access channel, interfering with entry of fluconazole, resulting in decreased fluconazole susceptibility (222). Interestingly, we observed the *UPC2cas5*Δ/Δ mutant cells to be larger than those of the parent strain and also to be elongated and clumped together. As a result, susceptibility testing for this strain was inconsistent due to the extreme variability in optical density versus cell density, and therefore could not be reported. The disruption of *CAS5* in each remaining background did not markedly impact the MICs at 24 hours, but did result in a reduction in MIC at later time points by all methods. The MFCs at 48 hours in YPD for *ERG11cas5*Δ/Δ, *MRR1cas5*Δ/Δ and *TAC1cas5*Δ/Δ were all reduced from 8 μg/ml, >64 μg/ml, and >64 μg/ml in their background strains to 4 μg/ml, 8 μg/ml, and 16 μg/ml respectively (**Table 3-6**). At 48 hours the MICs by Etest for *ERG11cas5*Δ/Δ, *MRR1cas5*Δ/Δ and *TAC1cas5*Δ/Δ were all reduced from 1.5 μg/ml, 6 μg/ml, and 8 μg/ml in their background strains to 0.5 μg/ml, 1.5 μg/ml, and 6 μg/ml respectively (**Figure 3-4A**). When plated on YPD agar plates containing 10 μg/ml fluconazole, growth was slightly reduced in the presence of

Figure 3-3. Effect of fluconazole on *CAS5* in 12-99 by time-kill assay

12-99 or 12-99*cas5*Δ/Δ cells were diluted in YPD medium containing FLC (10 μg/mL) or the solvent, DMSO. After 0, 6, 12, and 24 hours, samples from each were diluted and plated for colony forming units.

Table 3-6. MICs and MFCs in YPD in background of strains expressing resistance mechanisms

Strain	Relevant Characteristics or Genotype	MIC (µg/ml)			MFC (µg/ml)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
SC5314	<i>CAS5/CAS5</i>	0.5	0.5	>64	>64	>64	>64
<i>ERG11</i> ^{K143R}	<i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT <i>CAS5/CAS5</i>	4	8	8	4	8	8
<i>ERG11cas5Δ/Δ</i>	<i>ERG11</i> ^{K143R} ::FRT/ <i>ERG11</i> ^{K143R} ::FRT <i>cas5Δ</i> ::FRT/ <i>cas5Δ</i> ::FRT	4	4	4	4	4	4
<i>MRR1</i> ^{P683S}	<i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT <i>CAS5/CAS5</i>	8	16	>64	>64	>64	>64
<i>MRR1cas5Δ/Δ</i>	<i>MRR1</i> ^{P683S} ::FRT/ <i>MRR1</i> ^{P683S} ::FRT <i>cas5Δ</i> ::FRT/ <i>cas5Δ</i> ::FRT	8	8	8	8	8	8
<i>TAC1</i> ^{G980E}	<i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT <i>CAS5/CAS5</i>	8	16	>64	>64	>64	>64
<i>TAC1cas5Δ/Δ</i>	<i>TAC1</i> ^{G980E} ::FRT/ <i>TAC1</i> ^{G980E} ::FRT <i>cas5Δ</i> ::FRT/ <i>cas5Δ</i> ::FRT	8	16	16	16	16	16

Figure 3-4. *CAS5* slightly impacts fluconazole susceptibility in background of strains expressing resistance mechanisms

(A) Effect of *CAS5* in isogenic resistant backgrounds on MIC and growth on YPD agar as determined by Etest. A confluent lawn of *C. albicans* was streaked prior to the addition of Etest strips and then incubated for 48 hours. (B) Effect of *CAS5* on the ability of isogenic strains to grow on solid media containing fluconazole. From 4-fold serial dilutions of *C. albicans* strains, 2 μ l aliquots were spotted onto YPD agar +/- 10 μ g/mL FLC and incubated for 48 hours.

fluconazole for the *cas5* Δ/Δ mutants compared to their respective backgrounds (**Figure 3-4B**).

Expression of *ERG11*, *CDR1*, *CDR2*, and *MDR1* When *CAS5* Is Disrupted in Resistant Backgrounds

One possible explanation for the enhanced fluconazole activity could be decreased expression of *ERG11* or genes encoding the Cdr1 and Cdr2 or Mdr1 efflux pumps. In order to determine if this was the case, we measured *ERG11*, *CDR1*, *CDR2*, and *MDR1* mRNA abundance by qRT-PCR in the strains containing a single resistance mechanism and their respective *cas5* Δ/Δ mutants (**Figure 3-5**). Interestingly, the *cas5* Δ/Δ mutant constructed in the SC5314 background showed a slight reduction in baseline *ERG11* expression. This was also the case for the *cas5* Δ/Δ mutants constructed in the *ERG11*^{K143R}, *MRR1*^{P683S}, *TAC1*^{G980E} and *UPC2*^{G648D} backgrounds. Disruption of *CAS5* did not result in decreased *CDR1* expression, except slightly in the *TAC1*^{G980E} background. Alternatively, disruption of *CAS5* increased *CDR2* expression in all backgrounds. Meanwhile *MDR1* expression also increased in the SC5314, *ERG11*^{K143R}, and *TAC1*^{G980E} backgrounds but decreased in the *MRR1*^{P683S} background. The significance of the increased expression of these transporter genes is unclear. These data suggest that the enhanced activity of fluconazole observed in resistant strains lacking *CAS5* is not due to decreased expression of transporters gene expression levels. Instead, the observed phenotypes may be associated with a reduction in *ERG* gene expression, particularly *ERG11*.

Comparison of the Gene Expression Profiles of Wild-Type Strain SC5314 and *cas5* Δ/Δ Exposed to Fluconazole

In order to identify genes whose expression in response to fluconazole is influenced by Cas5, we compared the transcriptional profiles of SC5314 and its *cas5* Δ/Δ derivative after treatment with or without 10 μ g/ml fluconazole for 6 hours. Genes were considered to be differentially expressed in response to fluconazole if their expression changed by ≥ 1.5 -fold in two independent experiments (**Table A-1** and **Table A-2**). Fluconazole inducible genes were also considered to be *CAS5*-dependent if their induction was abrogated in the deletion mutant and was ≥ 2.0 -fold (50%) less than compared to SC5314. Using these criteria, there were 209 genes upregulated by fluconazole whose induction was abrogated in the absence of *CAS5* (**Table 3-7**). The most common biological processes represented by these genes include transport, response to chemical stimulus, oxidation-reduction process, filamentous growth, lipid metabolic process and cell wall organization. Interestingly, there were also 6 genes involved in iron ion transport, binding, and homeostasis.

Figure 3-5. Expression of *CDR1*, *CDR2*, *ERG11*, and *MDR1* by qRT-PCR
 Expression levels for *ERG11*, *CDR1*, *CDR2*, and *MDR1* were measured in triplicate by qRT-PCR and compared to the expression levels in SC5314 in YPD. Error bars represent the standard error of the mean.

Table 3-7. Genes upregulated by at least 1.5-fold by fluconazole which are dependent upon Cas5

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>cas5</i> Δ/Δ+FLC/ <i>cas5</i> Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Lipid metabolic process	orf19.10	<i>ALK8</i>	9.0	8.1	3.0	3.5	0.3	0.4
	orf19.2248	<i>ARE2</i>	1.7	1.6	0.7	0.7	0.4	0.4
	orf19.2670		1.6	1.8	0.4	0.4	0.3	0.2
	orf19.2909	<i>ERG26</i>	2.4	1.7	0.9	0.9	0.4	0.5
	orf19.4897	<i>SFH5</i>	2.4	2.4	1.3	1.2	0.5	0.5
	orf19.4982		2.1	2.3	0.9	0.6	0.4	0.3
	orf19.5640	<i>PEX5</i>	2.2	2.6	0.9	0.9	0.4	0.3
	orf19.5751	<i>ORM1</i>	3.3	3.6	1.6	1.7	0.5	0.5
	orf19.6594	<i>PLB3</i>	2.1	3.4	1.1	0.9	0.5	0.3
Cell wall organization	orf19.212	<i>VPS28</i>	1.7	2.1	0.5	0.5	0.3	0.3
	orf19.5302	<i>PGA31</i>	17.6	4.1	2.1	1.5	0.1	0.4
	orf19.5644		2.3	3.5	1.1	1.0	0.5	0.3
	orf19.6102	<i>RCA1</i>	2.5	3.4	1.2	1.2	0.5	0.4
	orf19.6420	<i>PGA13</i>	15.6	30.6	2.4	2.3	0.2	0.1
	orf19.6481	<i>YPS7</i>	1.6	2.3	0.4	0.4	0.3	0.2
	orf19.719		1.8	3.3	1.0	1.0	0.5	0.3
Cellular membrane organization	orf19.3412	<i>ATG15</i>	2.0	2.6	0.7	1.0	0.4	0.4
	orf19.5644		2.3	3.5	1.1	1.0	0.5	0.3
	orf19.669	<i>PRM1</i>	2.2	2.8	0.6	0.4	0.3	0.1
Iron ion transport	orf19.1415	<i>FRE10</i>	2.0	4.2	0.9	2.3	0.5	0.5
	orf19.1932	<i>CFL4</i>	35.6	359	2.4	6.6	0.1	0.0

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/		cas5Δ/Δ+FLC/		Ratio (B/A)	
			SC5314 (A)		cas5Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.4215	<i>FET34</i>	2.1	5.2	0.2	0.5	0.1	0.1
	orf19.7219	<i>FTR1</i>	3.2	7.8	0.5	1.1	0.1	0.1
	Iron ion binding	orf19.1411		2.7	4.8	1.1	0.9	0.4
Iron ion homeostasis	orf19.1264	<i>CFL2</i>	2.0	6.0	0.6	0.5	0.3	0.1
Transport	orf19.111	<i>CAN2</i>	1.7	13.0	0.7	1.5	0.4	0.1
	orf19.1252	<i>YME1</i>	1.9	3.0	1.0	0.7	0.5	0.2
	orf19.1313	<i>CDR3</i>	1.9	2.1	0.9	1.0	0.5	0.5
	orf19.1352	<i>TIM22</i>	3.6	4.4	0.9	1.1	0.2	0.3
	orf19.1563	<i>ECM3</i>	1.5	1.5	0.4	0.6	0.3	0.4
	orf19.1867		2.6	4.2	0.7	0.6	0.3	0.2
	orf19.2073		1.5	1.6	0.6	0.5	0.4	0.3
	orf19.2292	<i>OPT4</i>	5.2	3.7	1.2	1.0	0.2	0.3
	orf19.2350		2.5	4.1	0.6	1.0	0.3	0.3
	orf19.2810	<i>AAP1</i>	18.9	19.6	7.5	8.8	0.4	0.4
	orf19.2946	<i>HNM4</i>	8.4	4.9	2.9	2.5	0.3	0.5
	orf19.3015	<i>ARX1</i>	2.7	3.9	1.3	1.7	0.5	0.4
	orf19.3195	<i>HIP1</i>	1.5	2.6	0.6	0.7	0.4	0.3
	orf19.3232		24.6	5.3	2.6	1.0	0.1	0.2
	orf19.3574	<i>MDJ2</i>	5.6	3.1	2.0	1.2	0.4	0.4
	orf19.4041	<i>PEX4</i>	4.9	4.3	1.3	1.5	0.3	0.4
	orf19.4090		2.1	1.7	1.1	0.8	0.5	0.5
	orf19.4372		1.8	3.0	0.6	0.6	0.3	0.2

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>cas5Δ</i> /Δ+FLC/ <i>cas5Δ</i> /Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.4384	<i>HXT5</i>	70.6	75.5	11.1	23.4	0.2	0.3
	orf19.4546	<i>HOL4</i>	4.3	5.1	1.7	2.3	0.4	0.4
	orf19.4682	<i>HGT17</i>	45	24.7	7.0	7.3	0.2	0.3
	orf19.4887	<i>ECM21</i>	2.7	3.1	0.8	1.0	0.3	0.3
	orf19.5539		1.6	1.6	0.7	0.8	0.5	0.5
	orf19.5902	<i>RAS2</i>	7.6	6.2	0.8	1.1	0.1	0.2
	orf19.5958	<i>CDR2</i>	2.1	2.5	0.9	1.0	0.4	0.4
	orf19.6117		1.5	2.3	0.6	0.4	0.4	0.2
	orf19.6249	<i>HAK1</i>	4.4	10.7	2.1	3.2	0.5	0.3
	orf19.6648	<i>SDA1</i>	3.2	6.8	1.6	3.2	0.5	0.4
	orf19.6993	<i>GAP2</i>	21.7	8.6	3.0	4.5	0.1	0.5
	orf19.7056		6.2	120.3	2.5	2.7	0.4	0.0
	orf19.7093	<i>HGT13</i>	30.7	13.2	2.0	1.5	0.1	0.1
	orf19.7094	<i>HGT12</i>	423.2	19.8	8.0	1.9	0.0	0.1
	Response to stress	orf19.1434		1.7	2.7	0.8	1.0	0.5
orf19.211			1.7	2.4	0.7	0.7	0.4	0.3
orf19.3239		<i>CTF18</i>	2.7	2.4	1.0	1.1	0.4	0.5
orf19.4383			2.8	2.6	0.9	0.8	0.3	0.3
orf19.5069			14.9	15.5	5.9	7.1	0.4	0.5
Response to chemical stimulus	orf19.2060	<i>SOD5</i>	33.2	14.2	5.4	6.1	0.2	0.4
	orf19.2838		2.5	2.9	0.8	0.7	0.3	0.3
	orf19.2876	<i>CBF1</i>	2.3	3.2	0.5	0.5	0.2	0.2
	orf19.3159	<i>UTP20</i>	3.8	8.9	1.9	3.5	0.5	0.4

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>cas5Δ</i> /Δ+FLC/ <i>cas5Δ</i> /Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Oxidation-reduction process	orf19.3736	<i>KAR4</i>	2.8	2.6	1.2	1.0	0.4	0.4
	orf19.4015	<i>CAG1</i>	3.1	3.6	0.8	0.7	0.3	0.2
	orf19.4317	<i>GRE3</i>	1.5	1.5	0.8	0.6	0.5	0.4
	orf19.4318	<i>MIG1</i>	1.7	2.1	0.7	1.0	0.4	0.5
	orf19.5326		1.5	1.9	0.6	0.7	0.4	0.4
	orf19.5591	<i>ADO1</i>	2.4	2.9	1.1	1.5	0.4	0.5
	orf19.6202	<i>RBT4</i>	3.3	6.2	1.6	2.2	0.5	0.3
	orf19.6881	<i>YTH1</i>	1.9	2.0	0.9	0.9	0.4	0.4
	orf19.7316		2.0	2.3	0.7	0.8	0.3	0.3
	orf19.7384	<i>NOG1</i>	3.7	7.2	1.2	2.1	0.3	0.3
	orf19.1117		5.7	2.3	0.7	1.1	0.1	0.5
	orf19.1473		35.0	15.0	3.8	4.8	0.1	0.3
	orf19.2108	<i>SOD6</i>	7.9	10.6	1.6	1.1	0.2	0.1
	orf19.3538	<i>FRE9</i>	4.7	14.9	2.5	3.6	0.5	0.2
	orf19.3707	<i>YHB1</i>	3.0	2.8	0.9	0.6	0.3	0.2
	orf19.4747	<i>HEM14</i>	1.7	2.0	0.6	0.4	0.3	0.2
	orf19.4871	<i>ERO1</i>	1.9	1.9	0.9	0.6	0.5	0.3
	orf19.5879		3.3	2.9	1.5	1.2	0.5	0.4
	orf19.638	<i>FDH1</i>	11.7	19.5	4.4	3.9	0.4	0.2
	orf19.6837	<i>FMA1</i>	1.5	1.5	0.7	0.7	0.5	0.4
	orf19.7111.1	<i>SOD3</i>	40.5	69.2	9.4	15	0.2	0.2
	orf19.7314	<i>CDG1</i>	7.5	6.0	1.0	0.6	0.1	0.1
	orf19.742	<i>ALD6</i>	11.5	10.4	4.1	1.7	0.4	0.2
	orf19.7551	<i>ALO1</i>	1.5	2.8	0.7	0.6	0.5	0.2

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression				Ratio (B/A)	
			SC5314+FLC/ SC5314 (A)		<i>cas5</i> Δ/Δ+FLC/ <i>cas5</i> Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Filamentous growth	orf19.2397.3		2.0	4.3	0.9	1.2	0.5	0.3
	orf19.4055		1.9	2.5	0.9	0.7	0.5	0.3
	orf19.4815	<i>YTM1</i>	2.3	3.2	0.6	1.4	0.3	0.4
	orf19.4928	<i>SEC2</i>	2.0	2.1	0.4	0.4	0.2	0.2
	orf19.5741	<i>ALS1</i>	66.8	79.0	22.6	21	0.3	0.3
	orf19.5798	<i>LIG4</i>	1.8	2.6	0.9	0.8	0.5	0.3
	orf19.6595	<i>RTA4</i>	10.4	15.0	3.0	2.9	0.3	0.2
	orf19.6888		4.3	5.7	1.4	1.1	0.3	0.2
	orf19.7313	<i>SSU1</i>	4.5	4.8	1.3	1.1	0.3	0.2
	orf19.7374	<i>CTA4</i>	1.7	2.2	0.8	1.1	0.5	0.5
	orf19.7436	<i>AAF1</i>	2.0	4.4	1.0	1.2	0.5	0.3
	orf19.795	<i>VPS36</i>	2.0	2.5	0.6	0.6	0.3	0.3
Biofilm formation	orf19.6090		1.9	2.4	0.6	0.6	0.3	0.2
Pathogenesis	orf19.5542	<i>SAP6</i>	5.9	4.3	2.1	1.4	0.4	0.3
	orf19.5585	<i>SAP5</i>	3.7	3.0	0.9	1.0	0.2	0.3
	orf19.5716	<i>SAP4</i>	4.8	3.2	2.1	1.5	0.4	0.5
Organelle organization	orf19.3135		2.3	2.6	1.2	1.1	0.5	0.4
	orf19.4862.2	<i>PET100</i>	1.7	1.8	0.8	0.9	0.5	0.5
	orf19.6225.1		1.9	1.9	0.9	0.7	0.5	0.4
RNA metabolic process	orf19.1915	<i>MPP10</i>	3.8	4.5	2.1	2.5	0.5	0.5
	orf19.1923	<i>RRN3</i>	5.7	23.0	2.9	3.8	0.5	0.2

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Biological process	orf19.2115	<i>HAS1</i>	1.9	2.8	0.9	1.5	0.5	0.5
	orf19.2137		1.9	2.3	0.6	0.8	0.3	0.4
	orf19.3962		1.7	2.3	0.7	0.8	0.4	0.4
	orf19.501		2.4	3.3	1.0	1.4	0.4	0.4
	orf19.5614	<i>RPA135</i>	7.9	7.7	3.9	4.1	0.5	0.5
	orf19.7062		2.0	5.6	0.9	1.6	0.5	0.3
	orf19.2064	<i>PRB1</i>	1.9	3.0	0.9	0.8	0.5	0.3
	orf19.2242		1.7	1.5	0.7	0.6	0.4	0.4
	orf19.2710		2.6	1.8	1.0	0.9	0.4	0.5
	orf19.3547		5.3	5.9	1.1	1.7	0.2	0.3
	orf19.467	<i>WOR3</i>	32.3	7.1	3.0	2.9	0.1	0.4
	orf19.5045	<i>PTP2</i>	6.0	10.4	2.5	2.0	0.4	0.2
	orf19.6225	<i>PCL7</i>	1.5	2.0	0.8	0.8	0.5	0.4
Cellular protein modification	orf19.5236		1.7	2.2	0.5	0.4	0.3	0.2
	orf19.540		1.7	1.7	0.9	0.6	0.5	0.4
	orf19.7547		2.9	3.0	1.1	1.4	0.4	0.5
Carbohydrate metabolic process	orf19.4899	<i>GCA1</i>	4.8	4.4	0.9	1.2	0.2	0.3
	orf19.7434	<i>GLG2</i>	37.5	16.1	7.5	8.7	0.2	0.5
	orf19.7514	<i>PCK1</i>	6.0	8.6	1.9	1.4	0.3	0.2
Other	orf19.1369	<i>POT1-2</i>	2.9	2.3	1.2	1.0	0.4	0.4
	orf19.2046		1.5	2.2	0.5	0.4	0.3	0.2

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/		<i>cas5Δ</i> /Δ+FLC/		Ratio (B/A)	
			SC5314 (A)		<i>cas5Δ</i> /Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Unknown	orf19.278		2.5	2.7	1.4	1.5	0.5	0.5
	orf19.410.3	<i>RIB4</i>	5.6	5.7	0.6	0.5	0.1	0.1
	orf19.4551	<i>CTN1</i>	22.4	26.6	11.3	14.3	0.5	0.5
	orf19.5159	<i>DUG3</i>	1.8	3.6	0.9	0.7	0.5	0.2
	orf19.5206		5.2	4.0	1.4	1.7	0.3	0.4
	orf19.5454	<i>DAL1</i>	3.7	6.6	1.7	1.9	0.5	0.3
	orf19.6057		2.5	1.8	0.9	1.0	0.3	0.5
	orf19.6997	<i>ATO5</i>	2.5	2.6	0.8	0.7	0.3	0.3
	orf19.972		1.9	2.9	0.8	0.6	0.4	0.2
	orf19.1277		6.6	9.3	1.5	1.5	0.2	0.2
	orf19.1766		26.6	340.0	3.3	5.7	0.1	0.0
	orf19.1800		4.1	3.8	2.1	1.8	0.5	0.5
	orf19.1939		2.3	1.6	0.6	0.8	0.3	0.5
	orf19.2059		2.6	2.3	0.2	0.3	0.1	0.1
	orf19.2210		2.3	4.3	1.0	1.9	0.4	0.5
	orf19.2398		2.5	3.8	1.3	1.7	0.5	0.5
	orf19.2414		3.1	3.3	1.1	0.6	0.4	0.2
	orf19.2691		1.5	1.5	0.5	0.7	0.3	0.4
	orf19.2812		1.8	1.8	0.8	1.0	0.4	0.5
	orf19.2836		11.7	21.9	3.1	4.8	0.3	0.2
	orf19.2839	<i>CIRT4B</i>	1.6	2.6	0.4	0.3	0.2	0.1
	orf19.2878	<i>PGA15</i>	6.0	8.1	3.1	2.4	0.5	0.3
	orf19.296		3.1	1.7	1.5	0.9	0.5	0.5
	orf19.2962		2.3	2.2	0.7	0.8	0.3	0.4

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/ SC5314 (A)		<i>cas5Δ</i> /Δ+FLC/ <i>cas5Δ</i> /Δ (B)		Ratio (B/A)	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.3004		1.5	2.0	0.6	0.6	0.4	0.3
	orf19.3021		2.6	2.0	1.2	0.9	0.5	0.5
	orf19.3245		2.2	2.3	1.2	1.1	0.5	0.5
	orf19.3302		1.5	2.1	0.7	0.8	0.5	0.4
	orf19.3309		1.5	1.7	0.8	0.6	0.5	0.3
	orf19.3310		1.8	1.9	0.8	0.6	0.4	0.3
	orf19.3499		79.5	20.5	5.9	7.6	0.1	0.4
	orf19.3512	<i>CSP1</i>	2.6	2.8	0.9	1.2	0.4	0.4
	orf19.3621		3.2	5.2	0.7	0.7	0.2	0.1
	orf19.376		2.9	3.4	1.1	1.5	0.4	0.4
	orf19.3881		1.9	2.0	0.7	0.6	0.4	0.3
	orf19.3910		3.1	2.6	1.1	0.9	0.4	0.3
	orf19.4013		2.6	2.2	0.6	0.6	0.2	0.3
	orf19.4014		1.9	1.6	0.8	0.8	0.4	0.5
	orf19.4144		2.4	3.2	1.2	1.6	0.5	0.5
	orf19.4214		30.5	19.5	0.5	1.2	0.0	0.1
	orf19.4264		2.5	3.2	1.2	1.0	0.5	0.3
	orf19.4286		1.9	2.9	0.3	0.4	0.2	0.1
	orf19.4404	<i>PGA49</i>	7.3	1.6	1.1	0.8	0.2	0.5
	orf19.4450.1		3.6	4.5	1.0	0.7	0.3	0.2
	orf19.4539		1.8	1.7	0.9	0.5	0.5	0.3
	orf19.4569		1.8	2.0	0.8	0.8	0.4	0.4
	orf19.4596		3.5	4.9	1.0	1.1	0.3	0.2
	orf19.4607		5.6	7.0	0.5	0.3	0.1	0.0
	orf19.4608	<i>PDC12</i>	7.1	16.7	0.5	0.3	0.1	0.0

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/		<i>cas5Δ</i> /Δ+FLC/		Ratio (B/A)	
			SC5314 (A)		<i>cas5Δ</i> /Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.4658		5.0	6.6	2.5	3.1	0.5	0.5
	orf19.4691		16.6	24.8	4.2	3.4	0.3	0.1
	orf19.4872		8.2	19.2	1.2	1.3	0.1	0.1
	orf19.5019		2.8	3.4	1.4	1.8	0.5	0.5
	orf19.5282		2.4	2.7	0.5	0.4	0.2	0.2
	orf19.5626		3.6	4.0	1.5	1.4	0.4	0.3
	orf19.6084		123.7	19.7	11.8	9.8	0.1	0.5
	orf19.6186		12.1	13.3	2.4	2.0	0.2	0.1
	orf19.6248		3.1	12.1	0.7	1.2	0.2	0.1
	orf19.633		1.5	1.6	0.8	0.5	0.5	0.3
	orf19.6353		1.8	2.0	0.7	0.6	0.4	0.3
	orf19.6484		19.5	30.0	2.2	4.3	0.1	0.1
	orf19.6527		1.5	1.8	0.8	0.7	0.5	0.4
	orf19.6678		3.1	5.1	1.2	1.4	0.4	0.3
	orf19.6690		2.4	2.3	0.7	0.6	0.3	0.3
	orf19.670.2		47.4	152.7	10.8	16.1	0.2	0.1
	orf19.6766	<i>NOP13</i>	5.1	5.3	1.7	2.6	0.3	0.5
	orf19.6905		2.4	3.3	0.5	0.4	0.2	0.1
	orf19.6970		4.5	4.4	2.3	2.4	0.5	0.5
	orf19.6983		2.1	9.1	1.0	1.3	0.5	0.1
	orf19.7011		2.2	4.7	1.1	2.3	0.5	0.5
	orf19.7043		3.9	2.8	0.9	1.0	0.2	0.4
	orf19.7229	<i>IML2</i>	2.8	3.3	1.4	1.4	0.5	0.4
	orf19.7550	<i>IFA14</i>	2.3	1.7	1.0	0.7	0.4	0.4
	orf19.7567		2.5	2.4	1.1	0.8	0.4	0.3

Table 3-7. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression					
			SC5314+FLC/		cas5Δ/Δ+FLC/		Ratio (B/A)	
			SC5314 (A)		cas5Δ/Δ (B)			
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.893	<i>PGA17</i>	12.3	5.5	1.4	1.1	0.1	0.2
	orf19.951		2.3	2.4	0.4	0.4	0.2	0.2
	orf19.994		2.0	2.7	0.7	1.3	0.4	0.5

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).

Validation of Microarray Data by Real Time RT-PCR

In order to validate the differential expression of genes identified by microarray, we examined the mRNA abundance for 5 genes of interest using the same RNA isolated for the microarray experiments. *PGA13* was chosen because it is involved in cell wall organization and was also identified by Bruno et al. to be Cas5-dependent (134). In addition, four others were chosen based on their involvement in cell wall organization (*PGA31*), iron ion transport (*CFL4*, *FTR1*) and lipid metabolic process (*ERG26*). The correlation between the microarray data and the real time RT-PCR was good (**Figure 3-6**). The expressions of *CFL4*, *ERG26*, *FTR1*, *PGA13*, and *PGA31* were upregulated in the wild type SC5314 when treated with fluconazole, but could not respond to the same extent when *CAS5* was disrupted.

DISCUSSION

Identifying transcriptional networks that are central to azole antifungal susceptibility would be instructive for developing co-therapeutic strategies that would enhance their activity against *C. albicans*. Several signal transduction and transcriptional activation programs have been identified to respond to antifungal stress, but the transcription factors and their respective target genes have yet to be identified for many of these processes (98-102, 105, 236, 237). In the present study, we screened a library of *C. albicans* mutants disrupted for genes encoding TFs in order to identify those which exhibit increased susceptibility to fluconazole. We observed that disruption of *CAS5* resulted in a slightly enhanced MIC, but also resulted in a substantial reduction in fluconazole MFC at 24, 48, and 72 hours. Indeed disruption of *CAS5* in an azole susceptible strain prevented its regrowth in YPD medium in the presence of higher fluconazole concentrations after 72 hours, resulted in a clear zone of inhibition around a fluconazole Etest strip, and prevented growth on solid media containing a therapeutically relevant concentration of fluconazole (10 µg/ml). Time-kill analysis also demonstrated an enhanced effect of 10 µg/ml fluconazole against the *cas5Δ/Δ* mutant as compared to its parent strain. The disruption of *RPN4* did not display as strong of an effect, suggesting that *RPN4* influences fungistatic azole activity to a lesser extent, while *CZF1* disruption had no impact on susceptibility. As previously observed for disruption of *UPC2* (Chapter 2), *CAS5* disruption strongly impacts fluconazole susceptibility.

We then sought to determine if disruption of *MKC1*, involved in the CWI pathway, would have the same impact on fluconazole susceptibility as seen upon disruption of *CAS5*, as Cas5 has been postulated to be the transcriptional regulator of the CWI pathway (134). We observed that disruption of *MKC1* did have an effect on fluconazole susceptibility, but not as pronounced as was observed for disruption *CAS5*. Disruption of *MKC1* did result in a clear zone of inhibition around a fluconazole Etest strip, prevented its regrowth in YPD medium in the presence of higher fluconazole concentrations after 72 hours, and prevented growth on solid media containing a therapeutically relevant concentration of fluconazole (10 µg/ml). Time-kill analysis also demonstrated a slightly enhanced effect of 10 µg/ml fluconazole against the *mkc1Δ/Δ*

Figure 3-6. Validation of fluconazole-inducible and Cas5-dependent gene expression by qRT-PCR

Expression levels for *CFL4*, *ERG26*, *FTR1*, *PGA13* and *PGA31* were measured in triplicate by qRT-PCR and compared to the expression levels in SC5314 in YPD. Shown are the relative *n*-fold changes in gene expression in the in SC5314 and *cas5*Δ/Δ treated with fluconazole (FLC). Error bars represent the standard error of the mean.

mutant compared to its parent strain, but was still able to grow above its starting inoculum. This suggests that *MKC1* plays a smaller role in fluconazole susceptibility and Cas5p may represent a different or redundant pathway.

Our observations for *MKC1* are similar to those of others. Epp et al. observed a reduction in fluconazole MIC from 2 µg/ml to 1 µg/ml at 24 hours, >128 µg/ml to 2 µg/ml at 48 hours, but after 72 hours both its parent and the *mkc1*Δ/Δ mutant had an MIC of >128 µg/ml. This trend of regrowth after extended time periods was also seen when plated on YPD plates containing 2 µg/ml fluconazole. After 2 days (48 hours) the *mkc1*Δ/Δ mutant was unable to grow in the presence of fluconazole compared to the parent strain, but after 6 days the mutant showed signs of growth (244). LaFayette et al. also observed a decrease in ergosterol biosynthesis inhibitor MICs for *mkc1*Δ/Δ but its 48 hour MFC was comparable to wild type (106). Taken together, these findings suggest a partial role for Mkc1p in the susceptibility of *C. albicans* to the azoles.

Upon disruption of *CAS5* in fluconazole resistant clinical isolates, 12-99, G5, Gu5, and S2, which carry combinations of the four most common mechanisms of resistance, there was no marked change in susceptibility, except slightly in the G5 background. The disruption of *CAS5* in isogenic strains containing specific resistance mutations in *ERG11*, *MRR1*, or *TAC1*, resulted in slightly increased susceptibility. However, the strain containing the *UPC2*^{G648D} GOF mutation rendered irregular cell shape and size. This phenotype was also present when *CAS5* was disrupted from strains constructed to contain *UPC2*^{G648S} or *UPC2*^{A643V} alleles. This may suggest a relationship between the two TFs. When *UPC2* is constitutively active, ergosterol biosynthesis genes are upregulated and cellular ergosterol content increases (205), consequently impacting membrane fluidity which affects cell wall composition (245). *CAS5* is the putative transcriptional regulator of the cell wall integrity pathway (134), and upon its disruption in the presence of a *UPC2* GOF mutation, it is possible the cell cannot effectively regulate the morphology of its cell wall and membrane. Also, the similarity of Cas5p- and Upc2p-dependent genes (discussed below) suggests cross talk between the two transcription factors. Studies to examine such a relationship may uncover other currently unknown roles for each TF.

The strain containing the *ERG11*^{K143R} mutation had an MIC of 4 µg/ml at 24 hours, and 8 µg/ml at 48 and 72 hours in YPD. Although this background was not as highly resistant as others, its respective *CAS5* deletion mutant had a drop in both fluconazole MIC and MFC at 48 and 72 hours. The *MRR1*^{P683S} and *TAC1*^{G980E} resistant strains both had MICs of 8 µg/ml at 24 hours, 16 µg/ml at 48 hours and >64 µg/ml at 72 hours, and MFCs of >64 µg/ml at all time points in YPD medium. These values dropped moderately in the respective *cas5*Δ/Δ mutants. A halo of reduced confluent growth was observed around the Etest strip for the *MRR1*^{P683S} and *TAC1*^{G980E} constructed mutants, but not the *ERG11*^{K143R} background. Fitness defects have been shown to be associated with the introduction of resistance mutations and may explain the growth of *ERG11*^{K143R} when examined by Etest (225). Despite the absence of reduced confluent growth, the *cas*Δ/Δ mutants in each background showed increased susceptibility to fluconazole by Etest and a decreased ability to grow in the presence of fluconazole at 10 µg/ml.

Although the loss of *CAS5* in strains containing a single mechanism of resistance resulted in slightly enhanced susceptibility to fluconazole, this modest effect is not practical for clinical applications in overcoming resistance. The differences in susceptibility observed for the mutants constructed in the azole-resistant clinical isolates versus the isogenic strains may be due to the suggestion that clinical isolates have compensatory mutations mitigating any decrease in fitness resulting from such resistance mutations (225).

We then sought to determine if *CAS5* might influence fluconazole susceptibility through altered expression of the efflux pump genes *CDR1*, *CDR2*, and *MDR1*. The expression of *CDR1* was not affected in the absence of *CAS5*, except for slightly in the *TAC1*^{G980E} background. However, *CDR2* and *MDR1* expression was upregulated in most backgrounds, except for *MRR1*^{P683S} where *MDR1* expression was downregulated. The change in susceptibility for the *MRR1*^{P683S} background may be due to decreased *MDR1* expression, but *CDR2* expression is upregulated in this background. Therefore, the moderate effect on susceptibility observed upon disruption of *CAS5* does not appear to be due to altered expression of the efflux pumps.

Another hypothesis for the increased susceptibility to fluconazole of the *cas5Δ/Δ* mutants is changes in cell wall structure. Previous reports have shown that when the cell wall integrity pathway is disrupted, specifically by disrupting the protein kinase gene *MKC1*, the cell exhibits increased susceptibility to fluconazole (105, 237). In response to fluconazole, the upstream kinase Pkc1p phosphorylates Mkc1p, but is interrupted by its absence. Cas5p has been postulated to be the functional equivalent of the *Saccharomyces cerevisiae* cell wall integrity downstream transcriptional regulator ScRlm1p (134). As previous reports have suggested that cell wall integrity is critical for the fungistatic effect of sterol biosynthesis inhibitors (246), this implicates that when *CAS5* is disrupted the integrity of the cell wall is compromised and results in increased susceptibility. Indeed, expression of cellular cell wall integrity genes measured by microarray analysis was reduced in the *cas5Δ/Δ* mutant as compared to its parent strain SC5314 upon exposure to fluconazole. *PGA13*, *PGA31*, *RCA1*, *VPS7*, *VPS28*, orf19.5644, and orf19.719 genes are all involved in cell wall integrity. Interestingly, the gene ontology process categories of iron ion transport and iron ion homeostasis were also found to be dependent upon *CAS5*. This is consistent with the involvement of cell wall proteins in iron acquisition (247).

Previous studies by our group also identified a similar set of iron ion transport and iron ion homeostasis genes to be dependent upon *UPC2* (Chapter 2) (**Table 3-8**), suggesting crosstalk between the two transcription factors. Indeed upon disruption of *CAS5*, the baseline expression of *ERG11* was reduced in all resistant backgrounds as measured by real-time RT-PCR. Additionally, several genes involved in the lipid metabolic process were also identified to be Cas5-dependent, including *ERG26* of the ergosterol biosynthesis pathway, a process that is regulated by Upc2 (199, 221). *ERG26* encodes for C-4 sterol decarboxylase and has been found to be an essential gene in *C. albicans* (248). Therefore, inhibition of this step may represent a potential drug target.

These data suggest that the enhanced activity of fluconazole observed in both susceptible and resistant strains lacking *CAS5* may be due to deregulation of iron ion

Table 3-8. Genes upregulated by at least 1.5-fold by fluconazole which are dependent upon Cas5 and Upc2

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression								CAS5 Ratio (B/A)		UPC2 Ratio C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)							
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2				
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2				
Lipid metabolic process	orf19.2670		1.6	1.8	0.4	0.4	0.4	0.7	0.3	0.2	0.2	0.4		
	orf19.4982		2.1	2.3	0.9	0.6	0.7	0.8	0.4	0.3	0.3	0.4		
Iron ion transport	orf19.1415	<i>FRE10</i>	2.0	4.2	0.9	2.3	0.2	0.1	0.5	0.5	0.1	0.0		
	orf19.1932	<i>CFL4</i>	35.6	359.0	2.4	6.6	8.0	22.0	0.1	0.0	0.2	0.1		
	orf19.4215	<i>FET34</i>	2.1	5.2	0.2	0.5	0.0	0.1	0.1	0.1	0.0	0.0		
	orf19.7219	<i>FTR1</i>	3.2	7.8	0.5	1.1	0.1	0.0	0.1	0.1	0.0	0.0		
Iron ion homeostasis	orf19.1264	<i>CFL2</i>	2.0	6.0	0.6	0.5	0.2	0.8	0.3	0.1	0.1	0.1		
Transport	orf19.1352	<i>TIM22</i>	3.6	4.4	0.9	1.1	1.3	2.0	0.2	0.3	0.4	0.4		
	orf19.2292	<i>OPT4</i>	5.2	3.7	1.1	1.0	0.0	0.0	0.2	0.3	0.0	0.0		
	orf19.2350		2.5	4.1	0.6	1.0	0.9	0.6	0.3	0.3	0.4	0.1		
	orf19.3195	<i>HIP1</i>	1.5	2.6	0.6	0.7	0.7	0.8	0.4	0.3	0.5	0.3		
	orf19.3232		24.6	5.3	2.6	1.0	1.6	1.6	0.1	0.2	0.1	0.3		
	orf19.4384	<i>HXT5</i>	70.6	75.5	11.1	23.4	16.9	9.7	0.2	0.3	0.2	0.1		
	orf19.4682	<i>HGT17</i>	45.0	24.7	7.0	7.3	4.2	4.5	0.2	0.3	0.1	0.2		
	orf19.6249	<i>HAK1</i>	4.4	10.7	2.1	3.2	2.0	2.6	0.5	0.3	0.5	0.2		
	orf19.6993	<i>GAP2</i>	21.7	8.6	3.0	4.5	3.6	2.5	0.1	0.5	0.2	0.3		
Response to stress	orf19.1434		1.7	2.7	0.8	1.0	0.8	0.9	0.5	0.4	0.5	0.3		
	orf19.3239	<i>CTF18</i>	2.7	2.4	1.0	1.1	1.4	0.8	0.4	0.5	0.5	0.4		
	orf19.4317	<i>GRE3</i>	1.5	1.5	0.8	0.6	0.3	0.7	0.5	0.4	0.2	0.5		
	orf19.5902	<i>RAS2</i>	7.6	6.2	0.8	1.1	2.3	1.5	0.1	0.2	0.3	0.2		
Response to chemical stimulus	orf19.5591	<i>ADO1</i>	2.4	2.9	1.1	1.5	0.7	0.8	0.4	0.5	0.3	0.3		
	orf19.6102	<i>RCA1</i>	2.5	3.4	1.2	1.2	1.1	1.4	0.5	0.4	0.5	0.4		

Table 3-8. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.7374	CTA4	1.7	2.2	0.8	1.1	0.8	1.0	0.5	0.5	0.5	0.5
Oxidation-reduction process	orf19.1411		2.7	4.8	1.1	0.9	1.4	1.1	0.4	0.2	0.5	0.2
	orf19.2108	SOD6	7.9	10.6	1.6	1.1	1.1	1.6	0.2	0.1	0.1	0.1
	orf19.4747	HEM14	1.7	2.0	0.6	0.4	0.1	0.2	0.3	0.2	0.0	0.1
Filamentous growth	orf19.5741	ALSI	66.8	79.0	22.6	21.0	4.8	3.1	0.3	0.3	0.1	0.0
Biofilm formation	orf19.4899	GCA1	4.8	4.4	0.9	1.2	0.2	0.6	0.2	0.3	0.0	0.1
Other	orf19.410.3	RIB4	5.6	5.7	0.6	0.5	1.0	0.8	0.1	0.1	0.2	0.1
	orf19.467	WOR3	32.3	7.1	3.0	2.9	2.8	0.8	0.1	0.4	0.1	0.1
	orf19.6057		2.5	1.8	0.9	1.0	0.2	0.2	0.3	0.5	0.1	0.1
Unknown	orf19.1277		6.6	9.3	1.5	1.5	3.4	2.7	0.2	0.2	0.5	0.3
	orf19.2414		3.1	3.3	1.1	0.6	0.7	1.7	0.4	0.2	0.2	0.5
	orf19.2691		1.5	1.5	0.5	0.7	0.4	0.7	0.3	0.4	0.3	0.5
	orf19.2836		11.7	21.9	3.1	4.8	1.6	1.2	0.3	0.2	0.1	0.1
	orf19.2839	CIRT4B	1.6	2.6	0.4	0.3	0.7	0.6	0.2	0.1	0.4	0.2
	orf19.3499		79.5	20.5	5.9	7.6	10.6	10.1	0.1	0.4	0.1	0.5
	orf19.3881		1.9	2.0	0.7	0.6	0.5	0.5	0.4	0.3	0.2	0.2
	orf19.4013		2.6	2.2	0.6	0.6	0.3	0.4	0.2	0.3	0.1	0.2
	orf19.4144		2.4	3.2	1.2	1.6	0.9	0.9	0.5	0.5	0.4	0.3
	orf19.4658		5.0	6.6	2.5	3.1	2.4	1.1	0.5	0.5	0.5	0.2
	orf19.5019		2.8	3.4	1.4	1.8	0.9	1.3	0.5	0.5	0.3	0.4
	orf19.6186		12.1	13.3	2.4	2.0	2.8	2.7	0.2	0.1	0.2	0.2
	orf19.6690		2.4	2.3	0.7	0.6	0.6	0.7	0.3	0.3	0.3	0.3
	orf19.670.2		47.4	152.7	10.8	16.1	18.3	14.1	0.2	0.1	0.4	0.1

Table 3-8. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2		
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2		
	orf19.6905		2.4	3.3	0.5	0.4	1.2	1.4	0.2	0.1	0.5	0.4
	orf19.6970		4.5	4.4	2.3	2.4	2.0	1.8	0.5	0.5	0.4	0.4
	orf19.6983		2.1	9.1	1.0	1.3	0.4	0.0	0.5	0.1	0.2	0.0
	orf19.7567		2.5	2.4	1.1	0.8	0.2	0.5	0.4	0.3	0.1	0.2
	orf19.951		2.3	2.4	0.4	0.4	0.8	1.2	0.2	0.2	0.3	0.5

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).

transport and *ERG26* expression, in addition to the inability to upregulate genes involved in the cell wall integrity pathway. Delineation of which downstream targets influence azole susceptibility would identify potential targets for co-therapeutic strategies rendering enhanced activity to the azoles, and as a result may mitigate the development of resistance.

CHAPTER 4. DISCUSSION

Candida albicans is the most prevalent human fungal pathogen and causes a wide spectrum of diseases (65). Despite this there are few fundamentally different classes of clinical antifungals available for treatment, due to limited unique and important fungal targets which are not found in the host (61). Currently available antifungal drugs act primarily on the fungal cell wall and ergosterol found in the cell membrane. Perturbation of either of these structures or their components impacts the fungal cell's survival. Fungal cell walls display an electron-dense fibrillar outer layer composed of mannoproteins, and an inner layer of glucans and chitin conferring elasticity and tensile strength to the cell wall (62, 63). Ergosterol is found in the fungal cell membrane and regulates fluidity and ultimately membrane integrity (64). Both are exploitable drug targets as the cell wall is essential for survival, and human cells lack both cell walls and ergosterol.

The azole antifungals (such as fluconazole) are the most widely used and important ergosterol biosynthesis inhibitors (EBIs) for the treatment of *Candida* infections (65). This class of antifungals acts by inhibiting the product of *ERG11*, a critical cytochrome P450 enzyme of the ergosterol biosynthesis pathway, known as lanosterol 14 α -demethylase. Inhibition of this enzyme results in accumulation of sterol intermediates and altered cell membrane structure and function (79, 82). Despite their overall success in treatment, the azoles are fungistatic against *C. albicans* and therefore have limited efficacy against this organism, especially for immunocompromised patients, and the development of resistance can occur (61, 85, 206). The lack of lethality caused by sterol biosynthesis inhibition may be due to adaptive responses which circumvent azole activity and permit fungal survival in the presence of the azoles. Indeed, various pathways have been shown to be critical for the fungistatic activity of azole antifungals.

We hypothesized that the transcriptional regulators of these pathways are required for the response to the azoles in both susceptible and resistant strains. Identification of their downstream targets would be useful as novel targets for the development of cotherapeutic strategies to improve the efficacy of fluconazole treatment. By studying mutants of specific transcription factors we were able to identify those whose disruption results in enhanced susceptibility in both susceptible and resistant strains. Using *C. albicans* Affymetrix microarrays we were able to examine the genome-wide expression profile of these mutants and their background strains for fluconazole-inducible genes whose expressions are TF-dependent.

Liu et al. recently examined the genome-wide expression profile in response to ketoconazole exposure and observed an induction of genes involved in ergosterol biosynthesis (97). Many of these genes exist upstream from Erg11p, the azole target, and respond to ergosterol depletion by facilitating the production of alternate sterols including lanosterol, eburicol, obtusifolliol, 14 α -methyl fecosterol, and 14 α -methylergosta-8,24(28)-dien-3 β ,6 α -diol to be inserted into the cell membrane (107) (**Figure 4-1**). Integration of these sterols into the plasma membrane disrupts its integrity resulting in its

Figure 4-1. An alternate sterol biosynthesis pathway in *C. albicans*

Alternate sterols produced in response to azole inhibition. Genes in boldface have been shown to be influenced by iron. Dotted lines denote multiple enzymatic steps (232, 234, 235).

altered structure and function (79, 82, 108). Under these conditions, growth is inhibited but the organism remains viable (109).

The transcriptional regulator of the ergosterol biosynthesis pathway is Upc2, controlling inducible ergosterol genes expression in response to the azoles. Upon disruption of *UPC2*, the cells become hypersusceptible to fluconazole, *ERG* gene inducible transcription is abolished, and as a result ergosterol levels are reduced (199, 200). Our first objective was to better characterize the role of *UPC2* in fluconazole susceptibility in both azole-susceptible and azole-resistant strains. In addition to previous observations of reduced fluconazole MIC for a *upc2Δ/Δ* mutant as compared to its azole-susceptible parent strain, we observed a reduced MFC when disrupted from the azole-susceptible parent SC5314 as well as the highly azole-resistant parent 12-99, which carries the four most common mechanisms of resistance. Disruption in both backgrounds prevented its regrowth after 72 hours in the presence of higher fluconazole concentrations in YPD medium, resulted in a clear zone of inhibition around a fluconazole Etest strip, and prevented growth on solid YPD medium containing a therapeutically relevant concentration of fluconazole (10 µg/ml). An enhanced effect for fluconazole was also demonstrated by time-kill analysis for the *upc2Δ/Δ* mutants as compared to their respective parent strains. The same phenotype was also observed upon disruption of *UPC2* in isogenic strains containing resistance mutations in *ERG11*, *MRR1*, and *TAC1*. The *upc2Δ/Δ* mutants in each background showed increased susceptibility to fluconazole by Etest and an inability to grow on solid media containing fluconazole.

C. albicans Upc2 has been shown to bind the promoters of *CDR1* and *MDR1* (201). However, the observed effect on fluconazole susceptibility occurred without any change in expression of the efflux pump genes *CDR1*, *CDR2*, or *MDR1* and therefore is not the cause for how the disruption of *UPC2* overcomes drug resistance. *UPC2* disruption did result in reduced *ERG11* expression compared to both azole-susceptible and azole-resistant parent strains as measured by real-time RT-PCR, and inducible expression of *ERG6*, *ERG24*, and *ERG27* (as well as *ERG11*) was reduced in the *upc2Δ/Δ* mutant as compared to its parent strain SC5314 upon exposure to fluconazole as measured by microarray analysis. Such changes in expression would diminish the intracellular drug target and as well as the biosynthesis of alternative sterols, compromising the organism in the presence of fluconazole.

Interestingly, genes involved in iron transport (*CFL4*, *FET3*, *FET34*, *FRE10*, *FRP1*, *FTR1*) and homeostasis (*CFL2*, *CSA1*, *IRO1*, *RBT5*) were also found to be dependent upon *UPC2* for their induction in response to fluconazole. Before being taken up by the cell, ferric iron must be solubilized to its ferrous form by a cell surface ferric reductase (*FRE1*, also known as *FRE10*), and when iron availability is low it is then taken up by the high-affinity iron uptake system including a membrane permease (*FTR1*) and a multicopper oxidase (*FET3*) (249-251) (**Figure 4-2**). Disruption of *FTR1* and iron starvation have previously been shown to enhance fluconazole susceptibility in *C. albicans* (228-231) and the iron uptake system has been proposed as an alternative target for combination antifungal treatment (233). Iron deprived cells also have decreased amounts of both ergosterol and sterol intermediates when compared to iron sufficient

Figure 4-2. High-affinity ion uptake in yeast

Extracellular ferric iron is reduced to ferrous iron by a ferric reductase (Fre1). A multicopper oxidase (Fet3) oxidizes the iron back to its ferric form which is then transported into the cell by an iron permease (Ftr1) (252).

cells (228, 253). This may be due to the inactivity of enzymes in the ergosterol biosynthesis pathways which require iron for their function. *ERG11* and *ERG5* encode cytochrome P450 enzymes and their active sites contain a heme iron. Also, the expressions *ERG11* and *ERG3* have been shown to be iron regulated, and while *ERG25* expression is not regulated by iron, it is an iron containing gene identified to be essential to viability (253-256). Since many enzymes in the ergosterol biosynthesis pathway require iron, reduced expression of iron transport and homeostasis genes would impact the activity of ergosterol biosynthesis enzymes in response to fluconazole. Therefore, the enhanced effect of fluconazole upon the disruption of *UPC2* may be due to this effect in combination with decreased expression of the *ERG* genes. Indeed, a *upc2Δ/Δ* mutant has been shown to be highly susceptible to Malachite green, a compound which depletes intracellular iron (257).

We then sought out to identify other transcription factors that had similar effects on fluconazole susceptibility as *UPC2* by screening a library of mutants disrupted for genes encoding transcription factors (187). Our results in Chapter 3 showed that the TF Cas5 was identified to be hypersusceptible to fluconazole, and independent disruption of *CAS5* from the azole-susceptible parent SC5314 also exhibited increased susceptibility to fluconazole. The fluconazole MIC was slightly reduced and a substantial reduction was observed for the fluconazole MFC. Disruption of *CAS5* prevented regrowth after 72 hours in the presence of higher fluconazole concentrations in YPD medium, resulted in a clear zone of inhibition around a fluconazole Etest strip, and prevented growth on solid YPD medium containing a therapeutically relevant concentration of fluconazole (10 µg/ml). An enhanced effect for fluconazole was also demonstrated by time-kill analysis as compared to the parent strain. This phenotype was not as strong when disrupted in isogenic strains containing resistance mutations in *ERG11*, *MRR1*, and *TAC1* and had no effect on azole-resistant clinical isolates. Of note, the *cas5Δ/Δ* mutant derived from a strain containing resistance mutations in *UPC2* resulted in altered cell shape and size, suggesting a relationship between the two transcription factors, but was unable to be quantified due to cell density irregularities. The *cas5Δ/Δ* mutants in the *ERG11*, *MRR1*, and *TAC1* backgrounds showed slightly increased susceptibility to fluconazole by Etest and reduced inability to grow on solid media containing fluconazole. The effect of *CAS5* on fluconazole susceptibility was not shown to be due to decreased expression of the *CDR1*, *CDR2*, and *MDR1* efflux pump genes. Although the resulting modest effect on resistance is not practical for clinical application, it is important to note the enhanced effect in the susceptible background which would improve efficacy of treatment. As a result, it may also impact the development of resistance.

The *cas5Δ/Δ* mutants in the *ERG11*, *MRR1*, and *TAC1* backgrounds did exhibit a decrease in *ERG11* expression, adding evidence to a possible relationship between Cas5p and Upc2, as inducible *ERG11* expression is controlled by Upc2. Additionally, inducible expression of several genes involved in the lipid metabolic process (including *ERG26*) were reduced in the *cas5Δ/Δ* mutant as compared to its parent strain SC5314 upon exposure to fluconazole as measured by microarray analysis. *ERG26* encodes for C-4 sterol decarboxylase which is an essential enzyme in *C. albicans* for viability (248). As previously mentioned, decreased expression *ERG11* would diminish the target of the

azoles and decreased *ERG26* expression would impact the biosynthesis of alternative sterols, compromising the ability of this response mechanism to circumvent the activity of fluconazole. Another striking similarity to *UPC2* was the dependence of iron ion transport (*CFL4*, *FET34*, *FRE10*, *FTRI*), binding (orf19.1411), and homeostasis (*CFL2*) genes on Cas5 by microarray analysis. Many of these genes were previously identified to also be dependent upon Upc2 (Chapter 2). The disruption of cell membrane integrity by fluconazole also impacts cell wall integrity (258). Since cell membrane and wall proteins such as Cfl2p, Cfl4p, Fet3p, Fet34p, Fre10p, and Ftr1p are involved in iron acquisition (247), it is reasonable for a relationship to exist in regulating iron acquisition and cell wall maintenance genes.

Cas5 has been postulated to be the transcriptional regulator of the CWI pathway (239), which is required to maintain cell shape and integrity (134). The specificity of the CWI pathway to fungal cells and its role in responding to fluconazole activity makes it an attractive system for which novel co-therapeutic agents could be designed to act synergistically with fluconazole (103, 104). When the *cas5Δ/Δ* mutant was compared to its parent strains by microarray analysis, several genes involved in cell wall organization were identified to dependent upon Cas5 for their induction in response to fluconazole. Included were the genes *PGA13*, *PGA31*, *RCA1*, *VPS28*, *YPS7*, orf19.5644, and orf19.719. Previous studies also identified that the expression of *PGA13* was dependent upon Cas5 in response to caspofungin (134). *PGA13* encodes a GPI protein which is highly expressed during cell wall regeneration (259), and its deletion results in slightly enhanced susceptibility to fluconazole and caspofungin (260). The deletion of *VPS28* has also been shown to result in hypersusceptibility to both the azole (fluconazole, voriconazole) and echinocandin antifungals (caspofungin, micafungin) (261). Since many of these genes encode proteins contributing to cell wall integrity, it suggests that when *CAS5* is disrupted the integrity of the cell wall is compromised. This, combined with decreased iron uptake, which occurs at the cell surface, may account for the observed increase in fluconazole susceptibility.

Ciclopirox olamine was introduced more than 3 decades ago as a topical antifungal agent with a very broad spectrum of activity (262). Although its mechanism of action is poorly understood, the chelation of metal ions and the inhibition of iron-dependent enzymes have been shown to play a role (263). Upon exposure to ciclopirox olamine, the expression of genes encoding drug efflux pumps (*CDR1*, *CDR2*), iron permease (*FTRI*), iron transporter (*FTH1*), and iron reductase (*CFL1*) were induced, while the addition of iron(III) chloride reversed this effect. The iron chelator bipyridine also induced a similar pattern of gene expression (262). Additionally, no cases of resistance have been reported to ciclopirox olamine. This may be due to its fungicidal mode of action or an irreversible binding of the drug to intracellular structures, preventing its export by efflux pumps (264). To examine if resistance could be developed in vitro, cells were grown in the presence of ciclopirox olamine for 6 months. Expression of *CDR1* and *CDR2* increased, but there was no decrease in susceptibility or development of resistance (262). A later study showed similar gene expression changes in response to ciclopirox olamine, but mutants disrupted for *CDR1* and *CDR2*, as well as

a clinically azole-resistant isolate overexpressing *CDR1* and *CDR2*, had no change in susceptibility to ciclopirox olamine (265).

Further emphasis for targeting the iron uptake system in combination with antifungal treatment was provided by the observation that doxycycline acts synergistically with fluconazole (266). Doxycycline is an antimicrobial with iron-chelating activity (267-270). The combination of doxycycline with fluconazole resulted in fungicidal activity, and prevented the development of fluconazole resistance. It was also effective against a highly fluconazole-resistant clinical isolate, FH5, with an isochromosome 5L duplication resulting in 4 copies of *ERG11* and 4 copies of constitutively active *TAC1* (271). These effects were reversed upon the addition of iron to the growth medium. High levels of doxycycline alone did impact growth of the susceptible strain SC5314, but had not impact on the azole-resistant isolate. Interestingly, growth of an *S. cerevisiae* strain with constitutive *ERG11* overexpression was able to resume in the presence of these two drugs. However, overexpression of *ERG11* in *C. albicans* did not restore growth (266). Since the Erg11 protein binds heme, and azoles interact with the heme iron (272), iron depletion could decrease the activity of both Erg11 and the azoles. Further study is necessary in order to determine the extent of iron's role in fluconazole susceptibility.

By studying transcription factor mutants in both azole-susceptible and azole-resistant backgrounds, we were able to identify the involvement of high-affinity transport pathway in the response to azoles, as well as expanding upon pathways previously identified. We successfully identified *UPC2* and *CAS5* in the regulation of iron ion transport, binding, and homeostasis in addition to the role of Upc2 as the regulator of ergosterol biosynthesis and Cas5 of cell wall integrity in the presence of fluconazole. Disruption of the transcription factor, Upc2p, was also shown to overcome high levels of azole resistance. Our results demonstrate the importance of using TF mutants for identifying transcriptional pathways functioning in both susceptible and resistant response mechanisms to fluconazole.

The discovery of the role of iron transport and homeostasis may allow for the development of more efficient combination therapy with fluconazole. The combination of iron deprivation and cell wall disruption may be important for preventing the development of azole resistance from the time of therapy initiation. Additionally, targeting both high-affinity iron transport and ergosterol biosynthesis may be able to overcome the existence of resistance as well as hinder its development.

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APPENDIX A. *UPC2* SUPPLEMENTAL DATA

Table A-1. Genes upregulated by at least 1.5-fold by fluconazole in SC5314

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7094	<i>HGT12</i>	carbohydrate transport	423.2	19.8	8.0	1.9	13.5	140.0
orf19.4335	<i>TNA1</i>	anion transport	194.9	29.8	2.5	42.4	0.4	0.2
orf19.6084			123.7	19.7	11.8	9.8	62.0	51.5
orf19.4527	<i>HGT1</i>	carbohydrate transport	115.9	120.9	98.6	141.2	59.1	88.7
orf19.4476		cellular aldehyde metabolic process	104.5	102.1	114.2	83.9	419.7	228.8
orf19.3499			79.5	20.5	5.9	7.6	10.6	10.1
orf19.3923	<i>PGA37</i>		73.2	6.3	7.7	22.1	91.4	58.4
orf19.6311			72.8	23.5	382.3	60.5	98.1	13.7
orf19.4384	<i>HXT5</i>	high-affinity hexose transport	70.6	75.5	11.1	23.4	16.9	9.7
orf19.5741	<i>ALS1</i>	cell adhesion	66.8	79.0	22.6	21.0	4.8	3.1
orf19.3908			51.0	14.6	5.4	9.0	512.3	140.7
orf19.3668	<i>HGT2</i>	carbohydrate transport	48.3	35.5	10.1	19.8	20.1	12.3
orf19.670.2			47.4	152.7	10.8	16.1	18.3	14.1
orf19.4682	<i>HGT17</i>	carbohydrate transport	45.0	24.7	7.0	7.3	4.2	4.5
orf19.1438		oxidation-reduction process	42.4	13.5	36.3	29.8	17.4	9.8
orf19.2809	<i>CTN3</i>	acetyl-CoA metabolic process	41.1	36.9	33.4	30.6	12.6	11.5
orf19.7093	<i>HGT13</i>	carbohydrate transport	40.9	18.8	5.9	11.3	9.8	2.6
orf19.7111.1	<i>SOD3</i>	response to oxidative stress	40.5	69.2	9.4	15.0	46.5	99.8
orf19.7434	<i>GLG2</i>	glycogen biosynthetic process	37.5	16.1	7.5	8.7	47.7	39.9
orf19.1932	<i>CFL4</i>	siderophore-iron transport	35.6	359.0	2.4	6.6	8.0	22.0
orf19.1473		oxidation-reduction process	35.0	15.0	3.8	4.8	30.4	41.0
orf19.2060	<i>SOD5</i>	cellular copper ion homeostasis	33.2	14.2	5.4	6.1	14.2	28.1
orf19.467			32.3	7.1	3.0	2.9	2.8	0.8

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6993	<i>GAP2</i>	gamma-aminobutyric acid transport	30.6	10.6	3.5	10.0	6.0	3.0
orf19.4214			30.5	19.5	0.5	1.2	0.3	12.5
orf19.6486	<i>LDG3</i>		29.6	3.1	8.1	4.2	15.7	11.4
orf19.4773	<i>AOX2</i>	aerobic respiration	29.5	24.9	15.4	14.1	122.1	50.4
orf19.2652	<i>TEF4</i>	translational elongation	27.8	15.2	15.3	25.5	9.5	8.5
orf19.1344			27.1	12.6	13.1	32.5	53.0	6.3
orf19.3232		anion transport	24.6	5.3	2.6	1.0	1.6	1.6
orf19.4551	<i>CTN1</i>	acetyl-CoA metabolic process	22.4	26.6	11.3	14.3	17.4	75.7
orf19.1655	<i>PXP2</i>	fatty acid beta-oxidation	22.1	31.9	19.5	19.9	95.6	41.4
orf19.3982		sucrose catabolic process	21.1	5.4	2.5	3.2	44.9	44.0
orf19.2023	<i>HGT7</i>	glucose transport	20.8	21.6	9.4	7.8	0.5	0.4
orf19.5753	<i>HGT10</i>	carbohydrate transport	20.6	1.6	2.9	2.9	2.6	0.8
orf19.6484			19.5	30.0	2.2	4.3	240.3	97.9
orf19.344			19.0	6.9	13.6	8.9	152.4	53.0
orf19.2810	<i>AAP1</i>	amino acid transport	18.9	19.6	7.5	8.8	32.9	13.3
orf19.6919			18.1	4.5	1.4	10.1	21.7	54.8
orf19.5302	<i>PGA31</i>	fungal-type cell wall organization	17.6	4.1	2.1	1.5	9.9	3.4
orf19.7197		DNA replication initiation	17.0	6.6	2.0	7.3	11.6	22.6
orf19.3924			16.8	4.1	8.5	4.7	3.2	3.2
orf19.4691			16.6	24.8	4.2	3.4	4.0	59.6
orf19.4690		manganese ion transport	16.3	20.2	7.8	23.4	1.0	1.4
orf19.6420	<i>PGA13</i>	filamentous growth	15.6	30.6	2.4	2.3	87.4	117.0
orf19.5069		meiotic DNA recombinase assembly	14.9	15.5	5.9	7.1	28.3	16.1
orf19.740	<i>HAP41</i>	regulation of transcription from RNA polymerase II promoter	14.3	10.7	12.7	23.5	14.3	2.5
orf19.5037	<i>HRQ2</i>		13.5	9.7	8.4	12.3	14.4	4.8
orf19.7566		amino acid transport	13.4	7.0	6.9	11.2	11.3	5.3
orf19.3627			12.6	5.5	10.1	10.8	26.6	13.5

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.893	<i>PGA17</i>		12.3	5.5	1.4	1.1	32.2	3.5
orf19.6186			12.1	13.3	2.4	2.0	2.8	2.7
orf19.1765			11.7	7.6	4.4	6.9	22.8	13.6
orf19.638	<i>FDH1</i>	formate metabolic process	11.7	19.5	4.4	3.9	9.0	29.2
orf19.742	<i>ALD6</i>	ethanol metabolic process	11.5	10.4	4.1	1.7	3.5	6.8
orf19.3986	<i>PPR1</i>	regulation of transcription, DNA-dependent	10.9	5.3	9.9	10.3	10.7	3.2
orf19.7296			10.6	11.5	11.8	8.6	55.8	13.6
orf19.2717	<i>SAS10</i>	endonucleolytic cleavage of rRNA	10.4	2.5	4.4	3.5	4.7	14.9
orf19.6595	<i>RTA4</i>	filamentous growth	10.4	15.0	3.0	2.9	17.8	34.3
orf19.1763	<i>IFR1</i>	oxidation-reduction process	10.3	11.8	15.9	7.9	53.4	50.8
orf19.5307	<i>JEN2</i>	lactate transport	10.3	2.7	2.0	3.1	0.4	1.3
orf19.7504			10.1	4.9	12.6	25.3	23.1	5.3
orf19.6793			10.0	7.7	8.6	15.5	118.0	36.1
orf19.3733	<i>IDP2</i>	glutamate biosynthetic process	9.7	14.9	10.8	12.9	12.6	18.2
orf19.1363		ascospore formation	9.5	29.7	11.0	10.3	70.4	1188.0
orf19.7585	<i>INO1</i>	inositol biosynthetic process	9.2	20.5	21.8	19.6	0.9	0.8
orf19.2049			9.1	5.6	8.5	5.2	59.6	21.2
orf19.10	<i>ALK8</i>	fatty acid omega-oxidation	9.0	8.1	3.0	3.5	133.9	183.6
orf19.5634	<i>FRP1</i>	high-affinity iron ion transport	8.9	8.2	7.5	7.3	0.1	0.2
orf19.1070			8.6	8.0	5.4	4.6	2.4	1.6
orf19.4802	<i>FTH1</i>	high-affinity iron ion transport	8.5	9.9	5.9	7.1	14.3	6.5
orf19.2946	<i>HNH4</i>	choline transport	8.4	4.9	2.9	2.5	18.4	2.9
orf19.6844	<i>ICL1</i>	glyoxylate cycle	8.2	8.2	17.3	23.3	17.4	50.8
orf19.4872			8.2	19.2	1.2	1.3	25.8	10.2
orf19.2108	<i>SOD6</i>	oxidation-reduction process	7.9	10.6	1.6	1.1	1.1	1.6
orf19.1345	<i>LIP8</i>	lipid catabolic process	7.9	6.2	1.3	4.3	107.5	3.9
orf19.5614		cell wall organization	7.9	7.7	3.9	4.1	11.8	6.5

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3740	<i>PGA23</i>	response to drug	7.9	6.7	9.2	9.1	29.1	10.1
orf19.4706			7.7	6.2	15.1	12.7	17.5	6.6
orf19.5902	<i>RAS2</i>	Ras protein signal transduction	7.6	6.2	0.8	1.1	2.3	1.5
orf19.7314	<i>CDG1</i>	L-cysteine metabolic process	7.5	6.0	1.0	0.6	1.7	5.0
orf19.4404	<i>PGA49</i>		7.3	1.6	1.1	0.8	15.4	4.7
orf19.4608	<i>PDC12</i>		7.1	16.7	0.5	0.3	14.1	12.6
orf19.4274	<i>PUT1</i>	glutamate biosynthetic process	6.8	8.8	15.1	21.1	2.5	0.6
orf19.94			6.8	6.6	17.5	25.8	12.3	6.2
orf19.1826	<i>MDM34</i>	regulation of transcription, DNA-dependent	6.8	9.3	7.3	13.6	21.5	5.5
orf19.1277			6.6	9.3	1.5	1.5	3.4	2.7
orf19.5447	<i>HGT19</i>	carbohydrate transport	6.6	8.1	7.8	18.5	9.8	3.9
orf19.6828		rRNA processing	6.5	3.5	1.5	2.8	2.5	3.9
orf19.2747	<i>RGT1</i>	filamentous growth	6.4	4.6	5.3	43.8	97.4	10.4
orf19.7056		amino acid transmembrane transport	6.2	120.3	2.5	2.7	94.2	9.2
orf19.7131		carnitine biosynthetic process	6.2	6.8	6.2	7.8	3.6	1.8
orf19.5959	<i>NOP14</i>	response to drug	6.1	4.0	5.3	4.6	9.0	7.7
orf19.3672	<i>GAL10</i>	fungal-type cell wall biogenesis	6.0	7.3	14.2	6.9	2.7	3.8
orf19.5045	<i>PTP2</i>	osmosensory signaling pathway	6.0	10.4	2.5	2.0	23.0	20.2
orf19.3615			6.0	5.4	8.2	8.1	12.8	10.9
orf19.2878	<i>PGA15</i>		6.0	8.1	3.1	2.4	7.7	6.7
orf19.7514	<i>PCK1</i>	gluconeogenesis	6.0	8.6	1.9	1.4	9.3	13.0
orf19.5542	<i>SAP6</i>	adhesion to host	5.9	4.3	2.1	1.4	9.5	7.9
orf19.3684		ascospore formation	5.9	2.1	11.2	11.3	9.1	15.4
orf19.2247			5.9	5.6	5.5	1.3	88.0	0.7
orf19.2515			5.9	9.0	6.7	7.1	13.4	7.9
orf19.2952	<i>EXG2</i>	cell wall organization	5.8	4.1	2.6	3.0	7.0	4.2
orf19.7077		iron ion transport	5.8	5.5	4.1	4.1	90.1	64.1

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4082	<i>DDR48</i>	hyphal growth	5.8	5.7	9.3	6.9	1.9	1.3
orf19.1117		NADH regeneration	5.7	2.3	0.7	1.1	1.3	2.0
orf19.1923	<i>RRN3</i>	transcription from RNA polymerase I promoter	5.7	23.0	2.9	3.8	13.4	3.5
orf19.1237	<i>ARO9</i>	aromatic amino acid family catabolic process to alcohol	5.6	9.5	6.9	5.0	18.6	12.6
orf19.1069	<i>RPN4</i>	proteasomal ubiquitin-dependent protein catabolic process	5.6	6.0	5.7	5.5	20.2	12.7
orf19.6920			5.6	1.9	7.3	6.0	31.0	20.1
orf19.410.3		riboflavin biosynthetic process	5.6	5.7	0.6	0.5	1.0	0.8
orf19.3574	<i>MDJ2</i>	protein import into mitochondrial matrix	5.6	3.1	2.0	1.2	2.1	3.6
orf19.4607			5.6	7.0	0.5	0.3	5.8	24.3
orf19.5000	<i>CYB2</i>	lactate metabolic process	5.5	3.3	3.3	3.3	4.2	6.2
orf19.6249	<i>HAK1</i>	potassium ion transport	5.4	5.8	2.7	3.8	1.4	1.7
orf19.449		metabolic process	5.4	5.6	5.2	5.9	3.4	1.6
orf19.3934	<i>CAR1</i>	arginine catabolic process to ornithine	5.4	3.2	7.8	10.6	2.5	2.5
orf19.3547		regulation of transcription, mating-type specific	5.3	5.9	1.1	1.7	2.7	3.8
orf19.918	<i>CDR11</i>	multidrug transport	5.3	11.0	4.1	3.1	9.8	10.1
orf19.7263		cellular process	5.3	6.0	5.0	4.5	2.6	2.2
orf19.5206		box H/ACA snoRNP assembly	5.2	4.0	1.4	1.7	4.7	6.2
orf19.2292	<i>OPT4</i>	oligopeptide transport	5.2	3.7	1.1	1.0	0.0	0.0
orf19.4273		aerobic respiration	5.1	7.6	3.9	5.1	1.4	2.0
orf19.3931	<i>SFC1</i>	fumarate transport	5.1	5.2	9.9	13.9	6.9	3.4
orf19.6766	<i>NOP13</i>		5.1	5.3	1.7	2.6	4.7	9.8
orf19.3434	<i>TRY5</i>	positive regulation of cell-substrate adhesion	5.0	7.3	6.8	7.5	6.9	3.1
orf19.4070			5.0	8.9	3.0	2.5	1.8	1.9
orf19.3670	<i>GAL1</i>	galactose catabolic process	5.0	8.2	12.9	7.4	4.7	2.7
orf19.5094	<i>BUL1</i>	protein polyubiquitination	5.0	5.7	3.2	3.0	4.9	5.3
orf19.4658			5.0	6.6	2.5	3.1	2.4	1.1
orf19.5353			4.9	8.8	10.4	14.0	4.5	0.8

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4041	<i>PEX4</i>	protein autoubiquitination	4.9	4.3	1.3	1.5	3.5	6.8
orf19.6886		maturation of rRNA	4.9	4.9	2.5	4.6	16.5	16.7
orf19.5635	<i>PGA7</i>		4.9	4.1	2.8	3.0	0.5	0.4
orf19.677	<i>CHO1</i>	filamentous growth	4.9	5.5	7.6	9.8	16.7	3.3
orf19.1377	<i>IPK2</i>	arginine metabolic process	4.9	3.4	4.6	7.4	16.4	13.3
orf19.7552		endonucleolytic cleavage of rRNA	4.9	4.6	2.9	5.5	6.2	7.3
orf19.5716	<i>SAP4</i>	adhesion to host	4.8	3.2	2.1	1.5	6.4	4.8
orf19.3538	<i>FRE9</i>	siderophore-iron transport	4.7	14.9	2.5	3.6	12.6	4.3
orf19.4711			4.7	4.8	3.6	3.3	13.7	6.0
orf19.6840			4.7	3.0	12.1	18.1	34.7	10.0
orf19.4056	<i>GAT2</i>	filamentous growth	4.6	5.1	2.7	3.9	2.9	0.6
orf19.7221	<i>SET3</i>	filamentous growth	4.6	2.9	7.6	15.7	2.1	1.4
orf19.6970			4.5	4.4	2.3	2.4	2.0	1.8
orf19.7313	<i>SSU1</i>	filamentous growth	4.5	4.8	1.3	1.1	6.8	4.0
orf19.5850	<i>NOC2</i>	filamentous growth	4.5	4.8	2.1	3.5	8.7	9.5
orf19.6148		microtubule nucleation	4.4	47.3	4.6	5.2	2.0	13.8
orf19.4899	<i>GCA1</i>	biofilm formation	4.4	5.2	3.7	3.2	1.2	1.9
orf19.5874			4.4	6.6	31.0	10.4	14.5	8.2
orf19.7503	<i>CDA2</i>	ascospore wall assembly	4.4	2.4	3.8	1.5	6.4	14.0
orf19.6306		carnitine biosynthetic process	4.4	2.7	4.7	5.0	7.1	3.6
orf19.3369	<i>MOH1</i>		4.3	8.1	6.4	5.9	18.6	13.3
orf19.3902			4.3	4.5	2.8	1.6	10.4	32.6
orf19.6888		filamentous growth	4.3	5.7	1.4	1.1	8.8	11.4
orf19.2542			4.3	2.1	2.2	3.3	0.5	3.7
orf19.4546	<i>HOL4</i>	drug transport	4.3	5.1	1.7	2.3	6.9	4.1
orf19.7500	<i>PXA1</i>	fatty acid transport	4.3	5.2	4.3	6.3	8.2	4.7
orf19.3121	<i>GST1</i>	regulation of nitrogen utilization	4.2	2.7	5.8	19.0	1.5	7.8

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1715	<i>IRO1</i>	cellular iron ion homeostasis	4.2	8.1	2.7	2.3	2.2	1.4
orf19.4128			4.2	3.4	2.0	3.4	2.0	2.1
orf19.6169	<i>ATO1</i>		4.2	4.3	2.4	1.8	24.8	33.3
orf19.5125			4.2	4.5	3.5	2.8	10.6	9.1
orf19.6254	<i>ANT1</i>	ATP transport	4.2	2.3	2.8	3.2	5.4	4.0
orf19.711			4.1	4.1	9.8	6.2	8.9	5.2
orf19.1350		cell redox homeostasis	4.1	4.2	6.6	5.8	25.2	19.6
orf19.1800		protein targeting to vacuole	4.1	3.8	2.1	1.8	6.4	4.0
orf19.499		translational readthrough	4.1	4.2	11.2	14.9	24.8	5.4
orf19.1609		endonucleolytic cleavage of rRNA	4.1	5.0	3.4	3.5	3.8	6.6
orf19.695	<i>RGS2</i>	termination of G-protein coupled receptor signaling pathway	4.1	5.8	3.3	3.4	5.8	5.2
orf19.4894			4.1	3.8	7.6	6.6	7.0	8.9
orf19.7457			4.1	2.4	4.6	4.4	11.2	12.7
orf19.76	<i>SPB1</i>	maturation of rRNA	4.1	5.3	2.8	4.0	3.9	7.7
orf19.5735.3			4.0	3.1	4.1	3.4	2.9	2.5
orf19.7306		pyridoxal biosynthetic process	4.0	3.9	5.1	5.9	11.6	15.6
orf19.2706	<i>CRH11</i>	cell wall organization	4.0	3.3	4.1	2.7	3.9	2.3
orf19.3483		phosphatidylglycerol catabolic process	4.0	2.4	2.0	2.0	5.1	8.4
orf19.7449		mitochondrial genome maintenance	3.9	2.1	2.0	3.0	1.9	2.6
orf19.5759	<i>SNQ2</i>	filamentous growth	3.9	6.9	5.4	8.0	4.2	2.0
orf19.5205			3.9	4.9	1.1	0.6	1.9	2.2
orf19.7043			3.9	2.8	0.9	1.0	2.4	2.1
orf19.1488			3.9	2.2	2.6	3.8	2.1	3.5
orf19.6554		calcium ion homeostasis	3.9	3.8	6.8	7.5	7.6	3.9
orf19.2724			3.9	4.1	8.5	16.0	4.8	3.2
orf19.720	<i>GST3</i>	regulation of nitrogen utilization	3.8	3.4	11.8	13.2	58.6	32.7
orf19.1915	<i>MPP10</i>	endonucleolytic cleavage of rRNA	3.8	4.5	2.1	2.5	11.3	6.2

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6555		protein import into mitochondrial intermembrane space	3.8	3.4	2.9	4.8	2.4	1.2
orf19.90			3.8	3.1	2.6	3.6	4.3	2.4
orf19.4873			3.8	13.5	14.6	7.1	57.4	24.8
orf19.1704	<i>FOX3</i>	fatty acid beta-oxidation	3.8	2.7	5.0	7.0	6.9	4.3
orf19.7260		oxidation-reduction process	3.8	3.5	2.8	3.3	2.3	2.8
orf19.6000	<i>CDR1</i>	multidrug transport	3.8	6.1	2.6	4.7	6.8	3.3
orf19.286		autophagy	3.8	2.2	1.4	1.8	2.7	3.7
orf19.4779		drug transport	3.8	4.7	2.8	3.4	9.7	8.8
orf19.3829	<i>PHR1</i>	cell wall organization	3.8	5.0	2.8	3.8	14.9	7.0
orf19.3159	<i>UTP20</i>	endonucleolytic cleavage of rRNA	3.8	8.9	1.9	3.5	5.4	2.7
orf19.5585	<i>SAP5</i>	pathogenesis	3.7	3.0	0.9	1.0	1.4	2.3
orf19.3077	<i>VID21</i>	histone acetylation	3.7	4.3	2.7	3.4	3.8	2.8
orf19.7156	<i>FAA2-3</i>	lipid metabolic process	3.7	2.8	1.7	2.2	14.4	8.4
orf19.5565		valine metabolic process	3.7	2.2	9.7	5.5	7.0	11.5
orf19.7384	<i>NOG1</i>	ribosomal large subunit biogenesis	3.7	7.2	1.2	2.1	13.9	5.4
orf19.5454	<i>DAL1</i>	allantoin assimilation pathway	3.7	6.6	1.7	1.9	11.1	3.9
orf19.7635	<i>DRS1</i>	response to drug	3.7	5.2	2.0	5.3	16.5	6.7
orf19.6838		fatty acid catabolic process	3.7	2.1	2.5	3.2	6.2	7.5
orf19.3418		arginine catabolic process to ornithine	3.7	2.2	3.0	2.3	11.4	7.6
orf19.7053	<i>GAC1</i>	meiosis	3.7	5.8	4.6	4.5	10.4	2.1
orf19.5248	<i>MSO1</i>	ascospore-type prospore membrane assembly	3.7	2.7	2.8	3.8	3.5	3.2
orf19.4445		filamentous growth	3.7	4.1	2.6	3.3	39.0	56.8
orf19.7422		rRNA modification	3.7	3.9	2.0	2.5	4.1	5.4
orf19.1944	<i>GPR1</i>	filamentous growth	3.7	4.6	2.5	5.5	10.5	3.2
orf19.1562			3.7	4.5	3.9	4.6	1.7	1.3
orf19.4743	<i>AFG1</i>		3.7	4.6	2.8	2.5	9.3	5.9
orf19.3435			3.6	4.2	4.3	6.0	8.1	3.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1352	<i>TIM22</i>	protein import into mitochondrial inner membrane	3.6	4.4	0.9	1.1	1.3	2.0
orf19.1523	<i>FMO1</i>	cellular response to drug	3.6	5.6	2.5	4.3	4.3	5.7
orf19.6606			3.6	2.7	3.4	10.0	2.6	2.9
orf19.2859	<i>SRP40</i>	nucleocytoplasmic transport	3.6	2.9	3.3	4.9	1.3	4.3
orf19.1728			3.6	2.3	43.7	33.3	15.7	5.0
orf19.4450.1			3.6	4.5	1.0	0.7	2.4	10.6
orf19.7042			3.6	2.3	9.4	7.6	5.8	4.7
orf19.5626			3.6	4.0	1.5	1.4	3.6	2.3
orf19.2492	<i>STE3</i>	cytogamy	3.6	3.4	2.7	3.8	3.3	1.6
orf19.3710	<i>YHB5</i>	response to stress	3.5	4.0	12.2	12.4	8.2	4.5
orf19.1727	<i>PMC1</i>	cellular calcium ion homeostasis	3.5	3.6	5.0	7.9	8.3	3.0
orf19.5019			3.5	3.7	1.7	2.1	0.9	1.9
orf19.7300			3.5	4.8	4.4	3.5	9.7	16.9
orf19.2400		nuclear mRNA splicing, via spliceosome	3.5	1.5	0.9	1.2	1.5	2.3
orf19.5962	<i>SNF3</i>	carbohydrate transport	3.5	3.0	4.7	7.1	5.9	2.1
orf19.4596			3.5	4.9	1.0	1.1	5.6	5.2
orf19.4505	<i>ADH3</i>	fermentation	3.5	1.6	5.8	2.4	2.0	1.4
orf19.4450	<i>ZCF23</i>	regulation of transcription, DNA-dependent	3.5	3.3	3.5	8.7	22.9	2.3
orf19.3261	<i>ATO9</i>		3.4	3.7	1.1	2.8	2.5	2.9
orf19.2836		carbon utilization	3.4	5.9	3.7	4.3	1.6	1.5
orf19.661	<i>KRR1</i>	endonucleolytic cleavage of rRNA	3.4	3.0	3.2	4.6	4.0	4.8
orf19.7424	<i>NSA2</i>	maturation of rRNA)	3.4	2.8	2.1	3.1	2.6	4.0
orf19.6109	<i>TUP1</i>	filamentous growth	3.4	3.2	3.1	3.1	4.2	2.1
orf19.2504	<i>BMS1</i>	ribosome assembly	3.4	4.1	2.1	3.2	2.8	3.2
orf19.3646	<i>CTR1</i>	high-affinity iron ion transport	3.4	9.1	3.1	4.9	10.9	10.4
orf19.3606			3.4	3.2	2.1	3.7	2.8	2.3
orf19.2832	<i>INN1</i>	chromosome organization	3.4	3.9	2.9	3.7	10.3	10.2

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7298	<i>CHS2</i>	cell wall chitin biosynthetic process	3.4	4.0	4.9	7.1	10.5	3.5
orf19.6589		regulation of cell size	3.4	4.5	3.8	6.6	1.6	3.7
orf19.1646		endonucleolytic cleavage of rRNA	3.4	3.1	1.8	2.8	2.8	5.4
orf19.1424		oxalate transport	3.4	4.7	2.5	3.2	4.7	2.5
orf19.3815		ubiquitin-dependent protein catabolic process	3.3	4.2	3.2	4.3	9.2	6.2
orf19.1481	<i>HAP42</i>	regulation of transcription, DNA-dependent	3.3	4.1	3.8	6.0	2.7	2.0
orf19.5604	<i>MDR1</i>	multidrug transport	3.3	1.7	9.7	4.0	4.4	1.1
orf19.6202	<i>RBT4</i>	pathogenesis	3.3	6.2	1.6	2.2	5.7	2.2
orf19.6725	<i>FUM12</i>	fumarate metabolic process	3.3	6.4	3.8	6.4	5.7	2.5
orf19.2067		intracellular sequestering of iron ion	3.3	3.3	2.4	2.8	4.0	4.8
orf19.4077	<i>MIT1</i>	sphingolipid biosynthetic process	3.3	5.0	3.1	5.2	5.7	2.3
orf19.431	<i>ZCF2</i>	regulation of transcription, DNA-dependent	3.3	4.1	2.7	3.7	4.8	2.2
orf19.246			3.3	3.3	5.6	6.6	23.7	8.2
orf19.6313.2	<i>MVB12</i>	cellular response to alkalinity	3.3	1.5	3.7	4.6	0.5	1.6
orf19.5118	<i>SDS24</i>	cell separation during cytokinesis	3.3	6.6	3.0	2.5	7.3	2.4
orf19.1449			3.3	2.6	5.5	8.4	15.9	39.3
orf19.5879		phosphatidic acid biosynthetic process	3.3	2.9	1.5	1.2	3.3	2.8
orf19.5751	<i>ORM1</i>	response to drug	3.3	3.6	1.6	1.7	3.4	1.8
orf19.2568	<i>IFU5</i>		3.3	3.4	4.6	4.0	6.0	2.9
orf19.192			3.3	3.8	3.7	3.7	12.2	3.9
orf19.5113	<i>ADH2</i>	NADH oxidation	3.3	9.7	11.4	16.0	23.0	10.1
orf19.5862	<i>AFP99</i>		3.3	2.0	2.1	2.7	13.0	10.1
orf19.5345		ER-associated protein catabolic process	3.2	3.5	2.6	2.7	4.9	3.8
orf19.6648	<i>SDA1</i>	actin cytoskeleton organization	3.2	6.8	1.6	3.2	6.5	8.8
orf19.962			3.2	2.8	2.1	3.6	2.2	7.3
orf19.5010	<i>DIM1</i>	rRNA modification	3.2	2.3	2.5	6.8	3.6	4.7
orf19.6236		ribosomal small subunit biogenesis	3.2	3.2	2.7	5.0	3.1	5.7

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1578		endonucleolytic cleavage of rRNA	3.2	3.2	1.3	2.0	2.4	2.2
orf19.7469	<i>ARG1</i>	arginine biosynthetic process	3.2	2.8	11.5	17.1	7.6	5.7
orf19.4391			3.2	1.5	3.9	7.0	1.0	0.7
orf19.2831	<i>RPC31</i>	tRNA transcription from RNA polymerase III promoter	3.2	13.3	2.9	2.4	2.4	3.9
orf19.4883		signal transduction	3.2	3.5	4.4	4.6	22.5	12.4
orf19.7219	<i>FTR1</i>	high-affinity iron ion transport	3.2	7.8	0.5	1.1	0.1	0.0
orf19.4492		nuclear division	3.2	3.6	2.3	3.7	1.5	3.1
orf19.2712	<i>HCA4</i>	rRNA processing	3.2	3.3	4.1	3.7	3.2	5.1
orf19.4031		carbohydrate metabolic process	3.2	4.0	2.0	2.9	2.9	1.7
orf19.4979	<i>KNS1</i>	protein amino acid phosphorylation	3.2	3.3	2.2	3.2	22.9	19.0
orf19.857	<i>FMO2</i>	oxidation-reduction process	3.1	2.6	3.5	4.9	4.8	3.2
orf19.4015	<i>CAG1</i>	cellular morphogenesis during conjugation with cellular fusion	3.1	3.6	0.8	0.7	4.4	3.3
orf19.3984			3.1	6.2	5.6	6.0	8.0	5.6
orf19.1642		endonucleolytic cleavage of rRNA	3.1	2.6	6.4	3.4	3.3	5.9
orf19.2785	<i>ATP7</i>	ATP synthesis coupled proton transport	3.1	3.1	2.0	2.6	1.3	1.5
orf19.4593	<i>RGA2</i>	hyphal growth	3.1	4.2	2.9	4.9	5.7	3.3
orf19.2414			3.1	3.3	1.1	0.6	0.7	1.7
orf19.414		translation	3.1	3.0	4.9	12.3	1.6	4.8
orf19.6678			3.1	5.1	1.2	1.4	3.4	3.1
orf19.296			3.1	1.7	1.5	0.9	1.2	1.0
orf19.2943		amino acid transport	3.1	4.6	2.3	3.4	6.4	2.3
orf19.3910			3.1	2.6	1.1	0.9	7.6	5.3
orf19.1719	<i>SGA1</i>	glycogen catabolic process	3.1	2.5	3.7	3.3	4.0	1.5
orf19.3813			3.1	3.1	1.4	1.8	6.3	3.4
orf19.6248			3.1	12.1	0.7	1.2	25.0	3.1
orf19.113	<i>CIP1</i>	response to oxidative stress	3.0	3.7	10.2	5.3	11.2	6.5
orf19.6126	<i>KGD2</i>	2-oxoglutarate metabolic process	3.0	4.0	3.5	5.1	4.3	2.9

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.5799			3.0	2.5	13.8	11.3	6.7	1.8
orf19.6348		ubiquitin-dependent protein catabolic process	3.0	2.4	1.3	1.4	0.6	1.2
orf19.4191	<i>RLP24</i>	ribosomal large subunit biogenesis	3.0	3.6	1.9	3.0	1.2	4.9
orf19.288	<i>MET13</i>	methionine metabolic process	3.0	4.3	3.0	4.2	4.0	1.9
orf19.7380			3.0	3.2	1.8	2.5	1.6	1.3
orf19.3707	<i>YHB1</i>	nitric oxide catabolic process	3.0	2.8	0.9	0.6	1.3	2.0
orf19.1980	<i>GIT4</i>	phospholipid transport	3.0	2.1	1.5	1.8	3.4	3.1
orf19.24	<i>RTA2</i>	lipid translocation	3.0	5.5	9.4	11.1	2.0	1.1
orf19.872			3.0	2.0	2.1	4.1	3.6	2.4
orf19.3540	<i>MAK5</i>	rRNA processing	3.0	2.9	1.9	3.4	3.5	4.1
orf19.515			3.0	2.2	2.9	5.3	4.8	16.5
orf19.3544		oxidation-reduction process	3.0	3.2	2.5	2.6	7.2	4.7
orf19.4183	<i>MUC1</i>		3.0	3.5	4.8	3.6	9.9	3.1
orf19.3762			2.9	1.8	1.4	1.3	1.6	4.5
orf19.2905			2.9	3.2	2.9	2.3	11.9	14.5
orf19.2386		endonucleolytic cleavage of rRNA	2.9	1.7	3.0	4.9	2.9	6.4
orf19.5169			2.9	2.2	9.5	9.7	2.9	2.0
orf19.4793			2.9	2.0	1.5	2.9	2.6	2.3
orf19.1369		metabolic process	2.9	2.3	1.2	1.0	4.1	3.5
orf19.3661		protein deubiquitination	2.9	3.0	2.4	2.3	4.1	1.9
orf19.5026		regulation of transcription, DNA-dependent	2.9	3.2	5.1	10.3	7.0	2.6
orf19.2185	<i>NSA1</i>	ribosomal large subunit biogenesis	2.9	2.6	1.8	3.1	1.1	4.0
orf19.7547		protein ubiquitination	2.9	3.0	1.1	1.4	3.9	2.6
orf19.2167		ribosomal large subunit biogenesis	2.9	1.8	2.9	4.8	1.6	3.2
orf19.1774		oxidation-reduction process	2.9	2.7	3.2	6.0	17.3	2.3
orf19.6758		response to salt stress	2.9	1.9	3.7	2.6	2.9	2.8
orf19.4510	<i>IFA4</i>		2.9	3.7	2.2	2.2	2.5	2.7

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.376			2.9	3.4	1.1	1.5	7.6	5.5
orf19.35		protein phosphorylation	2.9	3.3	2.3	2.9	1.9	1.6
orf19.3736	<i>KAR4</i>	G1 phase of mitotic cell cycle	2.8	2.6	1.2	1.0	2.6	1.5
orf19.2752	<i>ADR1</i>	filamentous growth	2.8	4.4	5.5	11.9	8.2	3.9
orf19.4043		response to stress	2.8	4.5	1.6	1.7	5.4	3.0
orf19.3756	<i>CHR1</i>	nuclear mRNA splicing, via spliceosome	2.8	3.1	3.2	3.5	2.0	3.7
orf19.5295			2.8	1.8	2.1	2.0	22.1	16.6
orf19.4657		regulation of lipid biosynthetic process	2.8	2.2	3.5	6.1	1.4	0.6
orf19.7229	<i>IML2</i>		2.8	3.3	1.4	1.4	3.8	1.9
orf19.4479		endonucleolytic cleavage of rRNA	2.8	2.7	1.7	3.8	2.5	4.0
orf19.4383		response to DNA damage stimulus	2.8	2.6	0.9	0.8	1.4	2.2
orf19.3848			2.8	1.8	1.8	4.3	1.6	1.1
orf19.4211	<i>FET3</i>	high-affinity iron ion transport	2.8	6.2	1.6	1.7	0.7	0.2
orf19.287			2.8	2.9	1.7	2.3	1.4	1.5
orf19.3240	<i>ERG27</i>	ergosterol biosynthetic process	2.8	2.4	2.3	2.8	1.4	1.1
orf19.2285		response to drug	2.8	1.5	5.0	2.8	2.9	83.5
orf19.5547			2.8	2.4	2.0	1.8	0.8	1.1
orf19.7520	<i>POT1</i>	fatty acid beta-oxidation	2.8	2.3	4.8	6.9	3.6	5.1
orf19.1388		ribosomal large subunit biogenesis	2.8	2.1	2.7	5.2	1.3	6.9
orf19.4255	<i>ECM331</i>		2.7	5.7	5.4	5.7	5.0	1.5
orf19.1411		oxidation-reduction process	2.7	4.8	1.1	0.9	1.4	1.1
orf19.2154	<i>HXK1</i>	hyphal growth	2.7	2.8	1.4	2.2	3.1	1.6
orf19.3015	<i>ARX1</i>	ribosomal large subunit biogenesis	2.7	3.9	1.3	1.7	3.7	2.8
orf19.4887	<i>ECM21</i>	ubiquitin-dependent endocytosis	2.7	3.1	0.8	1.0	4.0	5.0
orf19.3239	<i>CTF18</i>	double-strand break repair via homologous recombination	2.7	2.4	1.0	1.1	1.4	0.8
orf19.4093	<i>PES1</i>	filamentous growth	2.7	3.5	2.3	2.3	0.6	1.3
orf19.2344	<i>ASR1</i>		2.7	1.7	10.9	7.9	7.1	2.5

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.2458		cellular response to glucose starvation	2.7	2.6	3.8	5.8	7.8	6.5
orf19.1714	<i>PGA44</i>		2.7	2.3	1.6	0.8	201.7	19.2
orf19.183	<i>HIS3</i>	histidine biosynthetic process	2.7	1.7	4.7	4.1	8.4	7.4
orf19.419			2.7	6.4	4.7	3.5	30.5	8.7
orf19.2319		rRNA processing	2.7	2.3	2.5	3.3	1.5	2.0
orf19.5991		ribosomal large subunit assembly	2.7	4.4	2.7	3.7	6.1	7.5
orf19.6674	<i>BTS1</i>	terpenoid biosynthetic process	2.7	2.1	1.3	1.4	3.3	2.3
orf19.4346		COPII vesicle coating	2.7	2.7	1.9	2.3	1.7	1.0
orf19.1809	<i>FOX2</i>	fatty acid beta-oxidation	2.7	4.0	6.8	7.7	6.0	5.7
orf19.6608			2.7	4.7	1.5	1.3	5.8	9.0
orf19.2090		maturation of SSU-rRNA	2.7	3.3	1.8	2.9	2.8	4.4
orf19.2768	<i>AMS1</i>	oligosaccharide catabolic process	2.7	3.9	2.1	1.5	2.9	2.7
orf19.5251	<i>ZCF30</i>	regulation of transcription from RNA polymerase II promoter	2.7	3.1	3.0	4.0	4.6	3.1
orf19.4961	<i>STP2</i>	amino acid import	2.7	3.2	1.6	2.4	2.8	1.9
orf19.4531		transport	2.7	4.4	5.5	12.3	1.5	0.6
orf19.4204			2.6	2.2	4.1	4.5	0.8	1.6
orf19.6955	<i>HBR3</i>	maturation of rRNA	2.6	2.3	2.3	4.3	6.8	6.4
orf19.2710		chromosome segregation	2.6	1.8	1.0	0.9	1.0	2.5
orf19.6941		lipid storage	2.6	3.6	2.8	2.8	7.2	6.1
orf19.6659	<i>GAP6</i>	amino acid transport	2.6	3.0	1.4	1.7	2.1	1.8
orf19.6830		metabolic process	2.6	2.7	4.4	6.5	8.9	5.3
orf19.921	<i>HMS1</i>	filamentous growth	2.6	2.8	2.0	2.6	0.8	1.3
orf19.517	<i>HAP31</i>	regulation of carbohydrate metabolic process	2.6	1.5	2.6	4.8	2.9	3.8
orf19.4114	<i>FAA2-1</i>	lipid metabolic process	2.6	2.4	3.5	3.5	6.9	6.4
orf19.6843		mitotic sister chromatid segregation	2.6	3.0	2.7	3.1	9.2	10.1
orf19.1596	<i>FGR28</i>	filamentous growth	2.6	2.9	1.3	1.8	1.8	1.9
orf19.4013			2.6	2.2	0.6	0.6	0.3	0.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3512		cell wall organization	2.6	2.8	0.9	1.2	8.0	6.6
orf19.3865	<i>RFXI</i>	negative regulation of transcription from RNA pol II promoter	2.6	2.6	6.5	5.9	21.5	9.7
orf19.7456			2.6	2.6	3.6	4.9	2.2	0.6
orf19.2942	<i>DIP5</i>	amino acid transport	2.6	2.5	1.8	2.8	3.6	2.0
orf19.2432	<i>HACI</i>	cell wall organization	2.6	4.3	2.5	2.8	13.2	5.4
orf19.4731		intra-Golgi vesicle-mediated transport	2.6	2.6	2.0	2.4	1.2	1.5
orf19.5299	<i>ECMI</i>	cell wall organization	2.6	4.8	2.7	3.5	1.5	5.3
orf19.7398		ribosomal large subunit biogenesis	2.6	2.4	2.4	3.9	3.0	3.8
orf19.3021			2.6	2.0	1.2	0.9	2.4	2.2
orf19.809		maturation of rRNA	2.6	1.8	2.2	3.4	2.9	7.1
orf19.5710		NLS-bearing substrate import into nucleus	2.6	3.2	4.0	2.3	3.5	2.2
orf19.5876			2.6	2.3	1.8	1.6	7.3	7.1
orf19.5459		mRNA polyadenylation	2.6	2.3	4.2	4.3	3.9	2.3
orf19.3080		mitotic sister chromatid cohesion	2.6	2.9	4.7	4.8	11.8	4.9
orf19.996		filamentous growth	2.6	3.3	3.5	3.5	7.0	4.3
orf19.6143		oxidation-reduction process	2.6	2.6	3.6	3.4	11.5	6.8
orf19.6999		oxidation-reduction process	2.6	1.7	2.2	2.6	4.0	1.4
orf19.496		mismatch repair	2.6	2.3	1.6	2.3	1.1	0.6
orf19.2059			2.6	2.3	0.2	0.3	2.3	17.6
orf19.1867		malate transport	2.6	4.2	0.7	0.6	15.1	62.1
orf19.3737			2.5	2.4	4.6	5.7	8.0	4.1
orf19.1865		reciprocal meiotic recombination	2.5	2.7	2.0	2.8	3.3	1.5
orf19.6605			2.5	2.1	3.8	4.1	5.7	4.4
orf19.281			2.5	2.2	1.5	1.9	3.8	3.2
orf19.5145	<i>SSP96</i>	oxidation-reduction process	2.5	1.7	1.2	2.6	18.0	9.3
orf19.6322	<i>ARD</i>	fatty acid catabolic process	2.5	2.8	2.7	2.9	3.7	4.7
orf19.5963		protein amino acid glycosylation	2.5	2.7	2.4	4.4	2.1	1.0

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4507			2.5	2.3	1.7	1.3	2.5	2.3
orf19.2350		filamentous growth	2.5	4.1	0.6	1.0	0.9	0.6
orf19.2978			2.5	1.7	4.5	5.9	1.1	1.3
orf19.2398			2.5	3.8	1.3	1.7	4.5	3.0
orf19.5023	<i>DAL7</i>	anion transport	2.5	4.2	3.9	4.0	6.2	5.5
orf19.4870	<i>DBP3</i>	rRNA processing	2.5	3.1	1.1	2.4	2.0	3.8
orf19.278			2.5	2.7	1.4	1.5	2.2	1.9
orf19.7267	<i>SAM35</i>	mitochondrion organization	2.5	2.1	2.0	2.3	5.2	3.1
orf19.1625			2.5	2.6	2.4	3.2	1.6	2.0
orf19.2772	<i>HOS3</i>	histone deacetylation	2.5	2.6	2.2	2.2	1.4	1.1
orf19.6102	<i>RCA1</i>	DNA metabolic process	2.5	3.4	1.2	1.2	1.1	1.4
orf19.4495	<i>NDH51</i>	biofilm formation	2.5	3.3	2.4	3.4	2.7	1.6
orf19.3008	<i>COQ4</i>	ubiquinone biosynthetic process	2.5	1.8	1.6	1.7	0.4	0.8
orf19.6997	<i>ATO5</i>	cellular response to alkalinity	2.5	2.6	0.8	0.7	2.7	2.9
orf19.4264			2.5	3.2	1.2	1.0	0.8	3.2
orf19.7115	<i>SAC7</i>	actin filament reorganization during cell cycle	2.5	3.5	8.6	7.9	6.9	3.1
orf19.4590	<i>RFX2</i>	adhesion to host	2.5	4.5	1.5	3.3	23.4	8.1
orf19.93		development of symbiont in host	2.5	2.4	2.4	2.8	1.8	2.5
orf19.6057		pantothenate biosynthetic process	2.5	1.8	0.9	1.0	0.2	0.2
orf19.4477	<i>CSH1</i>	biofilm formation	2.5	2.3	2.2	2.1	3.9	2.3
orf19.5938	<i>SEN1</i>	rRNA processing	2.5	5.2	1.5	2.5	8.0	1.9
orf19.2066.1	<i>ATP18</i>	ATP synthesis coupled proton transport	2.5	2.3	2.0	2.6	2.5	2.8
orf19.6709			2.5	2.6	3.1	2.8	7.3	4.8
orf19.7650	<i>LTV1</i>	response to osmotic stress	2.5	2.9	1.7	2.4	1.8	3.1
orf19.203	<i>STB3</i>	positive regulation of transcription from RNA polymerase II	2.5	3.6	3.5	2.9	7.0	3.1
orf19.2838		response to drug	2.5	2.9	0.8	0.7	1.6	0.9
orf19.7154	<i>UTP18</i>	endonucleolytic cleavage of rRNA	2.4	1.5	1.6	3.1	2.1	2.9

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6003		intracellular signal transduction	2.4	2.1	1.9	2.5	0.9	0.7
orf19.2909	<i>ERG26</i>	ergosterol biosynthetic process	2.4	1.7	0.9	0.9	0.7	1.0
orf19.3734	<i>GEF2</i>	transmembrane transport	2.4	4.0	1.5	1.6	10.1	4.1
orf19.3867	<i>RPL7</i>	exonucleolytic trimming of rRNA	2.4	2.0	1.3	2.9	1.2	2.7
orf19.5588	<i>PGA60</i>		2.4	3.3	1.3	1.9	3.1	1.3
orf19.2887			2.4	2.7	3.6	4.2	5.8	2.2
orf19.2954			2.4	2.9	1.5	2.1	1.8	1.7
orf19.6062.3		cristae formation	2.4	2.9	2.7	2.9	3.2	2.5
orf19.7610	<i>PTP3</i>	osmosensory signaling pathway	2.4	2.5	3.3	3.3	2.7	1.4
orf19.2707.1	<i>QCR9</i>	iron-sulfur cluster assembly	2.4	2.1	1.3	4.5	0.7	1.3
orf19.2190	<i>VRP1</i>	actin cortical patch localization	2.4	2.4	3.5	5.1	3.1	2.0
orf19.2623	<i>ECM22</i>	sterol biosynthetic process	2.4	4.0	1.8	2.3	4.1	1.9
orf19.3298	<i>CCH1</i>	calcium ion transport	2.4	3.5	2.0	1.6	7.6	3.2
orf19.5752		ubiquitin-dependent protein catabolic process	2.4	1.8	1.5	1.4	2.7	2.5
orf19.6790		mRNA splice site selection	2.4	3.0	3.5	5.5	7.1	1.3
orf19.5591	<i>ADO1</i>	purine base metabolic process	2.4	2.9	1.1	1.5	0.7	0.8
orf19.6905			2.4	3.3	0.5	0.4	1.2	1.4
orf19.804		transport	2.4	1.6	1.6	2.1	2.3	3.4
orf19.4522		response to drug	2.4	1.8	1.7	2.3	3.2	3.7
orf19.7680	<i>CTA26</i>	positive regulation of transcription, DNA-dependent	2.4	2.4	3.0	4.3	3.2	8.3
orf19.2459			2.4	3.0	1.9	2.5	2.9	2.2
orf19.4191.1		protein polyubiquitination	2.4	2.0	2.2	1.6	2.0	1.9
orf19.5368		ER-associated protein catabolic process	2.4	1.8	3.6	4.3	4.1	2.0
orf19.1949	<i>VPS1</i>	biofilm formation	2.4	2.6	3.4	2.8	5.6	2.4
orf19.3705	<i>PTC6</i>	protein amino acid dephosphorylation	2.4	2.5	1.7	1.9	2.1	1.7
orf19.3109			2.4	2.9	0.9	1.6	7.8	3.2
orf19.6043		aerobic respiration	2.4	2.2	3.1	2.8	4.7	2.6

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7281	<i>PDK2</i>	response to drug	2.4	2.0	4.3	3.9	1.9	1.6
orf19.3276	<i>PWP2</i>	cytokinesis	2.4	3.8	1.4	2.4	3.7	2.6
orf19.6514	<i>CUP9</i>	cellular copper ion homeostasis	2.4	2.3	2.4	3.1	4.9	2.2
orf19.3974	<i>PUT2</i>	glutamate biosynthetic process	2.4	3.4	3.0	3.3	3.1	1.2
orf19.6638	<i>PTC4</i>	protein dephosphorylation	2.4	2.1	1.2	2.1	0.9	1.2
orf19.6690			2.4	2.3	0.7	0.6	0.6	0.7
orf19.6417	<i>TSR1</i>	rRNA processing	2.4	3.6	1.5	3.5	5.2	3.1
orf19.4897		phospholipid transport	2.4	2.4	1.3	1.2	1.0	1.4
orf19.1307			2.4	2.4	2.0	1.7	8.5	6.1
orf19.4144			2.4	3.2	1.2	1.6	0.9	0.9
orf19.147	<i>YAK1</i>	filamentous growth	2.4	2.4	1.2	2.0	2.3	1.7
orf19.501		rRNA processing	2.4	3.3	1.0	1.4	3.2	2.3
orf19.6548	<i>ISU1</i>	cellular iron ion homeostasis	2.4	2.1	4.8	6.5	7.7	5.8
orf19.5282			2.4	2.7	0.5	0.4	1.9	5.4
orf19.7578		ER to Golgi vesicle-mediated transport	2.3	2.5	1.8	2.1	3.2	3.2
orf19.1546			2.3	1.5	1.8	1.5	0.4	0.9
orf19.6842	<i>TUS1</i>	fungus-type cell wall organization	2.3	3.0	1.6	1.6	3.8	2.6
orf19.4815	<i>YTM1</i>	filamentous growth	2.3	3.2	0.6	1.4	3.2	2.8
orf19.3135		ER-associated protein catabolic process	2.3	2.6	1.2	1.1	1.7	1.6
orf19.2938		mitochondrial respiratory chain complex III assembly	2.3	1.7	1.8	2.7	1.7	1.2
orf19.5961		proteasome regulatory particle assembly	2.3	2.0	1.3	1.2	1.7	3.1
orf19.2423	<i>ZCF11</i>	filamentous growth	2.3	2.1	2.5	3.8	2.1	1.9
orf19.6557			2.3	2.1	1.4	1.4	1.7	2.1
orf19.2527		single-species biofilm formation on inanimate substrate	2.3	2.3	1.7	3.0	3.0	4.8
orf19.2876	<i>CBF1</i>	chromosome segregation	2.3	3.2	0.5	0.5	0.4	2.0
orf19.6953	<i>IRS4</i>	fungus-type cell wall organization	2.3	2.0	4.5	5.7	14.3	7.6
orf19.7247	<i>RIM101</i>	cellular response to iron ion starvation	2.3	3.4	4.7	6.9	10.1	6.0

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3746	<i>IFC1</i>	oligopeptide transport	2.3	2.7	1.3	0.6	0.2	0.0
orf19.6376	<i>PTC5</i>	protein amino acid dephosphorylation	2.3	3.6	2.4	2.9	3.3	2.0
orf19.2210			2.3	4.3	1.0	1.9	8.0	3.8
orf19.3169		establishment of mitotic sister chromatid cohesion	2.3	2.2	1.5	1.5	2.6	3.2
orf19.6938	<i>MEU1</i>	glutamate biosynthetic process	2.3	2.3	1.8	2.8	2.7	1.4
orf19.6971	<i>DIE2</i>	protein amino acid N-linked glycosylation	2.3	2.1	1.2	1.4	1.7	1.3
orf19.6026	<i>ERG2</i>	ergosterol biosynthetic process	2.3	1.5	2.6	4.7	1.3	0.4
orf19.1557		protein methylation	2.3	2.0	1.1	1.1	2.5	3.0
orf19.1939			2.3	1.6	0.6	0.8	3.3	1.9
orf19.5552		regulation of transcription from RNA polymerase II promoter	2.3	3.7	1.7	1.8	3.5	2.7
orf19.345		response to oxidative stress	2.3	1.9	5.6	4.8	1.3	1.4
orf19.951			2.3	2.4	0.4	0.4	0.8	1.2
orf19.5713	<i>YMX6</i>	glucose catabolic process to ethanol	2.3	1.8	1.8	1.6	2.2	1.8
orf19.5611		ergosterol metabolic process	2.3	1.5	12.7	8.9	4.9	10.2
orf19.2917		rRNA processing	2.3	2.3	1.3	2.9	4.0	3.2
orf19.3921			2.3	2.1	1.2	1.6	1.8	2.0
orf19.6624		regulation of Rab GTPase activity	2.3	2.2	3.0	5.8	2.9	2.3
orf19.2962			2.3	2.2	0.7	0.8	33.6	13.7
orf19.1531		mRNA cleavage	2.3	1.8	1.1	1.5	2.2	2.0
orf19.3726			2.3	2.2	1.6	2.6	1.6	1.7
orf19.7550	<i>IFA14</i>		2.3	1.7	1.0	0.7	4.0	9.6
orf19.5644		ascospore wall assembly	2.3	3.5	1.1	1.0	2.7	1.9
orf19.6385	<i>ACO1</i>	citrate metabolic process	2.3	2.7	1.9	2.6	2.4	1.4
orf19.2079	<i>PHHB</i>	filamentous growth	2.3	2.9	1.3	1.3	1.8	5.7
orf19.2030			2.3	2.3	2.9	2.7	4.7	2.8
orf19.3732	<i>ERG25</i>	ergosterol biosynthetic process	2.3	1.5	4.4	6.5	7.9	2.4
orf19.4411	<i>HOS1</i>	chromatin organization	2.3	2.2	1.6	1.7	3.0	2.7

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.415		cristae formation	2.2	2.3	1.7	2.1	1.2	1.0
orf19.6411		phospholipid metabolic process	2.2	2.8	1.7	1.3	2.3	1.6
orf19.1839	<i>RPA190</i>	transcription from RNA polymerase I promoter	2.2	4.6	1.3	2.2	2.9	1.8
orf19.3675	<i>GAL7</i>	galactose catabolic process via UDP-galactose	2.2	1.9	2.0	1.8	2.0	0.9
orf19.899			2.2	1.9	1.8	1.9	4.2	3.2
orf19.2003	<i>HNMI</i>	choline transport	2.2	1.9	2.5	3.8	7.1	1.7
orf19.706	<i>NMD3</i>	ribosomal large subunit assembly	2.2	3.1	1.8	3.3	4.7	3.3
orf19.5131		negative regulation of gluconeogenesis	2.2	2.5	1.5	1.0	2.8	2.9
orf19.6578		phosphate transport	2.2	3.1	3.5	3.0	2.0	0.9
orf19.3150	<i>GRE2</i>	response to osmotic stress	2.2	1.6	3.9	2.9	2.4	2.5
orf19.7011		maturation of SSU-rRNA	2.2	4.7	1.1	2.3	4.8	3.9
orf19.944	<i>IFG3</i>	oxidation-reduction process	2.2	2.6	4.0	4.4	5.1	2.6
orf19.6713			2.2	3.7	2.7	2.5	0.9	0.3
orf19.6229	<i>CAT1</i>	hyphal growth	2.2	1.5	5.5	6.1	4.9	2.2
orf19.4970			2.2	4.9	2.5	1.6	228.3	47.3
orf19.7513	<i>ALK2</i>	oxidation-reduction process	2.2	3.5	4.5	6.4	40.9	20.2
orf19.4584	<i>PHO114</i>	phosphate metabolic process	2.2	1.5	2.3	2.1	2.4	1.9
orf19.2204.2		cell wall organization	2.2	2.6	1.4	1.5	2.8	1.8
orf19.3444		drug transport	2.2	3.3	1.6	1.2	2.2	3.1
orf19.2575			2.2	2.3	1.1	1.9	2.0	2.5
orf19.4795			2.2	2.6	3.4	4.8	11.5	5.1
orf19.3724		endonucleolytic cleavage of rRNA	2.2	1.7	3.0	4.0	1.4	2.7
orf19.7320	<i>LIP7</i>	lipid catabolic process	2.2	2.5	3.6	3.8	16.6	9.0
orf19.669	<i>PRM1</i>	plasma membrane fusion during cytogamy	2.2	2.8	0.6	0.4	1.2	3.2
orf19.3245			2.2	2.3	1.2	1.1	2.5	1.6
orf19.7472	<i>IFF4</i>	cell-substrate adhesion	2.2	2.3	1.6	1.7	4.3	2.2
orf19.318		iron-sulfur cluster assembly	2.2	2.0	2.9	4.5	2.0	1.6

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.2781		protein amino acid phosphorylation	2.2	2.1	4.6	2.5	27.0	4.6
orf19.7546		maturation of rRNA)	2.2	1.5	2.8	5.3	8.7	5.0
orf19.4591	<i>CAT2</i>	acetyl-CoA metabolic process	2.2	2.6	4.3	6.5	5.2	2.8
orf19.188			2.2	2.0	6.1	4.8	0.9	1.7
orf19.5640	<i>PEX5</i>	fatty acid beta-oxidation	2.2	2.6	0.9	0.9	2.2	2.3
orf19.7618		endonucleolytic cleavage of rRNA	2.2	1.6	1.3	3.1	9.9	7.4
orf19.6956	<i>DAL9</i>	anion transport	2.2	2.5	1.6	1.8	1.6	1.3
orf19.2091		oxidation-reduction process	2.2	2.3	1.9	3.4	0.8	0.9
orf19.732		fatty acid catabolic process	2.2	2.2	2.4	4.1	14.2	21.9
orf19.2726		actin cytoskeleton organization	2.2	1.5	6.0	5.0	7.2	3.7
orf19.1902	<i>NOC4</i>	endonucleolytic cleavage of rRNA	2.2	1.9	1.6	3.0	2.1	4.3
orf19.1701	<i>RIK1</i>	pyridoxine biosynthetic process	2.2	1.5	4.4	4.6	6.5	3.6
orf19.3863			2.2	28.3	6.2	35.3	157.2	36.4
orf19.7359	<i>CRZ1</i>	calcium-mediated signaling	2.1	3.3	3.3	6.4	5.9	2.6
orf19.3885		metabolic process	2.1	2.2	4.3	8.3	75.7	17.7
orf19.6594	<i>PLB3</i>	phosphatidylserine catabolic process	2.1	3.4	1.1	0.9	3.4	2.8
orf19.7204		oxidation-reduction process	2.1	2.5	2.1	2.3	3.3	4.3
orf19.5610	<i>ARG3</i>	arginine biosynthetic process	2.1	1.7	4.2	5.2	2.0	1.3
orf19.2175		positive regulation of apoptosis	2.1	2.7	1.8	1.4	1.4	2.3
orf19.6706	<i>GYP7</i>	vesicle-mediated transport	2.1	2.4	1.9	1.8	3.0	2.3
orf19.2176	<i>IFM3</i>	oxidation-reduction process	2.1	1.8	1.7	2.1	3.6	3.6
orf19.3621			2.1	3.5	1.3	0.7	3.7	3.1
orf19.5426		cell budding	2.1	2.5	2.3	2.4	5.9	4.3
orf19.7061		metabolic process	2.1	1.7	1.2	1.3	1.0	2.1
orf19.4012	<i>PCL5</i>	filamentous growth	2.1	6.0	3.4	3.2	13.1	7.5
orf19.5198	<i>NOP4</i>	rRNA processing	2.1	1.8	2.0	3.1	1.1	3.5
orf19.5601			2.1	2.0	2.5	2.2	1.6	2.6

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1682			2.1	3.0	1.7	2.1	1.5	1.3
orf19.4229		diadenosine polyphosphate catabolic process	2.1	2.0	3.0	2.9	3.3	4.4
orf19.2062	<i>SOD4</i>	evasion or tolerance	2.1	3.2	3.3	3.6	3.6	4.9
orf19.1440			2.1	2.6	1.7	2.4	0.8	0.9
orf19.7517	<i>CHT1</i>	cytokinesis, completion of separation	2.1	2.5	1.8	1.5	14.0	3.4
orf19.6811		iron ion transport	2.1	1.8	2.0	1.9	3.1	1.9
orf19.4982		lipid metabolic process	2.1	2.3	0.9	0.6	0.7	0.8
orf19.2192	<i>GDH2</i>	nitrogen compound metabolic process	2.1	1.9	1.5	2.6	2.4	1.1
orf19.3154			2.1	2.3	2.0	3.3	0.6	0.6
orf19.692			2.1	1.9	1.6	1.9	3.2	4.2
orf19.5958	<i>CDR2</i>	drug export	2.1	2.5	0.9	1.0	2.5	1.7
orf19.6983			2.1	9.1	1.0	1.3	0.4	0.0
orf19.124	<i>CIC1</i>	protein catabolic process	2.1	2.0	1.5	3.8	1.7	4.2
orf19.6132			2.1	2.0	1.7	2.1	1.4	1.7
orf19.1482	<i>HSK3</i>	mitotic spindle organization in nucleus	2.1	1.6	2.3	1.7	1.1	1.3
orf19.1300		filamentous growth	2.1	1.8	2.1	2.3	0.3	0.6
orf19.4090		fucose transport	2.1	1.7	1.1	0.8	3.8	4.5
orf19.5845	<i>RNR3</i>	DNA replication	2.1	2.7	4.5	5.3	1.4	1.2
orf19.4278		negative regulation of transcription from RNA pol II promoter	2.1	2.3	1.8	1.8	2.4	1.9
orf19.6902	<i>DBP7</i>	ribosomal large subunit assembly	2.1	2.0	1.0	2.0	9.9	10.3
orf19.6139	<i>FRE7</i>	siderophore-iron transport	2.1	4.9	9.5	7.8	37.7	25.9
orf19.2825		iron-sulfur cluster assembly	2.1	1.5	2.1	1.9	1.5	2.2
orf19.4862		vesicle-mediated transport	2.1	1.6	1.5	1.8	3.9	2.1
orf19.5089	<i>TERT</i>	telomere capping	2.1	2.3	1.8	1.6	3.1	1.4
orf19.272	<i>FAA21</i>	lipid metabolic process	2.1	3.0	6.6	8.3	11.5	7.4
orf19.1549			2.1	1.8	2.0	3.0	1.1	1.6
orf19.4215	<i>FET34</i>	high-affinity iron ion transport	2.1	5.2	0.2	0.5	0.0	0.1

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.5924	<i>ZCF31</i>	cellular response to drug	2.1	2.3	1.8	1.9	2.5	2.0
orf19.1426			2.1	2.8	2.5	3.5	2.6	1.0
orf19.6517	<i>RAD14</i>	nucleotide-excision repair, DNA damage recognition	2.1	1.7	2.7	3.2	2.8	2.6
orf19.26		misfolded or incompletely synthesized protein catabolic process	2.1	2.0	1.5	1.8	1.8	2.1
orf19.5492		nuclear mRNA splicing, via spliceosome	2.1	1.9	2.2	3.9	1.5	3.2
orf19.396	<i>EAF6</i>	histone acetylation	2.1	1.5	1.3	1.2	0.7	1.4
orf19.3611			2.1	2.2	2.1	3.1	1.1	1.4
orf19.7512		oxidation-reduction process	2.1	1.8	1.5	1.6	5.2	3.0
orf19.500		tRNA methylation	2.1	2.3	1.5	2.6	2.9	2.6
orf19.2202			2.1	1.8	5.5	6.4	15.1	10.1
orf19.1813	<i>FLC2</i>	heme transport	2.1	1.7	2.5	3.0	7.1	1.9
orf19.3669	<i>SHA3</i>	filamentous growth	2.1	2.4	1.6	2.9	4.5	2.5
orf19.5097	<i>CAT8</i>	filamentous growth	2.1	1.7	3.6	4.8	8.1	4.7
orf19.795	<i>VPS36</i>	hyphal growth	2.0	2.5	0.6	0.6	0.9	1.5
orf19.7385			2.0	2.4	1.3	1.7	85.7	31.9
orf19.4975	<i>HYR1</i>	single-species biofilm formation in or on host organism	2.0	1.8	1.4	1.5	4.0	2.7
orf19.5677	<i>DUR4</i>	urea transport	2.0	2.5	1.2	0.5	2.8	3.6
orf19.6268			2.0	1.8	1.9	2.0	1.9	1.5
orf19.7222	<i>PAM16</i>	protein import into mitochondrial matrix	2.0	1.6	1.6	2.1	1.8	2.1
orf19.7436	<i>AAF1</i>	cell adhesion	2.0	4.4	1.0	1.2	2.1	1.2
orf19.6178	<i>FBP1</i>	gluconeogenesis	2.0	2.0	1.7	1.7	2.3	4.1
orf19.3127	<i>CZF1</i>	filamentous growth	2.0	2.9	2.8	2.9	3.2	2.0
orf19.1114		mitochondrial respiratory chain complex IV assembly	2.0	1.9	2.5	3.3	3.5	3.6
orf19.6274	<i>PBR1</i>	biofilm formation	2.0	1.7	1.0	2.7	2.3	1.2
orf19.5270			2.0	2.3	1.4	1.8	2.5	5.4
orf19.2667	<i>RPF1</i>	maturation of rRNA	2.0	1.8	1.0	2.0	1.9	4.5
orf19.1270	<i>FRE3</i>	oxidation-reduction process	2.0	1.5	1.2	1.9	48.0	7.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1633	<i>UTP4</i>	maturation of rRNA	2.0	1.5	1.2	3.5	0.9	1.6
orf19.4121		fatty acid metabolic process	2.0	2.4	5.6	4.4	10.9	15.2
orf19.4356	<i>HGT3</i>	carbohydrate transport	2.0	1.9	2.6	2.1	3.4	1.5
orf19.1264	<i>CFL2</i>	cellular iron ion homeostasis	2.0	6.0	0.6	0.5	0.2	0.8
orf19.6518		oxidation-reduction process	2.0	2.6	2.9	2.3	83.1	56.9
orf19.512		ribosomal small subunit biogenesis	2.0	5.4	1.5	2.3	3.5	2.3
orf19.3578			2.0	1.5	1.7	4.1	4.1	5.8
orf19.5824			2.0	1.9	1.9	5.1	3.3	3.9
orf19.3151		cellular metabolic process	2.0	1.9	2.3	1.8	3.1	2.7
orf19.3478	<i>NIP7</i>	rRNA processing	2.0	1.5	0.7	1.9	0.9	1.5
orf19.1710	<i>ALH1</i>	oxidation-reduction process	2.0	1.9	1.9	2.7	0.7	0.8
orf19.6928	<i>SAP9</i>	fungal-type cell wall organization	2.0	2.3	3.2	2.6	2.1	1.2
orf19.3338			2.0	4.0	3.4	5.1	5.2	13.2
orf19.4928	<i>SEC2</i>	exocytosis	2.0	2.1	0.4	0.4	1.1	2.6
orf19.2397.3		filamentous growth	2.0	4.3	0.9	1.2	3.3	5.9
orf19.7282	<i>PEX13</i>	protein import into peroxisome matrix	2.0	1.6	2.6	2.0	2.3	2.1
orf19.7006		reciprocal meiotic recombination	2.0	3.1	1.5	2.1	4.2	2.1
orf19.4766	<i>ARG81</i>	arginine metabolic process	2.0	1.9	1.6	1.7	1.3	1.4
orf19.1598	<i>ERG24</i>	ergosterol biosynthetic process	2.0	1.6	2.2	2.2	0.9	0.7
orf19.5231.2	<i>ATP19</i>	ATP synthesis coupled proton transport	2.0	2.2	1.3	1.9	0.7	1.4
orf19.5815	<i>SCT2</i>	phospholipid biosynthetic process	2.0	2.3	1.3	1.1	2.8	1.5
orf19.1415	<i>FRE10</i>	iron ion transport	2.0	4.2	0.9	2.3	0.2	0.1
orf19.1030		meiotic recombination checkpoint	2.0	2.4	0.9	1.4	1.0	5.4
orf19.1224	<i>FRP3</i>	ammonium transport	2.0	1.5	0.9	1.3	2.5	1.4
orf19.5255	<i>PXA2</i>	fatty acid transport	2.0	1.5	3.3	5.6	8.9	4.3
orf19.2622	<i>YPT31</i>	exocytosis	2.0	1.7	2.1	3.2	1.4	1.8
orf19.1140			2.0	1.5	1.6	2.1	1.0	1.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3412	<i>ATG15</i>	multivesicular body membrane disassembly	2.0	2.6	0.7	1.0	3.2	2.5
orf19.4187		mitochondrial genome maintenance	2.0	1.8	1.5	1.2	3.2	4.3
orf19.7062	<i>RPA135</i>	transcription from RNA polymerase I promoter	2.0	5.6	0.9	1.6	3.8	2.7
orf19.6068	<i>SVF1</i>	response to oxidative stress	2.0	2.0	2.5	4.7	4.0	2.4
orf19.73		proteolysis	2.0	1.7	7.6	5.4	14.4	3.9
orf19.2998	<i>TSR2</i>	maturation of rRNA	2.0	2.4	1.5	2.6	2.5	3.5
orf19.2650			2.0	1.5	1.6	2.0	1.2	1.5
orf19.2016		ergosterol biosynthetic process	2.0	2.0	1.3	1.4	1.0	1.5
orf19.2570	<i>MCI4</i>	respiratory electron transport chain	2.0	1.7	1.2	2.0	1.5	2.1
orf19.7107		ribosomal large subunit biogenesis	2.0	2.2	0.6	1.2	3.2	3.3
orf19.7261		vesicle-mediated transport	2.0	2.3	1.8	1.8	2.7	1.9
orf19.994			2.0	2.7	0.7	1.3	3.4	1.7
orf19.7316		response to drug	2.0	2.3	0.7	0.8	2.6	2.2
orf19.5491	<i>ATP14</i>	ATP synthesis coupled proton transport	1.9	2.3	1.3	1.8	1.3	1.6
orf19.2005	<i>REG1</i>	glycogen metabolic process	1.9	2.2	2.3	3.3	3.1	1.8
orf19.4792		termination of G-protein coupled receptor signaling pathway	1.9	1.6	1.0	1.7	11.5	5.3
orf19.3617	<i>GTR1</i>	chromatin silencing at telomere	1.9	2.3	1.5	1.7	1.1	3.4
orf19.5170	<i>ENA21</i>	sodium ion transport	1.9	2.5	3.0	3.2	4.3	2.8
orf19.6225.1		mitochondrial respiratory chain complex IV assembly	1.9	1.9	0.9	0.7	0.7	1.6
orf19.5006.1		Golgi to plasma membrane transport	1.9	1.5	1.6	1.5	4.5	3.2
orf19.4746	<i>JIP5</i>	ribosomal large subunit biogenesis	1.9	1.7	1.9	4.4	1.0	2.2
orf19.2115			1.9	2.8	0.9	1.5	4.6	1.7
orf19.4055		filamentous growth	1.9	2.5	0.9	0.7	1.9	1.4
orf19.972		DNA repair	1.9	2.9	0.8	0.6	2.1	2.4
orf19.4014		budding cell bud growth	1.9	1.6	0.8	0.8	2.5	2.6
orf19.6881	<i>YTH1</i>	mRNA cleavage	1.9	2.0	0.9	0.9	0.8	1.5
orf19.6686	<i>ENP2</i>	rRNA processing	1.9	1.6	3.5	6.3	9.1	8.1

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4791			1.9	1.9	1.2	1.0	4.3	3.1
orf19.4016		aerobic respiration	1.9	2.8	1.7	2.4	1.6	1.3
orf19.1250		maturation of rRNA	1.9	1.5	1.5	2.3	2.2	3.6
orf19.6369	<i>RIO2</i>	maturation of rRNA	1.9	1.9	1.4	2.1	1.9	2.2
orf19.1770	<i>CYC1</i>	mitochondrial electron transport, ubiquinol to cytochrome c	1.9	2.2	1.4	1.7	0.6	0.7
orf19.4549	<i>FGR38</i>	filamentous growth	1.9	2.5	1.1	1.1	2.3	1.2
orf19.4941	<i>TYE7</i>	positive regulation of glycolysis	1.9	1.9	1.3	1.5	1.5	1.3
orf19.4401	<i>YVH1</i>	hyphal growth	1.9	1.8	1.1	2.3	2.7	3.8
orf19.909	<i>STP4</i>	regulation of transcription, DNA-dependent	1.9	1.5	4.0	4.6	3.2	3.1
orf19.1791		maturation of rRNA	1.9	2.5	1.7	3.0	0.9	1.5
orf19.2818		generation of catalytic spliceosome for second transesterification step	1.9	1.6	1.8	3.4	5.2	2.6
orf19.7150	<i>NRG1</i>	filamentous growth	1.9	1.8	0.8	1.3	1.4	0.7
orf19.7034		negative regulation of translation	1.9	2.1	3.3	3.7	1.5	1.1
orf19.6185			1.9	1.9	2.6	3.4	1.1	0.9
orf19.3778		ribosomal large subunit assembly	1.9	2.5	0.7	1.4	3.0	2.4
orf19.1313	<i>CDR3</i>	drug export	1.9	2.1	0.9	1.0	2.4	1.7
orf19.5553		response to oxidative stress	1.9	2.3	1.3	1.8	2.3	2.4
orf19.3241			1.9	2.0	1.9	1.8	3.4	2.2
orf19.2050		sterol metabolic process	1.9	2.2	1.2	1.3	2.9	1.8
orf19.925	<i>SAM51</i>	protein complex assembly	1.9	2.8	2.3	3.4	1.1	0.9
orf19.5367	<i>RDH54</i>	DNmeiotic sister chromatid segregation	1.9	2.2	2.1	2.3	2.1	0.9
orf19.151	<i>TPO5</i>	polyamine transport	1.9	1.9	0.9	1.1	2.8	3.3
orf19.2984	<i>MST1</i>	mitochondrial threonyl-tRNA aminoacylation	1.9	2.0	1.0	1.1	0.7	1.0
orf19.6852			1.9	1.7	1.3	1.2	1.0	1.4
orf19.2517		drug transport	1.9	1.6	2.0	1.8	2.3	2.0
orf19.4937	<i>CHS3</i>	cell wall chitin biosynthetic process	1.9	2.0	1.9	2.5	6.3	2.4
orf19.753		protein O-linked glycosylation	1.9	1.9	1.8	2.6	9.0	6.7

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3228		pseudohyphal growth	1.9	1.8	0.8	1.2	1.4	0.8
orf19.2906	<i>PGA41</i>		1.9	1.6	1.5	1.0	4.9	2.3
orf19.1570	<i>ERG7</i>	ergosterol biosynthetic process	1.9	1.8	1.5	1.4	1.7	1.3
orf19.6460	<i>PEX1</i>	protein import into peroxisome matrix, receptor recycling	1.9	1.8	2.4	2.7	1.9	1.7
orf19.6727	<i>RIT1</i>	charged-tRNA amino acid modification	1.9	2.2	1.1	1.1	1.9	3.4
orf19.1619		mRNA 3'-end processing	1.9	2.7	3.7	2.7	1.7	1.6
orf19.5203			1.9	2.2	2.7	2.7	1.4	1.4
orf19.2446		pantothenate biosynthetic process	1.9	2.0	2.4	2.4	2.6	2.8
orf19.6090		single-species biofilm formation on inanimate substrate	1.9	2.4	0.6	0.6	0.9	4.5
orf19.3769		zinc ion transport	1.9	1.6	1.7	2.4	6.4	3.5
orf19.6967	<i>USO6</i>	ER to Golgi vesicle-mediated transport	1.9	1.7	1.3	1.1	1.3	1.4
orf19.4286			1.9	2.9	0.3	0.4	2.3	3.8
orf19.3658		protein folding in endoplasmic reticulum	1.9	1.7	1.2	1.4	2.0	0.7
orf19.3536		protein amino acid N-linked glycosylation	1.9	1.5	2.1	4.1	2.0	1.0
orf19.3659		transcription from RNA polymerase II promoter	1.9	1.9	1.4	1.7	2.0	2.0
orf19.7567			1.9	2.1	1.1	1.0	0.2	0.2
orf19.2137		translation	1.9	2.3	0.6	0.8	0.9	2.3
orf19.4871	<i>ERO1</i>	protein thiol-disulfide exchange	1.9	1.9	0.9	0.6	1.8	1.7
orf19.2821			1.9	2.6	1.2	2.0	1.3	1.7
orf19.1317	<i>OSH3</i>	filamentous growth	1.9	1.9	2.6	2.9	4.3	3.4
orf19.6607			1.9	1.9	2.2	2.5	0.6	1.9
orf19.5921		transport	1.9	1.7	2.7	2.8	5.7	4.0
orf19.5847		transcription from RNA polymerase III promoter	1.9	2.7	1.4	2.4	4.6	2.5
orf19.6382		transport	1.9	2.7	1.4	1.8	7.3	2.3
orf19.2039	<i>MSF1</i>	mitochondrial phenylalanyl-tRNA aminoacylation	1.9	1.8	1.8	2.6	1.1	1.4
orf19.6165	<i>KGD1</i>	2-oxoglutarate metabolic process	1.9	3.6	2.7	3.5	3.2	2.0
orf19.367	<i>CNHI</i>	cell morphogenesis	1.9	2.1	1.4	1.5	3.1	2.0

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1687		maturation of 5.8S rRNA from tricistronic rRNA transcript	1.9	2.3	2.6	4.7	3.5	2.3
orf19.2609	<i>CET1</i>	mRNA capping	1.9	2.0	1.4	1.7	1.1	1.5
orf19.2064		regulation of transcription, DNA-dependent	1.9	3.0	0.9	0.8	3.3	3.0
orf19.1252	<i>YME1</i>	chronological cell aging	1.9	3.0	1.0	0.7	1.1	1.3
orf19.3131	<i>OYE32</i>	cell redox homeostasis	1.9	1.7	3.4	2.8	2.3	1.7
orf19.3881			1.9	2.0	0.7	0.6	0.5	0.5
orf19.6346		protein export from nucleus	1.9	1.5	1.6	1.6	0.8	2.1
orf19.719		fungus-type cell wall organization	1.8	3.3	1.0	1.0	1.5	1.3
orf19.4119	<i>SPO72</i>	autophagic vacuole formation	1.8	2.0	1.7	1.3	4.2	3.1
orf19.782		lipid homeostasis	1.8	1.7	2.1	2.6	4.9	2.7
orf19.7590		ATP synthesis coupled electron transport	1.8	2.8	2.0	3.6	1.2	0.8
orf19.518		tRNA methylation	1.8	1.9	1.3	2.1	1.1	1.0
orf19.6418		ribosomal large subunit biogenesis	1.8	2.3	1.1	1.6	3.2	4.4
orf19.2256			1.8	1.5	2.1	2.8	1.5	3.4
orf19.4569			1.8	2.0	0.8	0.8	2.1	2.5
orf19.4330			1.8	1.9	2.0	2.3	1.3	1.8
orf19.5848			1.8	2.1	1.8	2.4	7.1	4.3
orf19.3290		electron transport chain	1.8	2.0	1.8	2.3	1.0	1.1
orf19.1144			1.8	2.0	1.8	1.3	2.2	1.6
orf19.828		translation	1.8	1.5	1.8	2.1	0.2	0.4
orf19.4393	<i>CIT1</i>	acetyl-CoA catabolic process	1.8	1.9	4.2	6.6	2.3	1.1
orf19.913.2		aerobic respiration	1.8	1.9	1.5	2.4	0.7	1.6
orf19.2057		protein complex assembly	1.8	2.9	1.7	1.7	1.8	1.4
orf19.2590		biotin biosynthetic process	1.8	2.8	1.6	1.4	0.0	0.2
orf19.5232	<i>CSI2</i>	filamentous growth	1.8	1.7	1.3	2.6	1.3	2.0
orf19.3759		regulation of transcription from RNA polymerase II promoter	1.8	1.5	1.7	3.3	4.4	3.6
orf19.2812			1.8	1.8	0.8	1.0	1.6	1.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.2044	<i>PGA27</i>		1.8	2.0	1.5	2.2	3.1	1.6
orf19.1954	<i>PUS4</i>	tRNA modification	1.8	2.0	2.0	2.3	2.4	2.6
orf19.3573	<i>PEX6</i>	protein import into peroxisome matrix	1.8	1.7	2.9	2.5	2.6	1.3
orf19.3310			1.8	1.9	0.8	0.6	2.9	3.3
orf19.2674		regulation of transcription, DNA-dependent	1.8	20.1	1.1	1.2	1.2	1.5
orf19.5798	<i>LIG4</i>	filamentous growth	1.8	2.6	0.9	0.8	1.0	1.0
orf19.6319		protein deubiquitination	1.8	2.1	2.0	2.0	1.1	0.9
orf19.2565		mitochondrion inheritance	1.8	2.6	1.3	1.4	5.4	4.3
orf19.5629	<i>QCR7</i>	aerobic respiration	1.8	2.0	1.4	2.3	0.9	1.6
orf19.881	<i>DFG16</i>	filamentous growth	1.8	2.1	1.2	1.2	2.5	2.2
orf19.199			1.8	1.8	3.8	5.2	8.8	3.2
orf19.1888		ER-associated protein catabolic process	1.8	1.8	0.9	1.0	1.6	2.3
orf19.4372		transmembrane transport	1.8	3.0	0.6	0.6	3.5	3.3
orf19.409		mitochondrial respiratory chain complex IV assembly	1.8	1.8	1.5	2.1	2.1	2.1
orf19.4175	<i>TOK1</i>	cellular potassium ion homeostasis	1.8	3.4	2.3	2.5	4.3	4.8
orf19.6353			1.8	2.0	0.7	0.6	2.0	2.6
orf19.4488		ATP-dependent chromatin remodeling	1.8	2.0	2.1	2.6	0.9	1.4
orf19.1574		RNA polymerase II transcriptional preinitiation complex assembly	1.8	1.8	2.9	2.6	2.1	2.4
orf19.1631	<i>ERG6</i>	ergosterol biosynthetic process	1.8	1.7	3.5	4.9	0.4	0.5
orf19.2516		protein folding	1.8	1.7	3.3	3.5	1.5	2.0
orf19.6736		maturation of SSU-rRNA	1.8	1.5	1.6	2.1	0.5	1.1
orf19.1864			1.8	1.5	1.3	1.2	1.5	2.1
orf19.4539		small GTPase mediated signal transduction	1.8	1.7	0.9	0.5	1.5	2.3
orf19.2009	<i>PEX12</i>	protein import into peroxisome matrix	1.8	2.1	2.4	3.0	3.0	3.1
orf19.3065	<i>DAO1</i>	oxidation-reduction process	1.8	2.0	3.6	3.0	3.0	3.2
orf19.6047	<i>TUF1</i>	mitochondrial translation	1.8	2.8	1.5	1.4	0.3	0.3
orf19.874		misfolded or incompletely synthesized protein catabolic process	1.8	1.5	1.1	1.0	0.5	1.0

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.5159	<i>DUG3</i>	glutathione catabolic process	1.8	3.6	0.9	0.7	1.3	1.3
orf19.5412		cell wall organization	1.8	1.6	2.3	2.5	4.2	3.7
orf19.6152			1.8	1.6	3.6	3.8	3.0	2.4
orf19.6530		SRP-dependent cotranslational protein targeting to membrane	1.8	2.4	1.7	2.9	6.6	4.0
orf19.7561	<i>DEF1</i>	telomere maintenance	1.8	4.8	1.3	2.5	2.4	2.7
orf19.6209		monocarboxylic acid transport	1.8	1.6	2.0	2.0	2.6	1.6
orf19.5095		sterol transport	1.8	2.2	1.8	2.1	2.4	1.4
orf19.4885	<i>MIR1</i>	phosphate transport	1.8	2.7	1.4	2.1	1.9	1.3
orf19.2808	<i>ZCF16</i>		1.8	2.5	1.9	2.4	4.8	3.4
orf19.4051	<i>HTS1</i>	histidyl-tRNA aminoacylation	1.8	1.7	2.2	2.9	0.9	0.8
orf19.3811	<i>GYP1</i>	vesicle-mediated transport	1.8	1.6	0.9	1.2	0.8	0.9
orf19.5356		fungus-type cell wall organization	1.8	2.9	1.5	2.7	12.5	3.9
orf19.351			1.8	2.7	1.6	1.8	8.7	6.0
orf19.5392	<i>NGT1</i>	N-acetylglucosamine transport	1.8	2.0	1.6	1.6	44.3	29.7
orf19.5806	<i>ALD5</i>	acetate biosynthetic process	1.8	1.7	2.7	2.3	2.0	1.0
orf19.6558		ER to Golgi vesicle-mediated transport	1.8	1.8	1.8	2.8	2.6	2.3
orf19.75	<i>SEC5</i>	protein complex assembly	1.8	2.0	3.2	1.8	2.1	1.6
orf19.5238		metabolic process	1.8	2.5	1.8	1.9	2.8	4.2
orf19.4396		cristae formation	1.8	1.9	1.8	2.3	1.0	0.9
orf19.2646	<i>ZCF13</i>	aerobic respiration	1.8	3.0	2.3	3.6	3.2	2.8
orf19.2753	<i>ZCF15</i>	regulation of transcription, DNA-dependent	1.8	2.1	2.3	3.7	5.9	3.4
orf19.607		transcription-coupled nucleotide-excision repair	1.8	2.0	2.1	1.8	2.5	2.5
orf19.4054	<i>CTA24</i>	positive regulation of transcription, DNA-dependent	1.8	2.5	2.0	2.0	0.5	1.0
orf19.516		cell cycle	1.8	2.0	1.2	1.7	1.1	1.0
orf19.920	<i>RMT2</i>	arginine metabolic process	1.8	1.6	1.3	1.1	1.8	2.7
orf19.173		regulation of transcription, DNA-dependent	1.8	4.2	1.3	2.0	2.3	1.6
orf19.7534	<i>MIS12</i>	folic acid-containing compound biosynthetic process	1.8	5.2	1.3	2.5	3.2	1.7

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4066		phospholipid biosynthetic process	1.8	1.6	2.2	2.2	1.7	0.7
orf19.5662	<i>PEP7</i>	filamentous growth	1.8	1.6	1.0	0.7	3.1	4.8
orf19.7173			1.8	2.6	1.3	0.8	3.8	3.3
orf19.2699	<i>ABP1</i>	actin cortical patch assembly	1.8	1.9	5.2	3.8	2.4	1.1
orf19.255	<i>ZCF1</i>	regulation of transcription, DNA-dependent	1.8	5.7	3.3	4.7	9.2	2.3
orf19.209	<i>DFG10</i>	pseudohyphal growth	1.8	1.5	1.4	1.4	3.3	2.4
orf19.4275	<i>RAD9</i>	hyphal growth	1.8	1.5	1.1	2.7	1.1	1.4
orf19.6639		mitochondrial fission	1.8	1.5	2.6	3.3	2.1	1.8
orf19.1957	<i>CYC3</i>	hyphal growth	1.8	2.4	1.6	2.8	2.0	2.5
orf19.4862.2	<i>PET100</i>	aerobic respiration	1.7	1.8	0.8	0.9	0.4	1.4
orf19.212	<i>VPS28</i>	fungal-type cell wall organization	1.7	2.1	0.5	0.5	1.0	1.3
orf19.27			1.7	1.5	1.5	1.5	2.4	3.4
orf19.4758		oxidation-reduction process	1.7	2.8	2.3	2.9	1.5	1.3
orf19.6211		chromosome segregation	1.7	3.1	1.2	1.2	2.1	1.2
orf19.6698		mitochondrial asparaginyl-tRNA aminoacylation	1.7	1.6	1.4	1.5	0.7	0.9
orf19.1434		nucleotide-excision repair	1.7	2.7	0.8	1.0	0.8	0.9
orf19.1356		wobble position uridine thiolation	1.7	2.1	1.8	3.2	3.1	2.4
orf19.1753	<i>PUS7</i>	enzyme-directed rRNA pseudouridine synthesis	1.7	2.7	1.6	3.1	2.4	2.3
orf19.4511			1.7	1.8	1.5	1.7	2.8	1.6
orf19.2143		negative regulation of transposition, RNA-mediated	1.7	2.4	1.4	2.8	8.2	3.5
orf19.4614		peroxisome degradation	1.7	1.8	1.6	1.5	3.8	3.3
orf19.3962	<i>HAS1</i>	ribosomal small subunit biogenesis	1.7	2.3	0.7	0.8	1.1	2.4
orf19.6445	<i>ECII</i>	filamentous growth	1.7	1.5	3.3	3.0	2.5	4.0
orf19.5940	<i>ZCF32</i>	lysine biosynthetic process via aminoadipic acid	1.7	1.6	1.5	2.2	2.1	1.7
orf19.322			1.7	2.2	2.1	1.4	7.0	1.1
orf19.4864		triglyceride metabolic process	1.7	1.6	1.8	1.6	8.7	6.4
orf19.5236		protein amino acid glycosylation	1.7	2.2	0.5	0.4	1.0	1.8

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.254			1.7	2.6	2.3	2.6	2.9	1.7
orf19.4760		response to drug	1.7	1.5	2.8	4.2	1.5	2.9
orf19.7114	<i>CSA1</i>	cellular iron ion homeostasis	1.7	3.2	1.5	1.5	0.7	0.6
orf19.5384	<i>CHS8</i>	cell wall chitin biosynthetic process	1.7	1.8	1.7	1.9	4.2	2.3
orf19.7544	<i>TLO1</i>		1.7	1.6	2.2	1.7	0.9	1.3
orf19.1795	<i>PUF3</i>	aerobic respiration	1.7	2.1	1.6	1.8	1.5	1.1
orf19.3586		fungal-type cell wall organization	1.7	1.9	3.4	3.5	7.5	3.2
orf19.3342		protein deubiquitination	1.7	2.0	1.1	0.9	2.4	2.5
orf19.3605	<i>PEX17</i>		1.7	1.8	1.8	1.5	4.7	3.0
orf19.2387		prolyl-tRNA aminoacylation	1.7	1.5	1.4	2.0	0.7	0.7
orf19.2908		oxidation-reduction process	1.7	1.5	1.1	0.8	1.1	1.3
orf19.3221	<i>CPA2</i>	arginine biosynthetic process	1.7	3.9	2.7	3.5	5.2	2.2
orf19.3237		protein monoubiquitination	1.7	2.1	1.4	1.4	1.8	2.1
orf19.847	<i>YIM1</i>	mitochondrial protein processing during import	1.7	1.9	1.7	1.6	2.5	1.2
orf19.843		protein amino acid dephosphorylation	1.7	1.7	1.2	1.9	2.6	1.2
orf19.211		DNA repair	1.7	2.4	0.7	0.7	1.5	2.4
orf19.7092			1.7	1.5	1.4	1.5	1.0	1.0
orf19.5311			1.7	3.2	2.3	1.9	3.3	1.2
orf19.117		Golgi to plasma membrane transport	1.7	1.5	2.5	2.5	3.9	3.6
orf19.1801	<i>CBR1</i>	oxidation-reduction process	1.7	1.5	1.3	1.7	1.6	1.3
orf19.7374	<i>CTA4</i>	positive regulation of transcription from RNA pol II promoter	1.7	2.2	0.8	1.1	0.8	1.0
orf19.2248	<i>ARE2</i>	ergosterol metabolic process	1.7	1.6	0.7	0.7	1.1	1.2
orf19.6544	<i>LPI9</i>	chromosome segregation	1.7	2.3	1.9	1.9	2.0	1.5
orf19.7527			1.7	2.1	1.0	1.0	2.3	1.9
orf19.3689		fructose metabolic process	1.7	2.7	2.2	2.6	4.6	3.0
orf19.2791	<i>BBC1</i>	actin cytoskeleton organization	1.7	2.4	3.1	2.8	2.9	1.8
orf19.3171	<i>ACH1</i>	acetate metabolic process	1.7	2.0	1.9	2.4	3.9	0.9

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.3757	<i>ATP20</i>	ATP synthesis coupled proton transport	1.7	1.9	1.2	1.5	1.1	1.1
orf19.273		sterol biosynthetic process	1.7	1.5	1.3	1.7	2.2	1.4
orf19.2665	<i>MSN5</i>	protein export from nucleus	1.7	2.5	1.2	1.0	2.3	1.5
orf19.3029		endocytosis	1.7	1.8	3.4	3.0	2.6	2.7
orf19.5636	<i>RBT5</i>	cellular iron ion homeostasis	1.7	1.6	1.8	1.5	0.2	0.2
orf19.3233	<i>HSE1</i>	ascospore-type prospore assembly	1.7	1.5	1.9	2.1	4.9	3.9
orf19.2077	<i>ZCF9</i>	arginine metabolic process	1.7	1.6	1.1	0.9	3.1	3.2
orf19.6479	<i>SEC1</i>	vesicle docking during exocytosis	1.7	1.7	1.9	2.0	1.6	1.6
orf19.23	<i>RTA3</i>	fatty acid transport	1.7	2.7	4.8	5.4	2.6	1.1
orf19.5912	<i>MAK21</i>	ribosomal large subunit assembly	1.7	3.5	1.3	2.1	2.5	3.1
orf19.6741		fungus-type cell wall organization	1.7	1.7	1.7	2.5	0.9	1.1
orf19.1191		ER-associated protein catabolic process	1.7	2.7	1.6	1.9	7.0	2.3
orf19.3026	<i>MAS1</i>	mitochondrial protein processing during import	1.7	1.6	1.6	1.5	0.9	0.8
orf19.6187			1.7	1.7	2.1	3.0	0.9	0.7
orf19.5854.1		bile acid and bile salt transport	1.7	2.2	1.4	2.8	2.0	4.5
orf19.4357		misfolded or incompletely synthesized protein catabolic process	1.7	1.8	0.9	0.8	0.6	1.3
orf19.1210		amino acid transmembrane export from vacuole	1.7	2.7	1.5	1.2	2.2	1.2
orf19.540		cellular protein modification process	1.7	1.7	0.9	0.6	2.7	2.3
orf19.6794	<i>FESUR1</i>	oxidation-reduction process	1.7	2.1	1.6	2.1	2.0	1.5
orf19.7160		response to osmotic stress	1.7	1.9	1.1	1.5	1.9	3.3
orf19.6522		anion transport	1.7	2.2	1.5	1.4	5.4	3.3
orf19.2242	<i>PRB1</i>	proteolysis	1.7	1.5	0.7	0.6	2.5	2.5
orf19.1940		ubiquinone biosynthetic process	1.7	1.5	1.0	1.0	0.4	0.7
orf19.563	<i>RRP15</i>	maturation of rRNA	1.7	1.5	1.5	4.0	2.2	5.9
orf19.2479	<i>UGA4</i>	gamma-aminobutyric acid transport	1.7	2.9	2.6	6.6	18.2	9.3
orf19.1622	<i>YCG1</i>	meiotic chromosome condensation	1.7	2.1	2.3	3.9	1.3	1.7
orf19.6347		DNA replication	1.7	1.5	1.7	2.8	1.0	1.9

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7675		translation	1.7	1.9	1.8	2.6	0.8	0.8
orf19.7490		transmembrane transport	1.7	2.2	1.0	1.8	6.1	3.8
orf19.3565			1.7	1.7	2.5	2.1	1.6	1.5
orf19.5518			1.7	1.6	1.4	1.4	5.1	2.1
orf19.4950	<i>AKR1</i>	endocytosis	1.7	1.8	1.7	1.7	5.1	2.6
orf19.6726		RNA elongation from RNA polymerase II promoter	1.7	1.8	2.4	3.0	2.9	2.7
orf19.723	<i>BCR1</i>	filamentous growth	1.7	2.1	3.3	3.3	6.3	2.8
orf19.111	<i>CAN2</i>	amino acid transmembrane transport	1.7	13.0	0.7	1.5	1.2	3.5
orf19.4318	<i>MIG1</i>	carbon utilization	1.7	2.1	0.7	1.0	2.1	2.3
orf19.4747	<i>HEM14</i>	heme biosynthetic process	1.7	2.0	0.6	0.4	0.1	0.2
orf19.2346			1.7	2.1	2.0	2.0	4.8	2.3
orf19.3519	<i>SUA72</i>	transcription initiation from RNA polymerase II promoter	1.7	1.7	1.1	1.1	1.7	2.0
orf19.6990		response to stress	1.7	1.6	1.1	0.9	1.5	1.6
orf19.1367	<i>MTW1</i>	chromosome segregation	1.7	1.5	1.1	1.0	0.9	2.2
orf19.164			1.7	2.5	1.2	0.9	4.6	5.1
orf19.5884		rRNA metabolic process	1.7	2.8	1.3	2.2	4.8	2.9
orf19.3133	<i>GUT2</i>	NADH oxidation	1.7	1.8	1.1	1.1	2.2	1.6
orf19.4721		mRNA processing	1.7	1.5	1.0	1.0	0.7	1.9
orf19.7379	<i>FAA2</i>	lipid metabolic process	1.7	3.1	1.3	1.2	4.4	2.8
orf19.1785			1.7	2.2	1.4	1.2	2.9	2.1
orf19.2734		oxidation-reduction process	1.6	1.6	1.9	2.3	5.6	7.4
orf19.135	<i>EXO84</i>	Golgi to plasma membrane transport	1.6	2.2	1.9	2.6	2.8	2.0
orf19.69			1.6	2.3	1.3	1.4	0.8	1.4
orf19.5250			1.6	1.5	3.8	3.2	4.3	4.5
orf19.7529	<i>EPL1</i>	histone acetylation	1.6	2.5	1.0	1.1	2.0	1.5
orf19.2839	<i>CIRT4B</i>	regulation of transcription, DNA-dependent	1.6	2.6	0.4	0.3	0.7	0.6
orf19.1586	<i>FGR22</i>	filamentous growth	1.6	1.7	3.1	4.1	4.0	1.6

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4640	<i>PWP1</i>	rRNA processing	1.6	2.3	1.2	1.5	2.2	1.6
orf19.5419	<i>ATP5</i>	ATP synthesis coupled proton transport	1.6	1.9	1.3	1.6	1.3	1.3
orf19.1255	<i>ZCF5</i>	carbohydrate metabolic process	1.6	2.0	2.2	1.7	6.4	4.2
orf19.5029	<i>MODF</i>		1.6	2.1	1.3	1.3	2.0	1.5
orf19.285		mRNA stabilization	1.6	1.5	1.0	1.0	1.5	2.8
orf19.4788	<i>ARG5,6</i>	arginine biosynthetic process	1.6	2.8	2.8	3.1	4.0	2.9
orf19.2439.1		aerobic respiration	1.6	2.6	1.0	1.2	0.8	1.2
orf19.5543		Golgi to endosome transport	1.6	1.5	3.6	3.1	10.1	5.3
orf19.1124			1.6	2.0	7.9	6.7	7.8	4.6
orf19.2031	<i>VPS24</i>	protein targeting to vacuole	1.6	1.9	2.5	3.2	6.1	6.5
orf19.5537	<i>WSC2</i>	cellular cell wall organization	1.6	2.0	3.8	5.0	12.1	5.6
orf19.6652	<i>DBP8</i>	endonucleolytic cleavage of rRNA	1.6	2.5	1.9	3.0	3.8	4.1
orf19.6105	<i>MVD</i>	ergosterol biosynthetic process	1.6	1.9	1.6	1.4	1.5	0.9
orf19.7629			1.6	1.8	1.7	1.7	3.5	2.1
orf19.5660		protein ubiquitination	1.6	1.5	1.9	1.6	2.6	2.7
orf19.1698		actin cytoskeleton organization	1.6	1.9	1.5	1.8	2.1	0.8
orf19.4739	<i>MSS116</i>	RNA splicing	1.6	1.6	1.5	2.5	1.2	1.5
orf19.1467	<i>COX13</i>	aerobic respiration	1.6	1.7	1.0	1.2	0.6	1.1
orf19.5953		regulation of cell size	1.6	2.4	0.9	1.5	1.2	1.5
orf19.5705	<i>NAM2</i>	mitochondrial translation	1.6	2.0	1.3	1.9	0.7	0.7
orf19.6797			1.6	2.1	1.7	1.8	2.6	1.4
orf19.4906		G1/S transition of mitotic cell cycle	1.6	1.7	1.1	1.6	4.6	2.9
orf19.3706		oxidation-reduction process	1.6	1.5	1.4	1.5	3.3	2.1
orf19.7365		anti-apoptosis	1.6	1.5	1.5	1.6	1.9	1.7
orf19.1133	<i>MSB1</i>	cell adhesion	1.6	1.9	3.6	4.5	2.7	1.6
orf19.7521	<i>REP1</i>	response to drug	1.6	3.7	2.2	7.2	4.5	1.3
orf19.3223	<i>ATP3</i>	ATP synthesis coupled proton transport	1.6	2.1	1.2	1.3	1.5	1.5

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.166	<i>ASG1</i>	positive regulation of transcription from RNA pol II promoter	1.6	3.8	1.4	2.5	1.5	1.5
orf19.2104		iron-sulfur cluster assembly	1.6	1.6	2.5	3.1	2.1	2.0
orf19.1945	<i>AUR1</i>	sphingolipid metabolic process	1.6	2.3	1.3	2.1	7.1	3.8
orf19.6481	<i>YPS7</i>	response to pheromone during conjugation with cellular fusion	1.6	2.3	0.4	0.4	0.9	1.0
orf19.1223	<i>DBF2</i>	cytokinesis	1.6	1.9	2.4	2.6	0.9	0.9
orf19.5247			1.6	1.5	1.0	1.4	1.5	1.7
orf19.5767		nuclear mRNA splicing, via spliceosome	1.6	1.6	1.1	1.1	2.3	2.6
orf19.922	<i>ERG11</i>	ergosterol biosynthetic process	1.6	1.5	1.2	1.0	0.8	0.7
orf19.2881	<i>MNN4</i>	cell wall mannoprotein biosynthetic process	1.6	2.4	1.2	1.6	2.3	1.3
orf19.5535		anion transport	1.6	1.5	1.6	2.1	1.6	1.3
orf19.4159		transport	1.6	1.5	3.0	4.7	1.2	1.0
orf19.2314		rRNA processing	1.6	1.5	2.7	4.2	1.5	7.5
orf19.5296		mitochondrial respiratory chain complex IV assembly	1.6	1.6	1.1	1.3	0.7	1.3
orf19.5335	<i>SGS1</i>	double-strand break repair via homologous recombination	1.6	1.9	1.4	2.4	2.9	1.3
orf19.4478		mitochondrial aspartyl-tRNA aminoacylation	1.6	1.8	1.0	0.9	0.5	0.8
orf19.879		intracellular mRNA localization	1.6	2.2	1.4	1.1	2.5	2.6
orf19.59	<i>REI1</i>	budding cell bud growth	1.6	1.8	2.6	4.4	1.0	4.6
orf19.5139			1.6	1.8	1.2	1.4	3.2	1.4
orf19.6973		proteolysis	1.6	2.1	2.2	2.3	2.2	1.9
orf19.2296			1.6	2.2	2.6	3.0	1.7	0.8
orf19.3616	<i>ERG9</i>	ergosterol biosynthetic process	1.6	1.7	1.2	1.0	0.9	1.1
orf19.2343	<i>VPS23</i>	hyphal growth	1.6	1.8	2.5	1.7	2.8	2.5
orf19.6196		chromosome segregation	1.6	1.5	2.7	2.8	2.3	3.2
orf19.4363	<i>SGD1</i>	hyperosmotic response	1.6	1.7	1.9	3.7	2.1	2.8
orf19.4504		fermentation	1.6	2.7	3.7	3.1	4.5	6.1
orf19.399		sphingolipid metabolic process	1.6	2.0	1.2	1.1	3.8	2.4
orf19.7519			1.6	1.9	1.1	1.1	1.4	1.6

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1297		early endosome to Golgi transport	1.6	2.7	5.7	4.1	6.3	10.2
orf19.2663			1.6	1.9	1.0	1.2	1.2	0.9
orf19.2670		fatty acid biosynthetic process	1.6	1.8	0.4	0.4	0.4	0.7
orf19.5058	<i>SMI1</i>	cellular bud neck	1.6	2.1	1.6	1.3	0.7	1.2
orf19.781	<i>DUR31</i>	urea transport	1.6	1.6	2.0	3.0	6.4	3.3
orf19.3311	<i>IFD3</i>	cellular aldehyde metabolic process	1.6	1.6	2.1	2.2	2.7	3.3
orf19.3991		cellular lipid metabolic process	1.6	1.5	1.4	1.7	2.5	1.9
orf19.2410	<i>SYS3</i>	Golgi to plasma membrane protein transport	1.6	1.9	3.8	3.1	2.0	2.7
orf19.5539		retrograde vesicle-mediated transport, Golgi to ER	1.6	1.6	0.7	0.8	1.6	2.9
orf19.886	<i>PAN1</i>	axial cellular bud site selection	1.6	1.9	2.4	4.4	1.7	1.1
orf19.4688	<i>DAG7</i>	cellular response to drug	1.6	1.6	2.3	2.6	3.5	1.7
orf19.1464	<i>BMT5</i>	glycosphingolipid biosynthetic process	1.6	2.1	1.5	1.5	2.5	1.3
orf19.3360			1.6	2.0	0.9	1.0	1.8	2.1
orf19.2068			1.6	1.5	1.3	1.8	0.6	0.6
orf19.3508		nicotinamide nucleotide metabolic process	1.6	1.9	1.8	1.7	1.7	1.2
orf19.4896		transcription from RNA polymerase I promoter	1.6	2.0	2.2	3.5	1.3	2.5
orf19.6550			1.6	1.6	1.0	1.0	1.5	1.7
orf19.3535			1.6	1.8	1.4	2.2	8.0	5.5
orf19.6898.1			1.6	1.6	1.1	1.5	0.6	0.9
orf19.7599	<i>UTP5</i>	maturation of rRNA	1.6	1.5	1.3	2.6	2.1	1.7
orf19.7270			1.6	2.1	1.6	1.4	2.2	1.0
orf19.5892		protein monoubiquitination	1.6	2.7	1.3	1.0	3.1	2.4
orf19.6045		phosphatidylcholine biosynthetic process	1.6	1.9	0.9	1.0	1.9	1.6
orf19.5110	<i>OPY2</i>	cell cycle arrest in response to pheromone	1.6	2.3	2.9	3.5	13.6	4.9
orf19.4771		protein amino acid glycosylation	1.6	1.8	1.2	1.3	6.5	8.1
orf19.7350	<i>RCT1</i>		1.6	1.6	1.6	1.7	1.4	1.1
orf19.5989		mRNA cleavage	1.6	2.0	2.0	2.0	1.7	1.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.522		cellular chaperone-mediated protein complex assembly	1.6	1.7	2.0	2.2	1.1	1.1
orf19.2736	<i>HFL2</i>	negative regulation of transcription from RNA pol II promoter	1.6	2.1	1.1	1.3	1.6	1.4
orf19.6217	<i>PGA63</i>	ER to Golgi vesicle-mediated transport	1.6	2.8	2.5	3.3	1.6	0.6
orf19.260	<i>SLD1</i>	plasma membrane organization	1.6	1.5	1.3	1.5	1.0	1.4
orf19.1881		glycerophospholipid biosynthetic process	1.6	2.1	1.1	1.4	1.9	1.3
orf19.1648	<i>RAD50</i>	response to oxidative stress	1.6	1.7	2.2	1.7	1.8	1.4
orf19.5407	<i>SOF1</i>	maturation of rRNA	1.6	1.6	1.4	2.2	2.2	3.8
orf19.2644	<i>QCR2</i>	aerobic respiration	1.6	2.4	1.4	1.6	1.3	1.1
orf19.3579	<i>ATP4</i>	ATP synthesis coupled proton transport	1.6	1.8	1.4	2.0	0.9	1.4
orf19.418	<i>KOG1</i>	regulation of cell growth	1.6	2.0	1.4	1.6	3.0	1.4
orf19.6942	<i>ORC3</i>	DNA replication initiation	1.6	1.7	1.9	2.5	1.5	1.4
orf19.4670	<i>CAS5</i>	fungal-type cell wall organization	1.6	2.8	0.1	0.3	4.7	3.3
orf19.6643	<i>MCT1</i>	fatty acid metabolic process	1.6	1.5	3.0	3.0	2.6	1.6
orf19.5893	<i>RIP1</i>	aerobic respiration	1.6	2.2	1.4	1.9	0.7	0.8
orf19.3122	<i>ARR3</i>	arsenite transport	1.6	1.9	1.6	1.3	3.3	3.2
orf19.5005	<i>OSM2</i>	metabolic process	1.6	1.5	2.6	3.6	2.6	1.9
orf19.3553	<i>RPF2</i>	ribosomal large subunit assembly	1.6	1.8	1.1	2.2	1.0	1.5
orf19.603	<i>IMP4</i>	translation	1.5	1.6	1.5	3.2	2.4	3.5
orf19.7481	<i>MDH1</i>	tricarboxylic acid cycle	1.5	1.6	1.4	1.4	1.9	1.0
orf19.109		mitochondrial tyrosyl-tRNA aminoacylation	1.5	1.6	1.3	1.2	0.5	0.5
orf19.1374			1.5	3.1	1.3	1.4	3.8	4.9
orf19.3138	<i>NOPI</i>	rRNA methylation	1.5	2.4	1.2	1.5	0.7	0.7
orf19.1301			1.5	2.0	3.8	3.2	1.1	2.0
orf19.5500	<i>MAK16</i>	maturation of rRNA	1.5	1.9	3.5	6.1	0.9	6.1
orf19.5357	<i>AKL1</i>	actin cortical patch assembly	1.5	1.8	1.9	1.9	3.4	2.1
orf19.1515	<i>CHT4</i>	chitin catabolic process	1.5	1.5	1.0	0.7	5.5	4.8
orf19.5797	<i>PI-PLC</i>	filamentous growth	1.5	2.0	2.7	3.9	4.1	1.5

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5Δ</i> /Δ+FLC / <i>cas5Δ</i> /Δ		<i>upc2Δ</i> /Δ+FLC/ <i>upc2Δ</i> /Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.1589		regulation of transcription, DNA-dependent	1.5	2.3	1.5	3.2	4.7	4.9
orf19.7225			1.5	1.5	1.7	2.1	1.7	3.6
orf19.4750			1.5	1.8	2.3	4.2	2.4	1.1
orf19.5326		glucose metabolic process	1.5	1.9	0.6	0.7	1.3	3.4
orf19.6531	<i>NUC2</i>	oxidation-reduction process	1.5	2.0	1.7	2.2	1.2	1.0
orf19.3546	<i>PEX2</i>	protein import into peroxisome matrix	1.5	1.7	1.7	2.1	3.5	2.0
orf19.3841		autophagic vacuole formation	1.5	2.6	2.2	2.8	9.1	5.3
orf19.4418	<i>FMT1</i>	methionyl-tRNA aminoacylation	1.5	1.5	1.5	2.7	0.9	1.9
orf19.4106			1.5	2.1	3.2	4.6	4.7	3.5
orf19.2073		drug transmembrane transport	1.5	1.6	0.6	0.5	2.1	2.8
orf19.633		metabolic process	1.5	1.6	0.8	0.5	0.3	1.1
orf19.6464			1.5	1.8	1.0	1.2	3.3	2.4
orf19.7537	<i>BNR1</i>	cell separation during cytokinesis	1.5	2.3	1.8	2.3	4.4	2.8
orf19.2046	<i>POT1-2</i>	fatty acid beta-oxidation	1.5	2.2	0.5	0.4	1.6	1.9
orf19.5504			1.5	2.1	1.5	1.8	3.2	2.0
orf19.314		histone deacetylation	1.5	1.8	1.4	1.7	1.9	1.5
orf19.6194			1.5	1.6	1.2	1.3	0.6	0.9
orf19.7357		coenzyme A biosynthetic process	1.5	1.7	2.0	2.6	2.7	2.5
orf19.6117		transmembrane transport	1.5	2.3	0.6	0.4	3.3	3.3
orf19.2786		vesicle-mediated transport	1.5	2.2	1.4	1.7	1.6	1.6
orf19.1956		reciprocal meiotic recombination	1.5	1.8	1.7	2.5	0.8	1.0
orf19.2789			1.5	1.8	1.3	1.3	1.6	1.7
orf19.6014	<i>RRS1</i>	response to drug	1.5	1.7	1.3	2.3	2.7	4.2
orf19.5365			1.5	1.7	1.6	2.4	0.9	0.9
orf19.1658			1.5	1.7	2.5	3.5	3.3	2.1
orf19.6996		protein amino acid O-linked glycosylation	1.5	2.9	1.0	2.5	1.4	2.1
orf19.7664			1.5	1.7	0.7	1.6	1.3	2.4

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6837	<i>FMA1</i>	oxidation-reduction process	1.5	1.5	0.7	0.7	0.9	1.6
orf19.2608	<i>ADH5</i>	biofilm formation	1.5	1.7	1.7	1.5	1.2	1.2
orf19.2308		fructose 2,6-bisphosphate metabolic process	1.5	1.6	0.7	1.0	1.4	1.9
orf19.729.1	<i>RGD3</i>	intracellular signal transduction	1.5	2.0	1.0	0.8	2.3	2.2
orf19.3969	<i>SFL2</i>	filamentous growth	1.5	2.4	1.2	1.2	0.7	0.6
orf19.3782.2		cristae formation	1.5	1.8	1.1	1.5	0.6	0.5
orf19.6140	<i>FRE30</i>		1.5	4.8	9.5	6.1	23.3	15.4
orf19.1047	<i>ERB1</i>	rRNA processing	1.5	1.9	1.0	1.6	1.2	2.2
orf19.1373	<i>INP51</i>	fungal-type cell wall organization	1.5	1.8	2.1	2.4	3.7	2.7
orf19.2691			1.5	1.5	0.5	0.7	0.4	0.7
orf19.6989			1.5	2.5	1.6	1.4	3.2	3.7
orf19.6621	<i>MHP1</i>	filamentous growth	1.5	2.4	1.0	0.9	1.3	1.0
orf19.6527		conjugation with cellular fusion	1.5	1.8	0.8	0.7	4.4	2.7
orf19.1325	<i>ECM38</i>		1.5	1.9	1.6	1.2	1.9	1.5
orf19.7509.1	<i>ATP17</i>	ATP synthesis coupled proton transport	1.5	1.5	1.1	1.1	1.3	1.9
orf19.6601			1.5	3.0	4.1	7.2	6.2	8.4
orf19.649			1.5	2.2	1.2	1.5	3.0	2.3
orf19.6448			1.5	2.3	1.9	2.2	1.2	0.6
orf19.6512	<i>EXO70</i>	Golgi to plasma membrane transport	1.5	1.8	2.6	2.4	2.0	1.7
orf19.916		apoptotic process	1.5	1.7	1.8	1.8	3.7	2.3
orf19.4033	<i>PRP22</i>	generation of catalytic spliceosome for second transesterification step	1.5	1.8	1.9	2.3	2.6	2.8
orf19.1563	<i>ECM3</i>	cell wall organization	1.5	1.5	0.4	0.6	1.2	1.5
orf19.3461			1.5	1.6	2.2	2.9	1.7	1.1
orf19.1077	<i>ATM1</i>	cellular iron ion homeostasis	1.5	2.8	4.5	2.9	3.2	3.4
orf19.4918		regulation of transcription, DNA-dependent	1.5	1.7	1.7	1.9	2.4	1.5
orf19.4759	<i>COX5</i>	aerobic respiration	1.5	2.2	1.5	1.6	0.9	1.2
orf19.3758			1.5	1.6	1.0	0.9	1.6	2.0

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.5167	<i>IFM1</i>	mitochondrial translation	1.5	1.7	1.9	3.2	0.8	1.4
orf19.7551	<i>ALO1</i>	response to oxidative stress	1.5	2.8	0.7	0.6	1.7	1.3
orf19.6225	<i>PCL7</i>	regulation of cyclin-dependent protein kinase activity	1.5	2.0	0.8	0.8	1.5	1.3
orf19.5660.1		ATP synthesis coupled proton transport	1.5	1.8	1.1	1.6	1.2	1.6
orf19.1816	<i>ALS3</i>	biofilm formation	1.5	3.1	1.3	1.3	2.6	1.1
orf19.1981	<i>IMP2</i>	mitochondrial protein processing during import	1.5	1.5	1.8	2.3	1.3	2.0
orf19.7619		mitochondrion organization	1.5	1.9	2.1	2.6	3.6	3.5
orf19.191	<i>KIC1</i>	cell wall organization	1.5	1.9	1.2	1.2	1.7	1.3
orf19.4634		protein urmylation	1.5	3.2	2.1	2.4	3.2	3.8
orf19.7661	<i>HMI1</i>	mitochondrial genome maintenance	1.5	1.6	1.4	1.0	2.0	1.9
orf19.2309		RNA processing	1.5	1.6	1.8	4.0	1.2	1.3
orf19.6979		cellular manganese ion homeostasis	1.5	5.7	3.8	2.5	5.3	2.9
orf19.3827		misfolded or incompletely synthesized protein catabolic process	1.5	1.6	1.4	2.3	6.2	3.6
orf19.1371			1.5	1.6	1.3	1.7	0.8	0.6
orf19.1900		nucleolus organization	1.5	1.6	2.4	3.8	1.1	1.5
orf19.7187	<i>MAM33</i>	aerobic respiration	1.5	2.3	1.7	1.3	1.1	1.5
orf19.7027			1.5	2.2	0.7	1.3	2.4	2.2
orf19.4805		Golgi to plasma membrane transport	1.5	1.5	1.3	2.5	2.5	1.3
orf19.4127		actin cortical patch localization	1.5	1.8	1.8	1.8	1.6	1.2
orf19.783		intracellular protein transport	1.5	2.2	2.0	3.4	7.4	3.2
orf19.3309			1.5	1.7	0.8	0.6	3.2	2.8
orf19.738	<i>MYO5</i>	cell wall organization	1.5	2.0	1.9	1.9	2.7	1.4
orf19.4645	<i>BEM1</i>	cell budding	1.5	1.9	1.5	1.6	0.4	0.6
orf19.3201	<i>MTLA1</i>	cell differentiation	1.5	1.5	1.8	2.1	2.4	4.5
orf19.1671	<i>UTR2</i>	cell wall organization	1.5	1.6	1.9	2.5	3.8	2.6
orf19.3302		regulation of glycogen biosynthetic process	1.5	2.1	0.7	0.8	1.1	0.9
orf19.5619			1.5	2.9	1.4	1.6	5.7	3.8

Table A-1. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6297		tRNA pseudouridine synthesis	1.5	1.8	1.5	2.0	2.4	2.6
orf19.5134			1.5	2.2	0.9	2.0	1.7	6.0
orf19.2006.1	<i>COX17</i>	intracellular copper ion transport	1.5	1.6	1.8	1.2	0.7	1.0
orf19.5234	<i>RBD1</i>	filamentous growth	1.5	1.8	1.0	1.2	4.3	2.7
orf19.5168		maturation of SSU-rRNA	1.5	1.7	1.6	2.4	1.9	2.3
orf19.4764		mRNA 3'-end processing	1.5	1.5	1.9	1.8	2.5	1.1
orf19.7569	<i>SIK1</i>	rRNA processing	1.5	2.2	1.3	1.7	0.6	0.9
orf19.1776		coenzyme A biosynthetic process	1.5	1.5	1.6	1.9	0.6	1.0
orf19.3083		DNA recombination	1.5	1.8	1.0	1.6	0.8	0.3
orf19.4457	<i>BNI4</i>	filamentous growth	1.5	2.0	1.4	1.9	2.2	1.0
orf19.3603		multicellular organismal development	1.5	1.5	2.8	2.8	1.6	1.5
orf19.6070	<i>ENA2</i>	potassium ion transport	1.5	2.1	0.9	1.7	11.7	6.1
orf19.6854	<i>ATP1</i>	ATP biosynthetic process	1.5	2.0	1.2	1.0	1.2	1.0
orf19.3195	<i>HIP1</i>	amino acid transmembrane transport	1.5	2.6	0.6	0.7	0.7	0.8
orf19.1187	<i>CPH2</i>	filamentous growth	1.5	2.2	1.4	1.7	3.5	2.1
orf19.3004			1.5	2.0	0.6	0.6	0.9	1.4
orf19.4317	<i>GRE3</i>	response to oxidative stress	1.5	1.5	0.8	0.6	0.3	0.7
orf19.2443	<i>RGD1</i>	osmosensory signaling pathway	1.5	1.6	1.4	1.2	1.6	1.7

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).

Table A-2. Genes downregulated by at least 0.5-fold by fluconazole in SC5314

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.5673	<i>OPT7</i>	oligopeptide transport	0.0	0.1	0.2	0.2	0.0	0.0
orf19.2475	<i>PGA26</i>	filamentous growth	0.1	0.0	0.2	0.2	0.1	0.2
orf19.946	<i>MET14</i>	methionine metabolic process	0.1	0.1	1.1	2.5	0.4	0.2
orf19.689	<i>PLB1</i>	pathogenesis	0.1	0.0	0.0	0.0	0.0	0.0
orf19.2337	<i>ALP1</i>	basic amino acid transport	0.1	0.1	0.3	0.4	0.4	0.3
orf19.5017	<i>DUR32</i>	transmembrane transport	0.1	0.1	0.7	0.6	0.9	0.2
orf19.3475			0.1	0.1	0.4	0.4	2.0	2.1
orf19.2738	<i>SUL2</i>	sulfate transport	0.1	0.3	1.7	1.2	5.4	0.8
orf19.5399	<i>IFF11</i>	pathogenesis	0.1	0.1	0.0	0.0	0.0	0.0
orf19.1887		sterol metabolic process	0.1	0.1	0.4	0.5	0.6	0.5
orf19.7330		thiamin biosynthetic process	0.1	0.2	0.4	0.3	0.1	0.2
orf19.6679			0.1	0.3	1.0	2.8	1.0	1.5
orf19.386	<i>SAM4</i>	L-methionine biosynthetic process from S-adenosylmethionine	0.2	0.1	0.3	0.3	0.1	0.1
orf19.1442	<i>PLB4.5</i>	phosphoinositide metabolic process	0.2	0.3	0.4	0.3	0.0	0.0
orf19.769	<i>IFE1</i>	oxidation-reduction process	0.2	0.1	1.0	1.2	0.1	0.1
orf19.4526	<i>HSP30</i>	negative regulation of ATPase activity	0.2	0.1	2.6	1.2	0.8	1.5
orf19.4669	<i>AAT22</i>	aspartate catabolic process	0.2	0.1	0.3	0.4	0.0	0.0
orf19.385	<i>GCV2</i>	glycine catabolic process	0.2	0.3	1.2	2.5	0.9	0.1
orf19.1368			0.2	0.3	0.2	0.1	0.1	0.1
orf19.2618	<i>MET2</i>	homoserine metabolic process	0.2	0.2	0.8	0.9	0.6	0.3
orf19.5103			0.2	0.3	0.1	0.1	0.1	0.2
orf19.6770		actin cortical patch assembly	0.2	0.2	0.1	0.1	0.2	0.2
orf19.6086	<i>LEU4</i>	leucine biosynthetic process	0.2	0.3	0.6	0.8	0.1	0.1
orf19.7284	<i>ASR2</i>		0.2	0.1	1.7	1.7	0.3	0.3
orf19.7098		oxidation-reduction process	0.2	0.1	0.9	0.8	0.6	0.9
orf19.1447			0.2	0.3	1.0	2.7	0.7	0.3

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		cas5Δ/Δ+FLC /cas5Δ/Δ		upc2Δ/Δ+FLC/ upc2Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.768	<i>SYG1</i>	signal transduction	0.2	0.4	0.1	0.1	0.4	0.6
orf19.5870	<i>CTP1</i>	mitochondrial citrate transport	0.2	0.2	0.2	0.4	0.1	0.3
orf19.6570	<i>NUP</i>	nucleoside transport	0.2	0.1	0.7	0.3	0.1	0.1
orf19.7553			0.2	0.5	3.6	3.2	0.7	1.2
orf19.4664	<i>NAT4</i>	cellular developmental process	0.2	0.2	0.1	0.1	0.0	0.1
orf19.7586	<i>CHT3</i>	cellular bud site selection	0.2	0.3	0.2	0.2	0.0	0.0
orf19.3897			0.2	0.3	1.5	1.2	0.2	0.5
orf19.3916			0.2	0.2	0.9	0.6	0.4	0.5
orf19.3048			0.2	0.3	0.5	0.9	0.9	0.3
orf19.3106	<i>MET16</i>	methionine metabolic process	0.2	0.3	0.8	1.4	1.2	0.6
orf19.4456	<i>GAP4</i>	amino acid transport	0.2	0.3	0.5	0.7	0.9	0.6
orf19.923	<i>THR1</i>	homoserine metabolic process	0.2	0.2	0.2	0.2	0.1	0.2
orf19.4438	<i>RME1</i>	meiosis	0.2	0.4	0.4	0.3	0.0	0.2
orf19.6077			0.2	0.3	0.3	0.3	0.9	0.5
orf19.7199			0.3	0.2	0.1	0.2	0.1	0.2
orf19.4907		regulation of cell size	0.3	0.4	0.5	0.4	0.3	0.2
orf19.4651	<i>PGA53</i>		0.3	0.2	0.4	0.4	0.2	0.2
orf19.3664	<i>HSP31</i>	protein refolding	0.3	0.2	1.0	0.8	0.3	0.7
orf19.7310		reciprocal meiotic recombination	0.3	0.3	2.5	2.6	1.1	0.5
orf19.5992	<i>WOR2</i>	cellular developmental process	0.3	0.2	0.5	0.4	0.9	0.6
orf19.4889		drug transport	0.3	0.2	0.7	1.0	0.6	0.5
orf19.7025	<i>MCM1</i>	hyphal growth	0.3	0.4	0.5	0.6	0.5	0.7
orf19.4142			0.3	0.3	1.5	6.2	2.7	1.1
orf19.2602	<i>OPT1</i>	nitrogen utilization	0.3	0.2	0.8	0.5	0.1	0.1
orf19.1333	<i>SNG3</i>	response to drug	0.3	0.3	0.2	0.2	0.5	0.4
orf19.4375		chromatin silencing at rDNA	0.3	0.3	0.4	0.5	0.4	0.8
orf19.1075			0.3	0.5	1.5	1.4	2.5	1.4

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.2018		peptidyl-diphthamide biosynthetic process	0.3	0.3	2.1	4.1	2.0	2.3
orf19.5663	<i>RCH1</i>	calcium ion import	0.3	0.2	0.9	0.8	1.3	1.6
orf19.2246			0.3	0.1	0.6	0.8	0.7	2.1
orf19.3433	<i>OYE23</i>	oxidation-reduction process	0.3	0.5	0.5	0.2	0.1	0.1
orf19.473	<i>TPO4</i>	drug transport	0.3	0.3	0.9	1.0	0.5	0.4
orf19.5484	<i>SER1</i>	purine base biosynthetic process	0.3	0.3	0.8	0.9	0.4	0.5
orf19.5615	<i>AYR2</i>	phosphatidic acid biosynthetic process	0.3	0.2	0.3	0.2	0.2	0.2
orf19.21		ethanol metabolic process	0.3	0.3	0.2	0.2	0.3	0.8
orf19.5805	<i>DLD1</i>	aerobic respiration	0.3	0.5	1.1	1.5	0.9	0.5
orf19.1510	<i>SRD1</i>		0.3	0.4	0.8	1.4	1.8	0.8
orf19.1159			0.3	0.5	1.6	2.9	4.0	1.1
orf19.3915		glutathione catabolic process	0.3	0.4	0.4	0.5	0.4	0.3
orf19.6656	<i>DUR3</i>	urea transport	0.3	0.3	0.3	0.3	0.4	0.3
orf19.6636			0.3	0.2	0.4	0.4	0.2	0.2
orf19.1443	<i>PLB4</i>	phosphoinositide metabolic process	0.3	0.4	0.5	0.4	0.1	0.0
orf19.1182		Golgi to vacuole transport	0.3	0.3	0.7	1.0	0.8	0.5
orf19.1968.1		N-terminal protein amino acid acetylation	0.3	0.3	1.1	1.6	0.4	0.3
orf19.5032	<i>SIM1</i>	cell wall organization	0.3	0.4	0.6	1.0	0.8	1.0
orf19.1901	<i>MCM3</i>	DNA replication initiation	0.3	0.4	1.1	2.1	0.2	0.1
orf19.2396	<i>IFR2</i>	oxidation-reduction process	0.3	0.2	0.9	0.6	0.2	0.1
orf19.1253	<i>PHO4</i>	cellular response to phosphate starvation	0.3	0.2	1.0	1.5	0.8	0.6
orf19.2639		mitochondrial genome maintenance	0.3	0.2	1.0	2.5	0.1	0.6
orf19.4192	<i>CDC14</i>	hyphal growth	0.3	0.2	0.6	0.8	0.3	0.5
orf19.6817	<i>FCR1</i>	filamentous growth	0.3	0.4	0.1	0.1	0.3	0.2
orf19.4733	<i>YMC2</i>	transport	0.3	0.3	0.9	0.9	0.9	0.4
orf19.139	<i>TRA1</i>	histone acetylation	0.3	0.4	2.3	1.1	0.7	0.4
orf19.6580			0.3	0.3	0.5	0.5	0.1	0.2

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4816			0.3	0.2	0.4	0.3	0.5	0.6
orf19.1517	<i>ARO3</i>	aromatic amino acid family biosynthetic process	0.3	0.3	0.7	1.1	0.2	0.2
orf19.5316	<i>FGR29</i>	filamentous growth	0.3	0.5	0.9	0.9	0.7	0.5
orf19.5519	<i>GCV1</i>	glycine metabolic process	0.3	0.2	1.0	1.0	1.1	0.6
orf19.2452			0.3	0.2	0.2	0.5	0.2	0.1
orf19.1116			0.3	0.2	0.2	0.1	0.2	0.3
orf19.1390	<i>PMI1</i>	fungal-type cell wall organization	0.3	0.3	0.4	0.4	0.3	0.2
orf19.5267			0.3	0.3	0.2	0.3	0.0	0.0
orf19.2989	<i>GOR1</i>	glyoxylate catabolic process	0.3	0.3	0.5	0.4	0.2	0.2
orf19.1697		cytoplasmic translation	0.3	0.4	1.0	1.9	0.5	0.9
orf19.2600	<i>SPC98</i>	microtubule nucleation	0.4	0.4	1.6	3.7	1.7	1.3
orf19.655	<i>PHO84</i>	phosphate transport	0.4	0.5	0.6	0.5	0.1	0.6
orf19.6001	<i>SAP3</i>	adhesion to host	0.4	0.2	0.1	0.1	0.1	1.1
orf19.5496	<i>AVT1</i>		0.4	0.4	0.3	0.5	0.5	0.7
orf19.335		chromosome segregation	0.4	0.3	0.6	0.6	0.7	0.8
orf19.1621	<i>GPA2</i>	hyphal growth	0.4	0.5	0.7	0.8	0.9	1.0
orf19.4718	<i>TRP5</i>	tryptophan biosynthetic process	0.4	0.4	0.8	1.1	0.3	0.5
orf19.4068			0.4	0.4	1.1	1.1	0.5	0.4
orf19.6022	<i>NRM1</i>	cellular response to hydroxyurea	0.4	0.4	1.9	2.5	0.3	0.2
orf19.1258			0.4	0.2	0.9	1.2	1.0	0.4
orf19.1756	<i>GPD1</i>	glycerol metabolic process	0.4	0.4	0.2	0.2	0.1	0.2
orf19.7218	<i>RBE1</i>	pathogenesis	0.4	0.3	0.2	0.4	0.0	0.0
orf19.3648		DNA repair	0.4	0.3	1.0	1.2	0.4	0.4
orf19.7166			0.4	0.1	0.5	0.6	0.3	0.4
orf19.5051			0.4	0.2	1.3	2.3	0.6	0.9
orf19.3230	<i>BOI2</i>	budding cell apical bud growth	0.4	0.4	1.0	1.4	1.5	0.9
orf19.6951		calcium-mediated signaling	0.4	0.5	0.7	0.9	1.1	0.5

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC/ <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7190	<i>OGG1</i>	base-excision repair, AP site formation	0.4	0.4	3.5	5.1	1.6	0.6
orf19.2335		response to drug	0.4	0.3	0.3	0.3	0.2	0.2
orf19.6782	<i>BMT1</i>	beta-1,2-oligomannoside metabolic process	0.4	0.4	1.0	0.8	0.1	0.1
orf19.5760	<i>IHD1</i>		0.4	0.5	0.1	0.3	0.1	0.4
orf19.3160	<i>HSP12</i>	cell adhesion	0.4	0.3	1.7	1.4	0.5	0.5
orf19.3618	<i>YWP1</i>	cell-cell adhesion	0.4	0.3	0.7	0.7	0.1	0.0
orf19.6146	<i>CLG1</i>	cell cycle	0.4	0.5	0.6	1.0	1.5	1.0
orf19.2187	<i>ALG7</i>	aerobic respiration	0.4	0.4	0.4	0.7	0.5	0.4
orf19.4520		D-gluconate metabolic process	0.4	0.2	0.6	0.6	0.5	0.6
orf19.4390			0.4	0.3	1.1	1.8	0.3	0.2
orf19.3146			0.4	0.4	0.7	0.7	0.2	0.2
orf19.568	<i>SPE2</i>	pantothenate biosynthetic process	0.4	0.2	1.4	2.0	2.1	1.4
orf19.6424		G2/M transition of mitotic cell cycle	0.4	0.2	0.3	0.5	0.3	0.9
orf19.5789	<i>ADE8</i>	adenine biosynthetic process	0.4	0.2	1.3	1.9	0.6	0.6
orf19.6691		drug transmembrane transport	0.4	0.4	0.6	1.3	1.8	1.4
orf19.7564	<i>DPB2</i>	DNA repair	0.4	0.3	1.1	2.6	0.6	0.3
orf19.5612	<i>BMT4</i>	beta-1,2-oligomannoside metabolic process	0.4	0.3	0.7	0.6	1.0	0.6
orf19.6562	<i>RNH35</i>	DNA replication, removal of RNA primer	0.4	0.3	0.2	0.4	0.2	0.3
orf19.3810		folic acid and derivative biosynthetic process	0.4	0.3	1.1	1.9	1.2	0.7
orf19.4783			0.4	0.2	0.3	0.2	0.2	0.3
orf19.3099	<i>TRP4</i>	tryptophan biosynthetic process	0.4	0.4	0.8	1.9	0.2	0.3
orf19.2529		GPI anchor biosynthetic process	0.4	0.4	0.9	1.4	0.8	0.6
orf19.3430			0.4	0.4	0.7	0.9	0.3	0.3
orf19.4910	<i>FGR41</i>	filamentous growth	0.4	0.4	0.1	0.2	0.0	0.0
orf19.3469		regulation of transcription involved in G1/S phase	0.4	0.4	0.6	0.8	0.9	0.6
orf19.4241			0.4	0.3	0.5	0.6	0.6	0.7
orf19.5369		heme biosynthetic process	0.4	0.4	0.7	0.8	0.7	0.7

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		cas5Δ/Δ+FLC /cas5Δ/Δ		upc2Δ/Δ+FLC/ upc2Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6008			0.4	0.4	0.4	0.5	0.1	0.1
orf19.1757		regulation of transcription, DNA-dependent	0.4	0.2	1.6	3.3	0.7	0.6
orf19.3591	<i>APE3</i>	vacuolar protein catabolic process	0.4	0.4	0.6	0.7	0.9	0.5
orf19.7637	<i>YHB4</i>	response to stress	0.4	0.5	0.3	0.2	1.0	1.5
orf19.3139		oxidation-reduction process	0.4	0.3	0.3	0.3	0.1	0.3
orf19.1167		sulfur compound catabolic process	0.4	0.1	1.4	1.1	0.3	0.2
orf19.7106	<i>VPS70</i>	protein targeting to vacuole	0.4	0.5	1.0	1.1	3.9	1.0
orf19.5455		microautophagy	0.4	0.2	0.4	0.3	0.3	0.3
orf19.7667	<i>IAH1</i>	filamentous growth	0.4	0.4	0.5	0.5	0.2	0.4
orf19.4195.1	<i>FCA1</i>	cytosine metabolic process	0.4	0.3	0.2	0.3	0.2	0.4
orf19.4177	<i>HIS5</i>	histidine biosynthetic process	0.4	0.5	1.1	1.2	0.9	0.9
orf19.7149		endocytosis	0.4	0.4	0.9	0.9	0.4	0.3
orf19.6977	<i>GPI1</i>	GPI anchor biosynthetic process	0.4	0.5	0.4	0.3	0.2	0.4
orf19.4666			0.4	0.5	1.0	1.8	1.1	1.1
orf19.5280	<i>MUP1</i>	sulfur amino acid transport	0.4	0.4	0.5	0.6	0.3	0.4
orf19.4499	<i>RIM2</i>	mitochondrial genome maintenance	0.4	0.3	0.7	1.0	0.3	0.6
orf19.5495			0.4	0.5	1.3	1.4	1.3	0.7
orf19.1049			0.4	0.4	0.7	1.0	0.4	0.5
orf19.5057			0.4	0.3	5.8	1.9	1.8	0.7
orf19.1332	<i>SNG4</i>		0.4	0.3	0.4	0.4	0.7	0.6
orf19.3192	<i>STI1</i>	protein complex assembly	0.4	0.5	0.6	0.8	0.5	0.5
orf19.2041		chromatin remodeling	0.4	0.5	1.0	1.1	0.5	0.4
orf19.2936			0.4	0.3	1.2	1.6	1.9	0.7
orf19.1559	<i>HOM2</i>	methionine metabolic process	0.4	0.3	0.5	0.4	0.3	0.5
orf19.5136			0.4	0.5	0.1	0.1	0.1	0.3
orf19.1971	<i>TRY3</i>	positive regulation of cell-substrate adhesion	0.4	0.5	0.5	0.3	0.2	0.7
orf19.3104	<i>YDC1</i>	ceramide metabolic process	0.4	0.3	0.7	0.6	1.0	0.5

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.6010	<i>CDC5</i>	signal transduction during filamentous growth	0.4	0.3	1.0	1.3	0.2	0.2
orf19.7023		chromatin remodeling	0.4	0.3	0.5	0.5	0.1	0.4
orf19.5071	<i>NRP1</i>		0.4	0.5	0.6	1.7	1.0	0.8
orf19.4284	<i>BUR2</i>	mitotic sister chromatid segregation	0.4	0.3	1.7	4.8	0.4	2.5
orf19.34	<i>GIT1</i>	phospholipid transport	0.4	0.1	0.7	0.1	0.2	0.0
orf19.3016		meiotic chromosome segregation	0.4	0.3	0.8	0.9	0.9	1.0
orf19.7324	<i>THI13</i>	thiamin biosynthetic process	0.4	0.5	1.0	0.8	0.8	0.8
orf19.5839	<i>PDR17</i>	sterol biosynthetic process	0.4	0.3	0.9	1.7	0.9	0.6
orf19.3394			0.4	0.4	0.4	0.4	0.8	0.7
orf19.6489	<i>FMP45</i>	cellular cell wall organization	0.4	0.3	3.1	2.1	32.8	9.5
orf19.5745	<i>ALS9</i>	cell adhesion	0.4	0.4	0.2	0.1	0.3	0.3
orf19.3688		cell wall mannoprotein biosynthetic process	0.4	0.5	1.3	1.5	0.7	1.4
orf19.3252	<i>DAL81</i>	positive regulation of cell adhesion	0.4	0.3	1.3	1.6	0.8	0.9
orf19.4026	<i>HIS1</i>	histidine biosynthetic process	0.4	0.4	0.7	0.7	0.4	0.7
orf19.103	<i>KAR5</i>	karyogamy involved in conjugation with cellular fusion	0.4	0.4	1.0	1.5	0.7	2.6
orf19.6948	<i>CCC1</i>	cellular calcium ion homeostasis	0.4	0.3	0.7	0.7	1.7	0.9
orf19.3446		protein import into nucleus	0.4	0.3	0.5	0.6	0.2	0.7
orf19.514	<i>SNP3</i>	nuclear mRNA splicing, via spliceosome	0.4	0.2	0.4	0.5	0.1	0.3
orf19.7479	<i>NTH1</i>	trehalose catabolic process	0.4	0.5	0.3	0.3	0.3	0.5
orf19.687			0.4	0.2	0.8	1.3	0.7	0.5
orf19.908	<i>FEN12</i>	fatty acid elongation	0.4	0.3	0.5	0.8	0.3	0.3
orf19.3211	<i>RCF3</i>	leading strand elongation	0.4	0.4	0.8	1.2	0.3	0.4
orf19.708		microtubule nucleation	0.4	0.4	0.7	0.9	0.5	0.4
orf19.348	<i>SKN2</i>	1,6-beta-glucan biosynthetic process	0.4	0.3	1.4	1.3	3.2	2.3
orf19.756	<i>SAP7</i>	pathogenesis	0.4	0.4	0.9	0.6	13.6	6.6
orf19.3322	<i>DUT1</i>	DNA replication	0.4	0.4	0.8	0.8	0.3	0.2
orf19.1168	<i>ZCF3</i>	filamentous growth	0.4	0.1	1.5	2.2	0.5	1.0

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4447	<i>YMC1</i>	mitochondrial transport	0.4	0.4	0.4	0.6	0.4	0.5
orf19.7279		nitrogen compound metabolic process	0.4	0.2	1.0	1.3	0.4	0.3
orf19.927		telomere maintenance	0.4	0.2	1.3	1.8	1.0	1.6
orf19.2686		nitrogen compound metabolic process	0.4	0.5	1.1	1.1	0.7	0.4
orf19.6786			0.4	0.0	3.0	9.2	0.7	1.5
orf19.5242	<i>CDC6</i>	DNA replication initiation	0.4	0.5	0.7	2.2	0.1	0.1
orf19.5736	<i>ALS5</i>	cell adhesion	0.4	0.3	0.7	1.1	3.0	2.2
orf19.7100		amino acid transport	0.4	0.4	0.6	0.8	1.0	0.5
orf19.1832	<i>FCY23</i>	vitamin transport	0.4	0.4	1.1	2.7	0.7	0.4
orf19.4301		chromatin silencing at rDNA	0.4	0.4	1.0	2.8	1.1	0.2
orf19.7293	<i>MPS1</i>	mitotic cell cycle spindle assembly checkpoint	0.4	0.5	1.6	4.5	0.8	0.7
orf19.321		amino acid transport	0.4	0.5	0.4	0.4	0.9	0.6
orf19.6416		cellular response to oxidative stress	0.4	0.4	2.2	3.3	3.8	2.0
orf19.4280			0.4	0.5	0.3	0.4	0.2	0.5
orf19.333	<i>FCY2</i>	drug transport	0.4	0.4	0.2	0.3	0.1	0.2
orf19.398			0.4	0.4	1.3	1.2	1.2	1.3
orf19.4998	<i>ROB1</i>	cellular amino acid catabolic process	0.4	0.4	0.3	0.4	0.1	0.3
orf19.7344		chromosome segregation	0.4	0.4	1.8	3.5	0.6	1.2
orf19.7427			0.4	0.3	0.7	1.1	1.9	1.3
orf19.1308		drug transport	0.4	0.3	1.1	1.6	1.0	0.5
orf19.660			0.4	0.4	2.0	3.5	1.8	2.1
orf19.1318			0.4	0.2	0.8	1.3	0.6	0.6
orf19.4161		DNA repair	0.4	0.4	2.4	4.3	0.6	0.3
orf19.4665			0.4	0.4	0.6	0.7	0.7	0.6
orf19.7554		drug transport	0.5	0.3	1.1	2.0	10.9	11.4
orf19.1240			0.5	0.5	0.6	0.5	0.7	0.5
orf19.6784	<i>PGA32</i>	cellular cell wall organization	0.5	0.4	0.9	1.4	0.4	0.2

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.28		thiamin pyrophosphate transport	0.5	0.3	0.7	0.8	0.5	1.0
orf19.2951	<i>HOM6</i>	methionine metabolic process	0.5	0.4	0.6	0.6	0.5	0.6
orf19.1238	<i>TUB4</i>	microtubule nucleation	0.5	0.4	1.1	1.3	0.7	0.8
orf19.7083	<i>DCC1</i>	mitotic sister chromatid cohesion	0.5	0.4	0.9	1.6	0.5	0.5
orf19.2336			0.5	0.4	0.6	0.8	0.6	0.9
orf19.5074		protein sumoylation	0.5	0.4	1.0	1.4	0.7	0.5
orf19.1948			0.5	0.4	0.8	0.8	0.4	0.4
orf19.4046			0.5	0.5	0.5	0.4	0.7	1.0
orf19.3788	<i>SPC34</i>	mitotic spindle organization in nucleus	0.5	0.3	1.2	1.2	0.8	1.3
orf19.1613	<i>ILV2</i>	branched chain family amino acid biosynthetic process	0.5	0.5	0.5	0.7	0.2	0.2
orf19.3042			0.5	0.4	1.0	3.3	1.1	3.5
orf19.4040	<i>ILV3</i>	branched chain family amino acid biosynthetic process	0.5	0.5	0.4	0.3	0.1	0.2
orf19.1847	<i>ARO10</i>	aromatic amino acid family catabolic process to alcohol	0.5	0.4	0.8	0.6	0.5	0.1
orf19.5917.3		mRNA export from nucleus	0.5	0.4	0.4	0.4	0.2	0.2
orf19.2849	<i>AQY1</i>	response to osmotic stress	0.5	0.5	0.6	0.5	0.0	0.0
orf19.6177			0.5	0.3	0.8	0.9	0.2	0.4
orf19.6858	<i>EDC3</i>	cytoplasmic mRNA processing body assembly	0.5	0.4	1.8	2.0	0.6	1.1
orf19.547		DNA replication, removal of RNA primer	0.5	0.3	1.8	8.1	1.3	0.5
orf19.1796		metabolic process	0.5	0.4	0.3	0.3	0.3	0.5
orf19.6671	<i>LAP4</i>	vacuolar protein catabolic process	0.5	0.3	0.9	0.7	0.8	0.6
orf19.6189		cellular modified amino acid biosynthetic process	0.5	0.5	0.5	0.6	1.0	1.1
orf19.4233	<i>THR4</i>	threonine metabolic process	0.5	0.5	0.5	0.4	0.2	0.4
orf19.2547			0.5	0.2	1.7	2.7	0.9	1.0
orf19.704	<i>SOL3</i>	tRNA processing	0.5	0.3	0.7	0.5	0.4	0.4
orf19.6936	<i>RAD53</i>	hyphal growth	0.5	0.3	0.9	1.3	0.7	0.6
orf19.1626		peptidyl-lysine modification to hypusine	0.5	0.4	0.3	0.4	0.1	0.3
orf19.6751		tRNA methylation	0.5	0.3	0.7	1.6	0.5	1.3

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7244		regulation of transcription, DNA-dependent	0.5	0.5	0.8	0.9	0.3	0.6
orf19.6291		chromosome organization	0.5	0.4	2.0	2.5	1.0	0.7
orf19.802	<i>UGA1</i>	gamma-aminobutyric acid catabolic process	0.5	0.5	1.5	2.0	0.5	0.6
orf19.105	<i>HAL22</i>	methionine biosynthetic process	0.5	0.5	0.9	0.7	0.7	0.5
orf19.1802		translational termination	0.5	0.5	0.2	0.2	0.1	0.2
orf19.7375		nuclear mRNA splicing, via spliceosome	0.5	0.3	1.3	1.8	1.1	2.1
orf19.7358	<i>SAM50</i>	protein complex assembly	0.5	0.1	0.7	1.2	0.5	0.4
orf19.5647	<i>SUB2</i>	RNA elongation from RNA polymerase II promoter	0.5	0.5	0.4	0.4	0.3	0.4
orf19.2637		response to unfolded protein	0.5	0.3	0.5	0.6	0.4	0.4
orf19.4809	<i>ERG12</i>	ergosterol biosynthetic process	0.5	0.4	0.5	0.9	0.5	0.5
orf19.6365	<i>PTP1</i>	protein amino acid dephosphorylation	0.5	0.4	0.4	0.5	0.9	1.1
orf19.2969	<i>RAD16</i>	nucleotide-excision repair, DNA damage recognition	0.5	0.5	0.4	0.4	0.3	0.7
orf19.5825	<i>NCB2</i>	negative regulation of transcription from RNA polymerase II	0.5	0.2	0.7	0.7	0.4	0.6
orf19.7152		cysteine biosynthetic process	0.5	0.5	0.8	0.7	0.7	0.6
orf19.6692	<i>MNN7</i>	protein amino acid glycosylation	0.5	0.4	0.9	1.1	0.9	0.6
orf19.3972		ER to Golgi vesicle-mediated transport	0.5	0.4	1.2	1.6	1.3	1.0
orf19.5528	<i>MOB1</i>	mitosis	0.5	0.5	0.3	0.2	0.5	0.6
orf19.3914		translational initiation	0.5	0.3	0.9	1.0	0.3	0.4
orf19.3089		cardiolipin metabolic process	0.5	0.3	1.2	1.9	0.9	1.0
orf19.5841			0.5	0.5	0.7	0.8	0.8	1.0
orf19.5919	<i>MEA1</i>	actin cortical patch localization	0.5	0.3	1.1	1.4	0.8	0.5
orf19.5665		dehydro-D-arabinono-1,4-lactone biosynthetic process	0.5	0.5	0.8	1.0	1.0	0.7
orf19.6755	<i>DLD2</i>	aerobic respiration	0.5	0.5	0.6	0.4	0.4	0.5
orf19.1358	<i>GCN4</i>	filamentous growth	0.5	0.5	0.6	0.6	0.2	0.3
orf19.5034	<i>YBP1</i>	response to oxidative stress	0.5	0.5	0.7	0.9	0.8	0.9
orf19.3870	<i>ADE13</i>	purine base metabolic process	0.5	0.4	1.0	0.9	0.2	0.2
orf19.4148			0.5	0.3	0.8	0.7	0.2	0.3

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4612			0.5	0.2	0.2	0.3	0.8	3.7
orf19.3141		ER to Golgi vesicle-mediated transport	0.5	0.5	1.1	1.7	0.7	0.6
orf19.2763		meiotic telomere clustering	0.5	0.4	1.7	1.7	0.8	0.9
orf19.1115	<i>GUK1</i>	GMP metabolic process	0.5	0.5	0.7	1.1	0.2	0.2
orf19.6399	<i>ATSI</i>	budding cell bud growth	0.5	0.4	0.9	0.9	1.5	1.5
orf19.4774	<i>AOX1</i>	aerobic respiration	0.5	0.4	1.3	1.2	1.6	1.1
orf19.6237	<i>RAC1</i>	filamentous growth	0.5	0.3	0.8	1.1	0.5	0.6
orf19.2092		methionine biosynthetic process	0.5	0.5	0.6	0.8	0.8	0.7
orf19.6737			0.5	0.4	1.0	1.2	0.9	0.6
orf19.5572		protein amino acid N-linked glycosylation	0.5	0.5	0.4	0.4	1.1	0.8
orf19.5648			0.5	0.3	0.8	1.2	0.5	0.7
orf19.3471		regulation of mitotic metaphase/anaphase transition	0.5	0.4	0.7	0.5	0.3	0.7
orf19.1986	<i>ARO2</i>	aromatic amino acid family biosynthetic process	0.5	0.5	0.5	0.6	0.2	0.3
orf19.939	<i>NAM7</i>	intracellular mRNA localization	0.5	0.5	1.2	1.4	0.9	0.7
orf19.6628		chromosome segregation	0.5	0.4	1.2	1.1	0.3	0.9
orf19.3364			0.5	0.4	0.7	0.7	0.4	0.5
orf19.3965		double-strand break repair via homologous recombination	0.5	0.5	0.5	0.8	0.7	0.6
orf19.3449.2		cardiolipin biosynthetic process	0.5	0.3	0.7	0.7	0.3	0.4
orf19.2693	<i>GST2</i>	regulation of nitrogen utilization	0.5	0.3	1.1	0.7	0.5	0.3
orf19.3964	<i>ASH2</i>	chromatin silencing at telomere	0.5	0.4	1.1	1.9	0.8	0.7
orf19.2213			0.5	0.4	1.8	3.0	0.7	1.4
orf19.1235	<i>HOM3</i>	methionine metabolic process	0.5	0.5	0.3	0.6	0.2	0.4
orf19.6952		gene silencing by RNA	0.5	0.5	0.5	0.4	0.4	0.6
orf19.1852			0.5	0.4	0.6	1.0	0.3	0.4
orf19.6167	<i>AYR1</i>	phosphatidic acid biosynthetic process	0.5	0.5	0.6	0.9	0.8	0.6
orf19.390	<i>CDC42</i>	cell budding	0.5	0.5	0.8	1.0	0.9	0.7
orf19.7499		nicotinate nucleotide salvage	0.5	0.5	0.3	0.4	0.4	0.5

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7545			0.5	0.5	0.6	1.0	0.8	1.0
orf19.1600			0.5	0.3	0.8	2.5	1.0	0.4
orf19.6929			0.5	0.3	0.4	0.4	0.2	0.3
orf19.7484	<i>ADE1</i>	adenine biosynthetic process	0.5	0.3	0.8	0.8	0.3	0.3
orf19.3894		response to drug	0.5	0.2	2.5	5.1	2.9	3.8
orf19.7615		ER to Golgi vesicle-mediated transport	0.5	0.2	1.4	3.3	1.4	1.2
orf19.4523		folic acid and derivative biosynthetic process	0.5	0.3	0.4	0.3	0.4	1.0
orf19.7017	<i>YOX1</i>	regulation of mitotic cell cycle	0.5	0.4	2.2	3.1	2.0	1.3
orf19.6400		inositol metabolic process	0.5	0.5	1.2	1.5	1.3	1.2
orf19.2533.1		cotranslational protein targeting to membrane	0.5	0.3	0.9	1.1	0.4	0.6
orf19.3459		ascospore formation	0.5	0.5	0.8	0.7	1.0	0.7
orf19.7097		response to drug	0.5	0.4	0.9	1.3	0.7	0.8
orf19.4880			0.5	0.5	1.3	1.2	1.0	0.5
orf19.6976		folic acid transport	0.5	0.3	2.1	2.5	1.0	0.4
orf19.3395		drug transport	0.5	0.4	0.3	0.2	0.6	0.4
orf19.2870			0.5	0.4	4.1	4.6	1.5	1.1
orf19.1959		protein deubiquitination	0.5	0.3	1.3	2.3	0.3	1.1
orf19.4580			0.5	0.3	0.3	0.2	0.1	0.5
orf19.2546	<i>TRP2</i>	tryptophan biosynthetic process	0.5	0.5	0.6	0.6	0.2	0.4
orf19.6875	<i>VPS35</i>	protein retention in Golgi apparatus	0.5	0.5	0.8	0.7	0.6	0.5
orf19.5211	<i>IDP1</i>	glutamate biosynthetic process	0.5	0.5	0.9	0.9	0.3	0.3
orf19.6017			0.5	0.4	1.4	1.2	0.4	0.6
orf19.6316		L-amino acid transport	0.5	0.3	0.8	0.8	0.8	0.5
orf19.6885	<i>SPO7</i>	meiosis	0.5	0.5	1.0	1.5	1.7	1.3
orf19.1782			0.5	0.3	0.5	0.6	0.2	0.3
orf19.3872			0.5	0.4	0.5	0.5	1.1	1.0
orf19.2267	<i>RFA2</i>	DNA strand elongation during DNA replication	0.5	0.5	0.9	0.8	0.4	0.3

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.7454	<i>TAF60</i>	chromatin organization	0.5	0.4	1.1	1.3	0.7	0.7
orf19.4036	<i>APM1</i>	filamentous growth	0.5	0.5	1.2	1.8	1.2	0.9
orf19.2581		cellular metabolic process	0.5	0.5	0.7	1.3	0.4	0.6
orf19.4749			0.5	0.5	1.0	1.1	0.8	0.3
orf19.4616	<i>POL30</i>	base-excision repair	0.5	0.5	0.6	0.6	0.2	0.2
orf19.3442		oxidation-reduction process	0.5	0.5	0.5	0.4	0.5	0.8
orf19.985			0.5	0.4	1.4	1.9	0.6	0.7
orf19.2040		hydrogen peroxide-mediated programmed cell death	0.5	0.3	1.2	1.4	1.1	1.4
orf19.7638	<i>PRO1</i>	proline biosynthetic process	0.5	0.5	0.3	0.3	0.5	1.0
orf19.4205.1		nuclear mRNA splicing, via spliceosome	0.5	0.4	0.4	0.6	0.4	0.6
orf19.2461	<i>PRN4</i>		0.5	0.3	1.4	1.2	1.6	2.0
orf19.2949		intracellular signal transduction	0.5	0.5	1.6	2.3	1.0	0.6
orf19.6650			0.5	0.4	1.1	1.5	1.8	2.1
orf19.7095			0.5	0.5	0.3	0.4	0.5	0.5
orf19.6635			0.5	0.4	1.1	1.0	0.9	1.7
orf19.1913			0.5	0.4	0.5	0.7	0.4	0.7
orf19.5628		dicarboxylic acid transport	0.5	0.3	0.2	0.2	0.3	0.5
orf19.4022	<i>SDH4</i>	succinate metabolic process	0.5	0.5	0.5	1.0	0.3	0.6
orf19.3761	<i>CDC54</i>	DNA replication initiation	0.5	0.5	2.1	3.6	0.3	0.1
orf19.3679		nicotinamide nucleotide metabolic process	0.5	0.4	0.6	0.5	0.6	0.6
orf19.4553			0.5	0.5	1.0	1.3	1.2	0.7
orf19.511		nicotinamide riboside metabolic process	0.5	0.4	0.6	0.8	0.4	0.6
orf19.7175	<i>HLJI</i>	ER-associated protein catabolic process	0.5	0.5	0.7	1.0	0.8	0.8
orf19.95			0.5	0.5	1.6	3.2	0.9	0.4
orf19.3040	<i>EHT1</i>	medium-chain fatty acid biosynthetic process	0.5	0.5	0.3	0.2	0.2	0.3
orf19.5505	<i>HIS7</i>	histidine biosynthetic process	0.5	0.4	1.0	1.1	0.4	0.5
orf19.4748		nuclear mRNA splicing, via spliceosome	0.5	0.4	0.8	0.9	0.4	0.9

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.434	<i>PRD1</i>	proteolysis	0.5	0.5	0.6	0.4	0.4	0.4
orf19.7631	<i>SLD5</i>	DNA-dependent DNA replication	0.5	0.4	1.6	1.4	1.0	0.5
orf19.6779	<i>PRO2</i>	proline biosynthetic process	0.5	0.4	1.5	1.8	0.9	0.8
orf19.1973	<i>HAP5</i>	filamentous growth	0.5	0.3	1.2	1.4	0.4	0.6
orf19.1681			0.5	0.5	0.9	0.8	3.0	3.6
orf19.3844	<i>MRP8</i>	translation	0.5	0.5	0.6	0.5	0.7	1.3
orf19.306	<i>USO5</i>	ER to Golgi vesicle-mediated transport	0.5	0.5	1.8	2.0	1.3	1.7
orf19.4270		protein amino acid O-linked glycosylation	0.5	0.5	1.4	1.2	1.1	0.9
orf19.993			0.5	0.2	0.9	0.9	0.4	0.3
orf19.2898		protein targeting to vacuole	0.5	0.4	1.5	1.8	6.3	3.1
orf19.3534	<i>RHO3</i>	hyphal growth	0.5	0.4	0.9	1.2	1.3	0.9
orf19.4817	<i>RAM2</i>	protein geranylgeranylation	0.5	0.3	0.5	0.5	0.3	0.6
orf19.244	<i>DCG1</i>	nitrogen compound metabolic process	0.5	0.3	1.0	0.9	0.6	0.4
orf19.7370			0.5	0.4	1.5	2.4	2.5	1.6
orf19.4575			0.5	0.3	0.5	0.8	0.2	0.2
orf19.7304			0.5	0.5	0.9	0.9	0.8	0.4
orf19.3715	<i>ASF1</i>	DNA replication-dependent nucleosome assembly	0.5	0.5	1.0	2.1	0.3	0.2
orf19.424	<i>TRP99</i>	response to oxidative stress	0.5	0.4	1.0	1.1	0.6	0.7
orf19.2923		drug transport	0.5	0.5	0.5	0.4	0.9	0.7
orf19.6813			0.5	0.3	1.0	1.0	0.4	0.2
orf19.2087	<i>SAS2</i>	chromatin silencing at telomere	0.5	0.4	1.3	2.2	0.7	0.7
orf19.6563			0.5	0.5	1.0	1.3	1.7	1.1
orf19.3043		lipid metabolic process	0.5	0.4	0.9	1.0	0.9	0.8
orf19.4796		negative regulation of translation	0.5	0.4	1.0	1.5	0.3	0.5
orf19.1999			0.5	0.4	0.5	0.3	0.6	1.1
orf19.1828	<i>BUD16</i>	cellular bud site selection	0.5	0.3	1.3	3.1	1.0	0.9
orf19.5487	<i>CDC46</i>	DNA replication initiation	0.5	0.4	0.9	2.3	0.2	0.1

Table A-2. (Continued)

orf19 Designation	CGD Name	Process ^a	Change in Fold Expression					
			SC5314+FLC /SC5314		<i>cas5</i> Δ/Δ+FLC / <i>cas5</i> Δ/Δ		<i>upc2</i> Δ/Δ+FLC/ <i>upc2</i> Δ/Δ	
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
orf19.4024	<i>RIB5</i>	riboflavin biosynthetic process	0.5	0.4	0.8	1.0	0.5	0.7
orf19.5363		dolichol-linked oligosaccharide biosynthetic process	0.5	0.5	0.5	0.6	0.5	0.6
orf19.7414	<i>ALS6</i>	cell adhesion	0.5	0.4	0.7	1.1	0.7	0.6
orf19.1795.1		protein targeting to ER	0.5	0.5	0.2	0.3	0.3	0.5

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).
Red indicates those identified to be fluconazole repressed and dependent on Cas5 or Upc2.

APPENDIX B. CAS5 SUPPLEMENTAL DATA

Table B-1. TF disruptant clones displaying a reduction in FLC MIC in RPMI, rescreened in YPD

Clone ID	CGD	orf19 Designation	MIC (µg/ml)		MFC (µg/ml)	
			RPMI	YPD	RPMI	YPD
WT	--	--	0.25	0.5	>4	>64
TF3	<i>RPN4</i>	orf19.1069	0.0625	0.25	0.125	32
TF4	--	orf19.1253	SG ^a	0.5	SG	>64
TF33	<i>CAS5</i>	orf19.4670	0.125	0.25	4	2
TF45	<i>GAL4</i>	orf19.5338	0.125	0.5	>4	>64
TF59	<i>ACE2</i>	orf19.6124	0.03125	0.5	2	>64
TF77	<i>UPC2</i>	orf19.391	0.03125	0.125	1	1
TF82	<i>STP4</i>	orf19.909	0.125	0.5	>4	>64
TF95	<i>NDT80</i>	orf19.2119	0.03125	0.5	>4	>64
TF103	<i>HFL1</i>	orf19.3063	NG ^a	NG	NG	NG
TF104	<i>CZF1</i>	orf19.3127	0.0625	0.5	0.25	8
TF126	<i>RIM101</i>	orf19.7247	0.0625	0.5	>4	4
TF127	<i>ISW2</i>	orf19.7401	0.0625	0.25	>4	16
TF155	<i>DAL81</i>	orf19.3252	0.0625	0.5	4	>64
TF158	<i>RAP1</i>	orf19.1773	0.03125	0.5	>4	>64
TF159	--	orf19.1757	0.03125	0.5	>4	>64
TF160	--	orf19.2743	0.03125	0.5	>4	>64
TF163	<i>RFX1</i>	orf19.3865	0.0078125	0.25	>4	>64
TF164	<i>RGT1</i>	orf19.2747	0.0625	0.5	>4	>64
TF165	<i>PPR1</i>	orf19.3986	0.03125	0.25	>4	>64

^a Abbreviations: Slow growth (SG), No growth (NG)

Table B-2. Genes downregulated by at least 0.5-fold by fluconazole which are dependent upon Cas5 and Upc2

			Change in Fold Expression									
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)		CAS5 Ratio (B/A)		UPC2 Ratio (C/A)	
Process ^a	orf19 Designation	CGD Name	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Lipid metabolic process	orf19.1887		0.1	0.1	0.4	0.5	0.6	0.5	2.8	3.2	4.4	3.3
	orf19.3043		0.5	0.4	0.9	1.0	0.9	0.8	1.6	2.6	1.6	2.0
	orf19.3089		0.5	0.3	1.2	1.9	0.9	1.0	2.5	5.7	1.9	2.9
	orf19.3104	YDC1	0.4	0.3	0.7	0.6	1.0	0.5	1.8	2.0	2.3	1.6
Cell wall organization	orf19.5032	SIMI	0.3	0.4	0.6	1.0	0.8	1.0	1.8	2.7	2.5	2.6
	orf19.6489	FMP45	0.4	0.3	3.1	2.1	32.8	9.5	7.3	6.9	78.0	31.0
Iron ion transport	orf19.6948	CCCI	0.4	0.3	0.7	0.7	1.7	0.9	1.7	2.1	4.0	2.7
Drug transport	orf19.1308		0.4	0.3	1.1	1.6	1.0	0.5	2.4	5.8	2.3	1.6
	orf19.4889		0.3	0.2	0.7	1.0	0.6	0.5	2.6	4.3	2.3	2.2
	orf19.6691		0.4	0.4	0.6	1.3	1.8	1.4	1.6	3.7	4.7	4.0
	orf19.7554		0.5	0.3	1.1	2.0	10.9	11.4	2.5	6.1	24.1	34.1
Transport	orf19.1182		0.3	0.3	0.7	1.0	0.8	0.5	2.1	3.6	2.5	1.9
	orf19.2337	ALP1	0.1	0.1	0.3	0.4	0.4	0.3	3.2	7.5	5.0	6.1
	orf19.2738	SUL2	0.1	0.3	1.7	1.2	5.4	0.8	12.8	4.7	40.5	3.1
	orf19.2898		0.5	0.4	1.5	1.8	6.3	3.1	2.8	4.8	11.7	8.1
	orf19.3972		0.5	0.4	1.2	1.6	1.3	1.0	2.5	3.7	2.7	2.4
	orf19.4456	GAP4	0.2	0.3	0.5	0.7	0.9	0.6	1.9	2.2	3.6	1.8
	orf19.4733	YMC2	0.3	0.3	0.9	0.9	0.9	0.4	2.9	3.3	2.7	1.7
	orf19.5017	DUR32	0.1	0.1	0.7	0.6	0.9	0.2	6.8	4.6	8.7	1.7
	orf19.5663	RCH1	0.3	0.2	0.9	0.8	1.3	1.6	3.1	3.9	4.5	8.2
	orf19.6316		0.5	0.3	0.8	0.8	0.8	0.5	1.5	3.3	1.6	1.9
	orf19.6563		0.5	0.5	1.0	1.3	1.7	1.1	1.9	2.4	3.0	2.1

Table B-2. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio (C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2		
Response to stress	orf19.7106	<i>VPS70</i>	0.4	0.5	1.0	1.1	3.9	1.0	2.4	2.4	9.7	2.2
	orf19.7370		0.5	0.4	1.5	2.4	2.5	1.6	2.8	6.9	4.6	4.5
	orf19.7615		0.5	0.2	1.4	3.3	1.4	1.2	2.8	13.2	2.7	4.9
	orf19.4526	<i>HSP30</i>	0.2	0.1	2.6	1.2	0.8	1.5	15.6	12.6	5.2	15.9
	orf19.5034	<i>YBP1</i>	0.5	0.5	0.7	0.9	0.8	0.9	1.5	1.9	1.7	1.9
	orf19.6779	<i>PRO2</i>	0.5	0.4	1.5	1.8	0.9	0.8	2.7	4.1	1.7	1.8
	orf19.946	<i>MET14</i>	0.1	0.1	1.1	2.5	0.4	0.2	14.5	28.5	5.4	2.6
Response to chemical stimulus	orf19.3106	<i>MET16</i>	0.2	0.3	0.8	1.4	1.2	0.6	3.5	5.2	4.8	2.3
	orf19.3252	<i>DAL81</i>	0.4	0.3	1.3	1.6	0.8	0.9	3.1	4.6	1.8	2.6
	orf19.3894		0.5	0.2	2.5	5.1	2.9	3.8	5.0	24.2	5.7	17.7
	orf19.4177	<i>HIS5</i>	0.4	0.5	1.1	1.2	0.9	0.9	2.7	2.6	2.3	1.9
	orf19.5839	<i>PDR17</i>	0.4	0.3	0.9	1.7	0.9	0.6	2.2	5.3	2.1	2.0
	orf19.6671	<i>LAP4</i>	0.5	0.3	0.9	0.7	0.8	0.6	2.0	2.2	1.8	1.7
Oxidation- reduction process	orf19.2040		0.5	0.3	1.2	1.4	1.1	1.4	2.3	4.0	2.1	4.2
	orf19.3810		0.4	0.3	1.1	1.9	1.2	0.7	2.9	5.6	3.0	2.1
	orf19.5665		0.5	0.5	0.8	1.0	1.0	0.7	1.6	1.9	2.1	1.5
	orf19.7098		0.2	0.1	0.9	0.8	0.6	0.9	4.4	5.7	2.7	7.0
Filamentous growth	orf19.1253	<i>PHO4</i>	0.3	0.2	1.0	1.5	0.8	0.6	3.1	6.5	2.5	2.8
	orf19.1621	<i>GPA2</i>	0.4	0.5	0.7	0.8	0.9	1.0	2.0	1.6	2.6	1.8
	orf19.4036	<i>APM1</i>	0.5	0.5	1.2	1.8	1.2	0.9	2.3	3.5	2.3	1.7
	orf19.5992	<i>WOR2</i>	0.3	0.2	0.5	0.4	0.9	0.6	1.9	1.8	3.2	2.4
	orf19.7025	<i>MCMI</i>	0.3	0.4	0.5	0.6	0.5	0.7	1.9	1.7	1.8	1.8

Table B-2. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio (C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.7324	THI13	0.4	0.5	1.0	0.8	0.8	0.8	2.3	1.6	2.0	1.7
Organelle organization	orf19.103	KAR5	0.4	0.4	1.0	1.5	0.7	2.6	2.4	4.2	1.5	7.2
	orf19.1238	TUB4	0.5	0.4	1.1	1.3	0.7	0.8	2.4	3.0	1.6	1.9
	orf19.2600	SPC98	0.4	0.4	1.6	3.7	1.7	1.3	4.5	9.8	4.8	3.5
	orf19.2763		0.5	0.4	1.7	1.7	0.8	0.9	3.5	3.8	1.7	1.9
	orf19.3534	RHO3	0.5	0.4	0.9	1.2	1.3	0.9	1.7	3.0	2.4	2.3
	orf19.3688		0.4	0.5	1.3	1.5	0.7	1.4	3.0	3.3	1.6	3.0
	orf19.3788	SPC34	0.5	0.3	1.2	1.2	0.8	1.3	2.6	3.6	1.8	3.7
	orf19.6291		0.5	0.4	2.0	2.5	1.0	0.7	4.3	6.3	2.1	1.7
	orf19.6399	ATS1	0.5	0.4	0.9	0.9	1.5	1.5	1.9	2.6	3.0	4.2
	orf19.6885	SPO7	0.5	0.5	1.0	1.5	1.7	1.3	1.8	3.1	3.3	2.8
Cell cycle	orf19.1828	BUD16	0.5	0.3	1.3	3.1	1.0	0.9	2.4	10.9	1.8	3.3
	orf19.3016		0.4	0.3	0.8	0.9	0.9	1.0	1.9	3.0	2.1	3.6
	orf19.3230	BOI2	0.4	0.4	1.0	1.4	1.5	0.9	2.7	3.2	4.1	2.1
	orf19.5919	MEA1	0.3	1.1	1.4	0.8	0.5	2.3	4.4	1.6	1.6	
	orf19.7017	YOX1	0.5	0.4	2.2	3.1	2.0	1.3	4.4	8.2	4.0	3.5
DNA metabolic process	orf19.3964	ASH2	0.5	0.4	1.1	1.9	0.8	0.7	2.1	4.9	1.6	1.8
	orf19.547		0.5	0.3	1.8	8.1	1.3	0.5	3.9	25.0	2.9	1.6
	orf19.7190	OGG1	0.4	0.4	3.5	5.1	1.6	0.6	9.4	13.9	4.3	1.6
	orf19.7310		0.3	0.3	2.5	2.6	1.1	0.5	9.5	8.4	4.2	1.6
	orf19.927		0.4	0.2	1.3	1.8	1.0	1.6	3.1	8.4	2.3	7.1

Table B-2. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio (C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
Carbohydrate metabolic process	orf19.4270	<i>MNN13</i>	0.5	0.5	1.4	1.2	1.1	0.9	2.6	2.3	2.0	1.7
	orf19.5612	<i>BMT4</i>	0.4	0.3	0.7	0.6	1.0	0.6	1.7	2.1	2.5	2.1
	orf19.6400		0.5	0.5	1.2	1.5	1.3	1.2	2.4	3.0	2.5	2.3
	orf19.6416		0.4	0.4	2.2	3.3	3.8	2.0	5.0	7.4	8.5	4.4
	orf19.6692	<i>MNN26</i>	0.5	0.4	0.9	1.1	0.9	0.6	1.8	3.1	1.8	1.7
Other	orf19.1159		0.3	0.5	1.6	2.9	4.0	1.1	5.4	5.9	13.2	2.1
	orf19.1697		0.3	0.4	1.0	1.9	0.5	0.9	2.9	5.0	1.5	2.3
	orf19.1757		0.4	0.2	1.6	3.3	0.7	0.6	4.1	14.1	1.9	2.7
	orf19.2018		0.3	0.3	2.1	4.1	2.0	2.3	7.2	12.4	6.9	6.9
	orf19.4774	<i>AOXI</i>	0.5	0.4	1.3	1.2	1.6	1.1	2.7	3.0	3.3	2.7
	orf19.5369		0.4	0.4	0.7	0.8	0.7	0.7	1.8	1.9	1.9	1.7
	orf19.5519	<i>GCV1</i>	0.3	0.2	1.0	1.0	1.1	0.6	2.9	5.7	3.2	3.2
	orf19.568	<i>SPE2</i>	0.4	0.2	1.4	2.0	2.1	1.4	3.8	9.3	5.5	6.8
	orf19.5736	<i>ALS5</i>	0.4	0.3	0.7	1.1	3.0	2.2	1.5	3.2	6.8	6.3
	orf19.5789	<i>ADE8</i>	0.4	0.2	1.3	1.9	0.6	0.6	3.3	7.6	1.6	2.5
	orf19.6146	<i>CLG1</i>	0.4	0.5	0.6	1.0	1.5	1.0	1.6	2.3	4.0	2.2
	orf19.7375		0.5	0.3	1.3	1.8	1.1	2.1	2.7	6.7	2.2	7.5
	orf19.756	<i>SAP7</i>	0.4	0.4	0.9	0.6	13.6	6.6	2.2	1.5	31.7	15.7
Unknown	orf19.1075		0.3	0.5	1.5	1.4	2.5	1.4	5.1	2.7	8.9	2.8
	orf19.1258		0.4	0.2	0.9	1.2	1.0	0.4	2.5	5.2	2.7	1.6
	orf19.1510	<i>SRD1</i>	0.3	0.4	0.8	1.4	1.8	0.8	2.7	3.6	6.1	2.0
	orf19.1681		0.5	0.5	0.9	0.8	3.0	3.6	1.7	1.7	5.5	7.2
	orf19.2246		0.3	0.1	0.6	0.8	0.7	2.1	2.2	5.7	2.6	14.1
	orf19.2461	<i>PRN4</i>	0.5	0.3	1.4	1.2	1.6	2.0	2.7	4.5	3.1	7.6

Table B-2. (Continued)

Process ^a	orf19 Designation	CGD Name	Change in Fold Expression						CAS5 Ratio (B/A)		UPC2 Ratio (C/A)	
			SC5314+FLC/ SC5314 (A)		cas5Δ/Δ+FLC/ cas5Δ/Δ (B)		upc2Δ/Δ+FLC/ upc2Δ/Δ (C)					
			Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
	orf19.2547	USO5	0.5	0.2	1.7	2.7	0.9	1.0	3.5	12.1	1.9	4.7
	orf19.2870		0.5	0.4	4.1	4.6	1.5	1.1	8.0	11.2	3.0	2.8
	orf19.2936		0.4	0.3	1.2	1.6	1.9	0.7	2.8	4.9	4.6	2.2
	orf19.3042		0.5	0.4	1.0	3.3	1.1	3.5	2.1	7.6	2.4	8.0
	orf19.306		0.5	0.5	1.8	2.0	1.3	1.7	3.4	4.1	2.4	3.5
	orf19.335		0.4	0.3	0.6	0.6	0.7	0.8	1.7	2.3	2.1	3.1
	orf19.3475	SKN2	0.1	0.1	0.4	0.4	2.0	2.1	3.0	3.9	17.2	20.3
	orf19.348		0.4	0.3	1.4	1.3	3.2	2.3	3.3	4.9	7.5	9.0
	orf19.3916		0.2	0.2	0.9	0.6	0.4	0.5	4.0	2.9	1.8	2.1
	orf19.398		0.4	0.4	1.3	1.2	1.2	1.3	2.9	3.3	2.6	3.7
	orf19.4142		0.3	0.3	1.5	6.2	2.7	1.1	5.6	19.6	9.7	3.6
	orf19.4666		0.4	0.5	1.0	1.8	1.1	1.1	2.5	3.5	2.7	2.1
	orf19.5051	NRP1	0.4	0.2	1.3	2.3	0.6	0.9	3.6	9.9	1.7	4.0
	orf19.5057		0.4	0.3	5.8	1.9	1.8	0.7	14.2	6.0	4.5	2.4
	orf19.5071		0.4	0.5	0.6	1.7	1.0	0.8	1.5	3.7	2.5	1.8
	orf19.5841		0.5	0.5	0.7	0.8	0.8	1.0	1.5	1.5	1.8	1.9
	orf19.660		0.4	0.4	2.0	3.5	1.8	2.1	4.4	7.8	4.0	4.8
	orf19.6635		0.5	0.4	1.1	1.0	0.9	1.7	2.1	2.9	1.6	4.8
	orf19.6650	ARS2	0.5	0.4	1.1	1.5	1.8	2.1	2.1	3.4	3.5	5.0
	orf19.6679		0.1	0.3	1.0	2.8	1.0	1.5	6.5	8.7	6.8	4.5
	orf19.6737		0.5	0.4	1.0	1.2	0.9	0.6	2.0	3.3	1.8	1.7
	orf19.687		0.4	0.2	0.8	1.3	0.7	0.5	1.8	5.4	1.7	1.9
	orf19.7284		0.2	0.1	1.7	1.7	0.3	0.3	8.2	14.7	1.6	2.8
	orf19.7427		0.4	0.3	0.7	1.1	1.9	1.3	1.5	3.8	4.2	4.6
	orf19.7553		0.2	0.5	3.6	3.2	0.7	1.2	15.9	6.7	3.2	2.5

^a Descriptions are from the Candida Genome Database (<http://www.candidagenome.org>).

VITA

Erin M. Vasicek, daughter of Ronald and Sherrill Vasicek and David and Anna Buckler, was born in Plano, TX. In 1988, she and her family moved to Rochester, New York where she enrolled in the Greece Public School District. After graduating from Greece Athena High School in 2003, she attended the Ohio State University in Columbus, Ohio, earning her Bachelor of Science in Molecular Genetics in 2007. Later that year she then matriculated into the Integrated Program in Biomedical Science at the University of Tennessee Health Science Center in Memphis, Tennessee and expects to complete her Doctor of Philosophy in December 2013.