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Transcriptional Regulation of Azole Antifungal Resistance in *Candida albicans*

Abstract

Candida albicans is a pathogenic fungi found in the mucosa, gastrointestinal, and urogenital tracts of humans. Oropharyngeal candidiasis (OPC), an opportunistic mucosal infection caused by *C. albicans*, occurs most frequently in patients infected with human immunodeficiency virus (HIV). OPC is usually treated with azole antifungals, a class of antifungals that target ergosterol biosynthesis, at low doses over long periods of time. This course of treatment allows for the development of azole resistance.

Two major mechanisms of azole resistance exist in *C. albicans*, the up-regulation of genes encoding efflux pumps and the up-regulation of ERG11, a gene encoding the azole drug target lanosterol demethylase. The overexpression of efflux pumps remain the major contributor of azole resistance. The ATP binding cassette (ABC) transporter genes CDR1 and CDR2 have been shown to be regulated by a zinc cluster transcription factor Tac1p. A gain-of-function mutation in the gene encoding this transcription factor is responsible for the overexpression of CDR1 and CDR2. To identify the Tac1p regulon, we analyzed four matched sets of clinical isolates with gain-of-function mutations in TAC1 representing the development of CDR1- and CDR2-mediated azole resistance, using gene expression profiling. We identified genes that were consistently up-regulated coordinately with CDR1 and CDR2, including TAC1 itself. When a resistant strain disrupted for TAC1 was similarly examined, the expression of almost all of these genes returned to levels similar to those in the matched azole-susceptible isolate. Using chromatin Immunoprecipitation microarray (ChIP-chip) analysis, we found genes whose promoters were bound by Tac1p in vivo, including CDR1 and CDR2. Sequence analysis identified genes whose promoters contain the previously reported Tac1p drug-responsive element (DRE; CGGN4CGG), including TAC1. Our results show that Tac1p is constitutively bound to the promoter of its targets, including to its own promoter. They also suggest roles for Tac1p in regulating lipid metabolism (mobilization and trafficking) and oxidative stress response in *C. albicans*.

Constitutive overexpression of the MDR1 gene, which encodes an efflux pump of the major facilitator superfamily, is another cause of resistance to fluconazole in clinical *C. albicans* strains. The regulator of MDR1 gene expression has not been identified or characterized. Using genome-wide gene expression profiling, we have identified a gene encoding a zinc cluster transcription factor, designated as MRR1 (multidrug resistance regulator), that was coordinately up-regulated with MDR1 in drug-resistant clinical *C. albicans* isolates. The disruption of MRR1 in two resistant clinical isolates abrogated MDR1 expression and multidrug resistance. MRR1 alleles were also sequenced and two gain-of-function mutations were identified (P683S and G997V). The integration of the mutated alleles into the genome strain SC5314 resulted in the overexpression of MDR1 and in the increase of drug resistance. Microarray analysis of the resistant isolates, the disruption mutants and the reintegrated gain-of-function mutants allowed us to define a core group of genes regulated by Mrr1p. In addition to conferring multidrug resistance, Mrr1p also regulates genes that encode oxidoreductases that play a role in the response to oxidative stress.

The second major resistance mechanism is the constitutive up-regulation of ERG11. ERG11 encodes lanosterol demethylase, a cytochrome P-450 enzyme that is the target of the azole antifungals. In *C. albicans*, the zinc cluster transcription factor Upc2p has been shown to regulate the expression of ERG11 and other ergosterol biosynthesis genes. Overexpression of UPC2 increases azole resistance while disruption of UPC2 results in hypersusceptibility. Using genome-wide gene expression profiling, we found UPC2 and ergosterol biosynthesis genes to be coregulated with ERG11 in a fluconazole resistant clinical isolate. Sequence analysis of the susceptible and resistant UPC2 alleles revealed that the resistant isolate contained one UPC2 allele harboring a single nucleotide substitution, resulting in a G648D exchange. Introduction of the mutated UPC2 allele into the genome strain resulted in constitutive up-regulation of ERG11 and increased resistance to fluconazole. By comparing the gene expression profiles of the

fluconazole resistant isolate and of strains carrying wild-type and mutated UPC2 alleles, we identified target genes that are controlled by Upc2p. We show for the first time that a gain-of-function mutation in UPC2 leads to increased expression of ERG11 and imparts resistance to fluconazole in clinical isolates of *C. albicans*.

By studying genetically matched sets of azole susceptible and resistant clinical isolates, we identified genes co-regulated with known resistance genes. These core genes specific to particular resistance mechanisms allowed us to identify previously characterized but unknown transcriptional regulators that control this process. Gain-of-function mutations in these trans-regulatory elements are responsible for the development of azole resistance in these clinical isolates. The target genes of each of these transcription factors provide insight into their function in addition to their role in azole resistance.

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**TRANSCRIPTIONAL REGULATION OF AZOLE ANTIFUNGAL 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
Teresa T. Liu
May 2008

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DEDICATION

To my parents, Larry and Ginny Liu, for their love and encouragement, and
to my husband and best friend, Jeremy Dennis, for keeping me balanced.

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ABSTRACT

Candida albicans is a pathogenic fungi found in the mucosa, gastrointestinal, and urogenital tracts of humans. Oropharyngeal candidiasis (OPC), an opportunistic mucosal infection caused by *C. albicans*, occurs most frequently in patients infected with human immunodeficiency virus (HIV). OPC is usually treated with azole antifungals, a class of antifungals that target ergosterol biosynthesis, at low doses over long periods of time. This course of treatment allows for the development of azole resistance.

Two major mechanisms of azole resistance exist in *C. albicans*, the up-regulation of genes encoding efflux pumps and the up-regulation of *ERG11*, a gene encoding the azole drug target lanosterol demethylase. The overexpression of efflux pumps remain the major contributor of azole resistance. The ATP binding cassette (ABC) transporter genes *CDR1* and *CDR2* have been shown to be regulated by a zinc cluster transcription factor Tac1p. A gain-of-function mutation in the gene encoding this transcription factor is responsible for the overexpression of *CDR1* and *CDR2*. To identify the Tac1p regulon, we analyzed four matched sets of clinical isolates with gain-of-function mutations in *TAC1* representing the development of *CDR1*- and *CDR2*-mediated azole resistance, using gene expression profiling. We identified genes that were consistently up-regulated coordinately with *CDR1* and *CDR2*, including *TAC1* itself. When a resistant strain disrupted for *TAC1* was similarly examined, the expression of almost all of these genes returned to levels similar to those in the matched azole-susceptible isolate. Using chromatin Immunoprecipitation microarray (ChIP-chip) analysis, we found genes whose promoters were bound by Tac1p *in vivo*, including *CDR1* and *CDR2*. Sequence analysis identified genes whose promoters contain the previously reported Tac1p drug-responsive element (DRE; CGGN₄CGG), including *TAC1*. Our results show that Tac1p is constitutively bound to the promoter of its targets, including to its own promoter. They also suggest roles for Tac1p in regulating lipid metabolism (mobilization and trafficking) and oxidative stress response in *C. albicans*.

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The second major resistance mechanism is the constitutive up-regulation of *ERG11*. *ERG11* encodes lanosterol demethylase, a cytochrome P-450 enzyme that is the target of the azole antifungals. In *C. albicans*, the zinc cluster transcription factor Upc2p has been shown to regulate the expression of *ERG11* and other ergosterol biosynthesis genes. Overexpression of *UPC2* increases azole resistance while disruption of *UPC2* results in hypersusceptibility. Using genome-wide gene expression profiling, we found *UPC2* and ergosterol biosynthesis genes to be coregulated with *ERG11* in a fluconazole resistant clinical isolate. Sequence analysis of the susceptible and resistant *UPC2* alleles revealed that the resistant isolate contained one *UPC2* allele harboring a single nucleotide substitution, resulting in a G648D exchange. Introduction of the mutated *UPC2* allele into the genome strain resulted in constitutive up-regulation of *ERG11* and increased resistance to fluconazole. By comparing the gene expression profiles of the fluconazole resistant isolate and of strains carrying wild-type and mutated *UPC2* alleles, we identified target genes that are controlled by Upc2p. We show for the first time that a gain-of-function mutation in *UPC2* leads to increased expression of *ERG11* and imparts resistance to fluconazole in clinical isolates of *C. albicans*.

By studying genetically matched sets of azole susceptible and resistant clinical isolates, we identified genes co-regulated with known resistance genes. These core genes specific to particular resistance mechanisms allowed us to identify previously characterized but unknown transcriptional regulators that control this process. Gain-of-function mutations in these *trans*-regulatory elements are responsible for the development of azole resistance in these clinical isolates. The target genes of each of these transcription factors provide insight into their function in addition to their role in azole resistance.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
<i>CANDIDA ALBICANS</i>	1
Epidemiology of Infection.....	1
Treatment.....	2
<i>C. albicans</i> Physiology	3
Genetics	4
AZOLE RESISTANCE.....	5
Emergence of Clinical Resistance	5
Molecular Mechanisms of Resistance	6
TRANSCRIPTIONAL REGULATORS.....	9
Transcription Factor Classes	9
Transcription Factors and Resistance.....	11
<i>C. albicans</i> Transcription Factors.....	13
CENTRAL HYPOTHESIS AND RESEARCH OBJECTIVES	14
CHAPTER 2: IDENTIFICATION OF THE TAC1P REGULON	16
INTRODUCTION.....	16
MATERIALS AND METHODS	17
Strains and Growth Media.....	17
Construction of <i>C. albicans</i> Expression Microarrays.....	17
RNA Preparation for Microarrays	19
cRNA Synthesis and Labeling.....	19
Oligonucleotide Array Hybridization and Analysis	19
Expression Microarray Data Analysis.....	20
Quantitative Real-Time PCR for Expression Data.....	20
Generation of an HA-Tagged Tac1p-Expressing Strain.....	22
<i>Candida albicans</i> Transformation	22
Genomic DNA Isolation and Southern Blot Analysis.....	22
Total Protein Preparation and Western Blotting.....	23
Chromatin Immunoprecipitation	23
DNA Labeling, Hybridization to Intergenic Microarrays and Data Analysis	24
Quantitative Real-Time PCR Confirmation of the Genome-Wide Location Data....	24

DRE Motif Analysis	26
RESULTS.....	26
Global Gene Expression Profile	26
Identification of Tac1p-Binding Sites <i>in vivo</i>	31
Identification of Potential Tac1p-Binding Motifs	36
Expression and Location Data Mining	43
DISCUSSION	44
 CHAPTER 3: GAIN-OF-FUNCTION MUTATIONS IN <i>MRR1</i> REGULATE THE EXPRESSION OF <i>MDR1</i>	50
INTRODUCTION.....	50
MATERIALS AND METHODS	51
Strains and Growth Conditions.....	51
DNA Microarray Analysis.....	51
RNA Preparation for Microarrays	51
cRNA Synthesis and Labeling.....	53
Oligonucleotide Array Hybridization and Analysis	53
Microarray Data Analysis.....	54
Quantitative Real-Time RT-PCR	54
RESULTS.....	56
Transcription Factor <i>MRR1</i> Is Coordinately Regulated with <i>MDR1</i> in Resistant Clinical <i>C. albicans</i> Isolates	56
Identification of Mrr1p Target Genes.....	56
DISCUSSION	62
 CHAPTER 4: GAIN-OF-FUNCTION MUTATIONS IN <i>UPC2</i> REGULATE THE EXPRESSION OF ERGOSTEROL BIOSYNTHESIS GENES AND IMPART FLUCONAZOLE RESISTANCE IN A CLINICAL ISOLATE OF <i>CANDIDA</i> <i>ALBICANS</i>	68
INTRODUCTION.....	68
MATERIALS AND METHODS	69
Strains and Growth Conditions.....	69
Construction of <i>C. albicans</i> Affymetrix® Expression Arrays	69
RNA Preparation for Microarrays	69
cRNA Synthesis and Labeling.....	71
Oligonucleotide Array Hybridization and Analysis	71

Microarray Data Analysis.....	71
Quantitative Real-Time PCR.....	72
Bioinformatics Analysis	72
RESULTS.....	72
Expression of the Transcription Factor <i>UPC2</i> Is Coordinately Up-Regulated with <i>ERG11</i> in Azole Resistant <i>C. albicans</i> Clinical Isolate S2	72
Resistant Isolate S2 Contains a Gain-of-Function Mutation in <i>UPC2</i>	74
Identification of <i>UPC2</i> Target Genes.....	74
DISCUSSION	81
CHAPTER 5: DISCUSSION.....	86
LIST OF REFERENCES.....	98
APPENDIX A: <i>TAC1</i> SUPPLEMENTAL DATA	116
APPENDIX B: <i>MRR1</i> SUPPLEMENTAL DATA.....	136
APPENDIX C: <i>UPC2</i> SUPPLEMENTAL DATA.....	157
VITA.....	172

LIST OF TABLES

Table 2-1.	Strains used in this study.....	18
Table 2-2.	Primers used for quantitative real-time PCR expression analysis.	21
Table 2-3.	Primers used for quantitative real-time PCR binding assays.....	25
Table 2-4.	Genes up-regulated in all four matched clinical isolate sets.....	28
Table 2-5.	Genes down-regulated in all four matched clinical isolate sets.....	30
Table 2-6.	List of Tac1p-enriched promoters (<i>P</i> -value < 0.001).	37
Table 2-7.	DRE motifs found in the Tac1p target promoters.....	41
Table 3-1.	<i>C. albicans</i> strains examined in this study.....	52
Table 3-2.	Primers used for quantitative real-time RT-PCR.....	55
Table 3-3.	Genes commonly down-regulated by <i>MRR1</i> deletion in F5 and G5.	59
Table 3-4.	Identification of Mrr1p target genes in F isolates by DNA microarray analysis.....	60
Table 3-5.	Identification of Mrr1p target genes in G isolates by DNA microarray analysis.....	63
Table 4-1.	<i>C. albicans</i> strains used in this study.....	70
Table 4-2.	Primers used for quantitative real-time PCR analysis.	73
Table 4-3.	Genes up-regulated by the G648D mutation in Upc2p.....	77
Table 4-4.	Genes down-regulated by the G648D mutation in Upc2p.....	79
Table 4-5.	Genes up-regulated in both isolate S2 and transformants expressing the <i>UPC2</i> ^{S2-1} allele with SRE motifs in their promoter.	80
Table A-1.	Genes up-regulated in at least one matched clinical isolate.	116
Table A-2.	Genes down-regulated in at least one matched clinical isolate.....	127
Table B-1.	Genes up-regulated in at least one clinical matched isolate overexpressing <i>MDR1</i>	136
Table B-2.	Genes down-regulated in at least one clinical matched isolate overexpressing <i>MDR1</i>	144
Table B-3.	Genes down-regulated in the absence of <i>MRR1</i>	152
Table C-1.	Genes up-regulated in either resistant isolate S2 or gain-of-function S2 allele in SC5314.....	157
Table C-2.	Genes down-regulated in either S2 or gain-of-function S2 allele in SC5314.....	163
Table C-3.	Genes down-regulated in the absence of <i>UPC2</i>	169
Table C-4.	Genes up-regulated in the absence of <i>UPC2</i>	170

LIST OF FIGURES

Figure 2-1.	Quantitative real-time RT-PCR analysis of selected genes differentially expressed in the microarray experiments.....	32
Figure 2-2.	Chromosomal tagging of Tac1p.....	34
Figure 2-3.	<i>In vivo</i> enrichment of Tac1p binding at the <i>CDR1</i> , <i>PDR16</i> and <i>TAC1</i> promoters using Q-PCR.....	40
Figure 3-1.	Identification of genes that are differentially expressed in <i>MDR1</i> overexpressing clinical <i>C. albicans</i> isolates.	57
Figure 3-2.	Quantitative real-time PCR analysis of <i>MDR1</i> and <i>MRR1</i>	65
Figure 4-1.	Quantitative real-time PCR for genes of interest.....	75
Figure 4-2.	Susceptibilities to fluconazole and terbinafine of wild-type and gain-of-function mutant <i>UPC2</i> strains by spot assay.	76
Figure 5-1.	Proposed model for Tac1p-mediated fluconazole resistance in <i>C. albicans</i>	88
Figure 5-2.	Proposed model for Mrr1p-mediated fluconazole resistance in <i>C. albicans</i>	91
Figure 5-3.	Proposed model for Upc2p-mediated fluconazole resistance in <i>C. albicans</i>	94

LIST OF ABBREVIATIONS

5-FOA	5-Fluoroorotic acid
ABC	ATP binding cassette
AIDS	Acquired immune deficiency syndrome
AmB	Amphotericin B
AP-1	Activator protein-1
ARE	Activator protein-1 recognition element
bZip	Basic leucine zipper
c-CRD	Carboxyl-terminal cysteine rich domain
cDNA	Complementary deoxyribonucleic acid
CDR	<i>Candida</i> drug resistance
ChIP	Chromatin Immunoprecipitation
CLSI	Clinical and Laboratory Standards Institute
CRD	Cysteine rich domain
cRNA	Complementary ribonucleic acid
C_T	Cycle threshold
DBD	DNA binding domain
DNA	Deoxyribonucleic acid
DRE	Drug response element
GCOS	GeneChip Operating System
GEO	Gene Expression Omnibus
GFP	Green fluorescent protein
GO	Gene ontology
GPI	Glycosylphosphatidylinositol
GST	Glutathione <i>S</i> -transferase
HA	Hemagglutinin
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
IP	Immunoprecipitation
MDR	Multidrug resistance
MFS	Major facilitator superfamily
MIC	Minimum inhibitory concentration
<i>MLT</i>	Mating type locus
MM	Mismatch
mRNA	Messenger ribonucleic acid
NBD	Nucleotide binding domain
n-CRD	Amino-terminal cysteine rich domain
OD	Optical density
OPC	Oropharyngeal candidiasis
ORF	Open reading frame
PDR	Pleiotropic drug resistance
PDRE	Pleiotropic drug resistance element
PM	Perfect match
Q-PCR	Quantitative real-time polymerase chain reaction

RFLP	Restriction fragment length polymorphism
<i>RLUC</i>	<i>Renilla</i> luciferase
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SBI	Sterol biosynthesis inhibitors
SC	Synthetic complete
SDS	Sequence Detection System
SNP	Single Nucleotide Polymorphism
SRE	Sterol response element
SREBP	Sterol response element binding protein
SV40	Simian virus 40
TMD	Transmembrane domain
VVC	Vulvovaginal candidiasis
yAP1	Yeast activator protein-1
YPD	Yeast peptone dextrose
YRE	Yeast activator protein-1 recognition element

CHAPTER 1: INTRODUCTION

CANDIDA ALBICANS

Epidemiology of Infection

Instances of fungal infections have increased dramatically in the past two decades due to the acquired immune deficiency syndrome (AIDS) epidemic, improved life-sustaining technologies, and anti-cancer therapies. Unlike other pathogenic fungi with environmental niches, *Candida* species are found as a commensal organism in the mucosa, gastrointestinal, and urogenital tracts of 60-80% of humans (1). The most common infections caused by *Candida* species are invasive or mucosal infections.

Invasive candidiasis can range in severity from bloodstream infections to infections of one or more organs. Candidemia, the most common form of invasive candidiasis, is the fourth leading cause of nosocomial bloodstream infections in the United States (2). Candidemia usually occurs in patients hospitalized with serious underlying conditions. Risk factors associated with candidemia include neutropenia, cancer chemotherapy, antimicrobial agents, and indwelling catheters (3). Neutropenic patients, patients undergoing cancer chemotherapy, and organ transplant patients are immunosuppressed and cannot fight off opportunistic infections. Use of broad spectrum antimicrobial agents promotes increased colonization of fungal cells due to a decrease in competing flora. Indwelling catheters facilitate candidemia and invasive candidiasis by disrupting the mucocutaneous barrier and allowing direct vascular access. Catheters also provide a surface for the organism to adhere and create a focus for infection. Candidemia has a high rate of morbidity and mortality, 30 to 70% among cancer patients and 26 to 75% among non-cancer patients (3).

Mucosal candidiasis occurs mainly in the form of vulvovaginal candidiasis or oropharyngeal candidiasis. In contrast to organs and tissues, mucosal surfaces tolerate the growth of a variety of potentially pathogenic and nonpathogenic indigenous flora (4). Disease occurs when the balance of indigenous flora is disturbed, allowing for an overgrowth of *Candida* species. Vulvovaginal candidiasis (VVC) occurs most frequently in women of childbearing age with an estimated 75% of all adult women having had at least one infection. *Candida* species can be found as a commensal in the vagina in 10 to 25% of all women (1). Other than pregnancy, infections can also occur due to antibiotic use, immunosuppressive therapy, hormone replacement therapy, or uncontrolled diabetes mellitus (4).

Oropharyngeal candidiasis (OPC) occurs in several patient populations. OPC occurs in elderly patients with dentures due to poor oral hygiene. The dentures provide a surface on which *Candida* can adhere, and the resolution of the infection occurs with the removal of the dentures (5). OPC occurs most frequently in patients infected with the human immunodeficiency virus (HIV) with an estimated 90% of patients developing OPC. The occurrences of OPC increase as the CD4⁺ cell counts decrease usually to a threshold less than 200 cells/mm³ (6). Although OPC has declined with the introduction of highly active antiretroviral therapy (HAART), the cost of

drug and the monitoring required makes widespread use of HAART difficult in third world nations where the majority of HIV-infected patients reside (7, 8).

OPC is usually classified as pseudomembranous or erythematous. Pseudomembranous OPC is characterized by smooth, white, papular lesions. Erythematous OPC is characterized by diffuse, macular erythema of the palate, oropharynx, buccal mucosa, and dorsal tongue. Hyphae and pseudohyphae found on the mucosal surface are generally associated with pseudomembranous OPC, whereas, hyphae are generally not seen on the mucosal surface in erythematous OPC (6).

In invasive candidiasis, 60 to 75% of infections are caused by *C. albicans* (3). In VVC, *C. albicans* causes 85 to 90% of infections, whereas in OPC, *C. albicans* causes 77 to 100% of infections (4, 6). Although the instances of non-*albicans* species being the causative agent of infection is increasing in both invasive and mucosal candidiasis, *C. albicans* remains the most prevalent etiological agent of *Candida* infection.

Treatment

The treatment of fungal infections is limited to a few classes of antifungal agents. These antifungal compounds must exploit the differences between host and fungal cells; however, this is difficult due to the many similarities between fungal and mammalian cells. They are both eukaryotes with similar organelles, DNA replication, and protein synthesis. A structural difference is the presence of a cell wall in fungal cells. This makes it an optimal target for antifungal agents. The cell wall protects against osmotic pressure and is composed of chitin, mannoproteins, glycosylphosphatidylinositol (GPI)- anchored proteins, and glucans. The echinocandins (caspofungin), the newest class of antifungals, are thought to inhibit β -1,3-glucan synthase. Most cell wall components are interconnected through β -1,3-glucans, making it an important component in cell wall structure (9). By inhibiting β -1,3-glucan synthase, the cell wall destabilizes and the cell is more susceptible to osmotic stress. Caspofungin is fungicidal and effective against a large number of fungal pathogens including *Candida*. However, it is only available for intravenous administration, and the cost limits the use in developing nations.

Another difference between mammalian and fungal cells is the membrane sterol composition. Sterols regulate the membrane fluidity and integrity of the cell. Mammalian cell membranes consist mainly of cholesterol; fungal cell membranes depend on ergosterol as the major membrane sterol. This difference makes ergosterol a prime target for antifungal drugs. The polyenes target the ergosterol in the plasma membrane directly. The polyenes are fungicidal agents that bind the ergosterol in the membrane forming pores and disrupting the proton gradient (10). Amphotericin B (AmB), the most effective polyene, is still widely used to treat systemic candidiasis. However, high doses of, or long term treatment with, AmB can cause nephrotoxicity. AmB is also administered intravenously, limiting the use of the drug to hospital settings. Newer liposomal formulations of AmB have decreased the toxicity, but the increased cost precludes the widespread use of the drug (11).

The biosynthesis of ergosterol is another major target of antifungal agents. Three antifungal drug classes function as ergosterol biosynthesis inhibitors, targeting different enzymes of the pathway. The allylamines act as noncompetitive inhibitors of squalene epoxidase encoded by *ERG1*, causing squalene accumulation and ergosterol depletion. Terbinafine, an allylamine, is used as a topical agent due to its fungicidal activity against dermatophytes; it is fungistatic against *Candida* species, so it is rarely used to treat *Candida* infections. The morpholines (fenpropimorph, amorolfine) inhibit two enzymes in the ergosterol biosynthesis pathway, C-14 sterol reductase, encoded by *ERG24*, and C-8 sterol isomerase, encoded by *ERG2*. Fenpropimorph is generally used as an agricultural fungicide. Amorolfine, fungicidal against dermatophytes but fungistatic against yeast pathogens, is only used as a topical agent to treat dermatophytes and VVC. The azoles are the largest class of antifungal agents, consisting of two subclasses, triazoles and imidazoles. The azoles inhibit C-14 lanosterol demethylase encoded by *ERG11* (5). Lanosterol demethylase is a cytochrome P-450 enzyme with a heme in its active site. The azoles function by binding the iron of the heme through an unhindered nitrogen. This prevents the activation of oxygen necessary for the demethylation of lanosterol (12). The inhibition of lanosterol demethylase causes the accumulation of toxic sterol, 14 α -methyl-3,6-diol, causing growth arrest (13). Although the azoles are fungistatic, they are most effective against a broad spectrum of fungal pathogens. Ketoconazole, an imidazole, was the first azole to be widely used due to its low toxicity. Fluconazole, a triazole, is now the most widely used azole antifungal agent due to its water solubility, low toxicity, oral administration, and the ability to treat both mucosal and systemic candidiasis (5, 10).

***C. albicans* Physiology**

C. albicans is a dimorphic fungus capable of several cell morphologies. At temperatures less than 35°C grown with glucose as a carbon source, *C. albicans* grows as a blastospore, or yeast cell. Blastospores are characterized as spherical, single cells that undergo mitotic cell division by budding. Budding occurs at one spot on the blastospore, and once nuclear division occurs, a septum is formed between the parent and the daughter cell. The cells separate completely to form two new cells. At temperatures greater than 35°C grown in the presence of serum, *C. albicans* starts to filament. *C. albicans* can form pseudohyphae and true hyphae under filamentous conditions. Hyphal growth occurs when a blastospore forms a germ tube that continues to extend apically. As the cell grows, mitosis occurs and a septum forms. However, unlike blastospores, the cells do not separate; instead apical growth continues as the hypha continues to elongate. The cell walls of true hyphae are parallel with no constrictions at the septum. Pseudohyphae are differentiated from true hyphae by the constrictions that occur at the septum of pseudohyphae. Pseudohyphae form from blastospores in a process similar to budding. As the daughter cell continues to grow, it takes on a more elongated shape like a hypha. After the septum forms, the cells remain attached. As the cells continue to undergo mitosis, the cells form a chain of blastospores resembling filaments (1).

The change in cell morphology is thought to be a virulence factor of infection. The reversible switch from blastospore to hypha is one method by which *C. albicans* is thought to penetrate the mucosal membrane and invade host tissue. The hyphal form expresses various cell

wall proteins that allow for better adhesion to epithelial and endothelial cells. Once in the bloodstream, the switch back to yeast cells allows for better dissemination (14).

The morphological changes and the ability to form true hyphae play a large role in the formation of biofilms. Biofilms generally occur on the surfaces of catheters, dentures, or other indwelling devices. Biofilms create a focus of infection in invasive candidiasis and also creates an environment that confers drug resistance (5). The *Candida* biofilm is a complex, multi-layered growth structure. A thin layer of blastospores adhere to the plastics, forming a thin biofilm. The blastospores then start forming a thick hyphal layer and excreting an extracellular matrix of proteins and polysaccharides. The ability to form hyphae allows for thicker biofilms and less penetration of drugs into the extracellular matrix. Biofilms formed by yeast cells alone are less adherent and thinner. *Saccharomyces cerevisiae* has the ability to form biofilms, but other than a thin basal layer of blastospores, no extracellular matrix is formed. This allows for easier antifungal penetration and less development of antifungal resistance (15).

Genetics

Genetic manipulation in *C. albicans* has been difficult because *C. albicans* is a diploid yeast with no haploid stage in its cell cycle. It has eight chromosomes with 6,354 putative genes (16). It also does not have a conventional sexual cycle. Although there is a mating type locus on chromosome 5 in *C. albicans*, meiotic recombination occurs very infrequently. Instead, reproduction is largely clonal (5).

The initial understanding of the *C. albicans* genome came from studying the better characterized yeast, *S. cerevisiae*. Although *C. albicans* and *S. cerevisiae* diverged 140-841 million years ago, many genes are highly conserved (14). Hybridization to and complementation of *S. cerevisiae* genes were two methods used to characterize functions of genes in *C. albicans*. Complementation studies in *S. cerevisiae* mutants isolated genes that could restore function; studies in wild-type *S. cerevisiae* isolates found genes that conferred new function. Hybridization studies using *S. cerevisiae* DNA arrays only revealed genes that were most highly conserved between the two species (5). With the sequencing of the *C. albicans* genome complete, DNA microarrays specific to *C. albicans* are now available. Gene expression patterns under various conditions can now be studied independent of sequence homology to *S. cerevisiae*. Actual *C. albicans* gene expression along with *S. cerevisiae* homology allows for better characterization of cell function and pathways.

Because of the diploid nature of *C. albicans*, it has historically been very difficult to isolate homozygous recessive mutants. Mutants were generated using chemical mutagens or ultraviolet light and selected on media containing drugs. This method often generated several undefined genomic alterations. *Candida*, as a genus, also has unconventional codon usage. The CUG codons are predominantly decoded as serines rather than leucines (17). This codon reassignment makes it difficult to use *S. cerevisiae* vectors and reporter constructs that depend on translation of CUG codons to leucine. Once specific auxotrophic strains were developed for *C. albicans*, gene disruptions could be performed more readily. The most frequently used auxotrophic marker used in *C. albicans* is *URA3*, a gene that encodes orotidine 5' phosphate

decarboxylase. The strain CAI4, derived from clinical isolate and genome strain SC5314, has disruptions in both alleles of *URA3* (18). The *URA3* marker allows for easy selection and counterselection of the correct transformants. The URA-blast method of gene disruption uses the selectable marker *URA3*. The cassette was constructed by flanking *URA3* with direct repeats of bacterial *hisG* sequences. The 5' and 3' regions of the gene are then cloned on either side of the *hisG* repeats. When the cassette is transformed into a *ura⁻* strain, homologous recombination occurs, replacing one allele of the gene with the cassette. Correct transformants are selected by growth on media lacking uridine. Counterselection then occurs when grown on plates containing 5-fluoroorotic acid (FOA). 5-FOA selects for *ura⁻* strains which arise through homologous recombination at the *hisG* repeats. The *URA3* gene encodes orotidine 5'-phosphate decarboxylase, a gene required for uracil biosynthesis. When a wild-type strain is grown on 5-FOA, the *URA3* gene product converts 5-FOA to 5-fluorouracil, a toxic gene product. Because of this conversion, only strains that spontaneously lose the *URA3* gene can grow on the selection media. Once the cassette has been excised from the first allele, a second round of transformations can occur to disrupt the second allele (19).

While auxotrophic strains have allowed for easier gene manipulation, the auxotrophs were derived from either the genome strain SC5314, or strain WO-1. Gene disruption in clinical isolates would be problematic unless the clinical isolate happened to have an auxotrophic marker. Two new methods of gene disruption independent of auxotrophy has allowed for the disruption of genes in any isolates regardless of genetic background. The first method relies on a dominant selection marker that confers resistance to mycophenolic acid fused to a site-specific recombinase *FLP* from *S. cerevisiae* modified to function in *C. albicans*. The *FLP* gene is controlled by the *SAP2* promoter, so *FLP* activity only occurs under inducing conditions (20, 21). The second method relies on a dominant selection marker that confers resistance to the antibiotic nourseothricin fused to the *FLP* recombinase under the control of the *MAL2* promoter (22). Both these methods effectively disrupt genes in isolates without requiring auxotrophic markers.

AZOLE RESISTANCE

Emergence of Clinical Resistance

Clinical resistance occurs when an infection persists or progresses regardless of antifungal treatment. Primary or intrinsic resistance occurs when the organism is resistant before treatment with an antifungal agent. Secondary resistance arises in response to treatment (10). The most common antifungal drug resistance occurs to the azoles due to their widespread use in treating fungal infections. Because of the fungistatic nature of the azoles, it is difficult to eradicate all of the *Candida* present in the infection particularly in the setting of an immunocompromised host, so relapses occur. The Clinical and Laboratory Standards Institute (CLSI) Subcommittee for Antifungal Susceptibility Testing has established standard breakpoints for fluconazole susceptibility. An isolate is considered sensitive with a minimum inhibitory concentration (MIC) <8 µg/ml; an isolate is resistant with an MIC ≥64 µg/ml. An isolate with an MIC of 8 to 64 µg/ml is considered susceptible-dose dependent (23). Infections due to such

isolates respond to treatment in the same manner as a susceptible strain provided a high enough dosage of fluconazole is administered.

Secondary fluconazole resistance in *C. albicans* most often occurs in either AIDS patients with OPC or less frequently in immunosuppressed patients with systemic candidiasis. Resistance to fluconazole in systemic candidiasis is usually due to intrinsic azole resistance as a result from infection with *C. glabrata* or *C. krusei*. Development of resistance in bloodstream infections by *C. albicans* is rare (8).

Fluconazole resistance in OPC is due to secondary resistance with resistant *C. albicans* strains recovered from a third of the patients with advanced AIDS. Recurrent OPC occurs most often in patients with CD4⁺ T cells <50/mm³ (24). Patients with OPC are usually treated with fluconazole at low doses (100 mg per day) over a long period of time either continuously or intermittently (8, 24). When a relapse of OPC occurs, the dose of fluconazole is increased (200, 400, 800 mg per day) with a maximum dosage of 800 mg per day. Before the use of HAART, fluconazole prophylaxis decreased the morbidity and mortality attributed to mucosal and invasive fungal infections. With the use of HAART, incidences of OPC have decreased. However, in patients undergoing HAART who develop OPC, resistance has been shown to occur. Of the patients undergoing HAART and fluconazole treatment (continuous or episodic), 36 to 45% developed fluconazole resistance (25). Although HAART has decreased the incidence of OPC, the cost of therapy in developing nations is too high to make it widely available; poor compliance also limits the effectiveness of HAART. Fluconazole prophylaxis is still necessary in resource-poor regions to control candidiasis, so development of resistance by *C. albicans* is still problematic.

Molecular Mechanisms of Resistance

Azole resistance in *C. albicans* occurs primarily due to the selection of resistance over time rather than replacement by a more resistant strain. Several genotyping techniques are used to determine if an azole resistant strain was derived from a specific azole sensitive parent. Pulsed-field gene electrophoresis (molecular karyotyping), restriction fragment length polymorphism (RFLP), and repetitive marker Ca3 probe-based fingerprinting are three methods used to determine the genetic relationship between a susceptible and a resistant strain. The karyotype and RFLP patterns between *C. albicans* of different patients are different, suggesting little relationship between strains. However, serially-collected isolates recovered from a single patient typically indicate that the resistant isolate has the same karyotype and RFLP patterns as the previously susceptible isolate (26, 27). Using these typing methods to identify matched isolate sets, specific mutations that cause azole resistance can be determined.

There are several known mechanisms by which *C. albicans* can develop resistance to azoles. A decrease in intracellular accumulation of azoles reduces the amount of drug that comes into contact with its target. Differences in azole accumulation in the cell can be due to the uptake or the efflux of the drug. Using tritium labeled fluconazole, [³H]fluconazole, differences in accumulation can be studied. In a susceptible isolate, fluconazole accumulation as a function of concentration does not occur in a linear fashion. This eliminates passive diffusion as a

mechanism of fluconazole uptake (28). Fluconazole sensitive isolates pretreated with sodium azide and then exposed to fluconazole show little difference in drug accumulation (28, 29). Sodium azide, a metabolic inhibitor, interferes with energy dependent pumps by decreasing the amount of ATP generated by the mitochondria. Because accumulation doesn't change, drug import is not energy dependent suggesting that fluconazole enters the cell through facilitated diffusion (28). In resistant isolates, [³H]fluconazole accumulation is significantly less than that of the susceptible parent isolate. The resistant isolates pretreated with sodium azide shows increased fluconazole accumulation (28, 29). Because fluconazole accumulation increases due to the disruption of energy dependent pumps and drug import seems to be energy independent, increased resistance due to decreased accumulation can be attributed to efflux pumps rather than impaired drug uptake.

One class of efflux pumps found to be overexpressed in resistant isolates of *C. albicans* is the ATP-binding cassette (ABC) transporters. ABC transporters can be found in all organisms, and most transporters are substrate specific. Multidrug resistance ABC transporters, however, have evolved to transport a wide variety of lipophilic molecules. Prokaryotes have ABC transporters that import and export various substrates; eukaryotic ABC transporters are predominately exporters (30). All ABC transporters are composed of two six-transmembrane domains (TMD) and two cytoplasmic nucleotide-binding domains (NBD) that contain the ABC. The first and most well characterized ABC multidrug transporter was mammalian P-glycoprotein, responsible for the development of resistance in cancer cells to chemotherapy drugs (31).

In *C. albicans*, ten efflux pumps have been identified as ABC multidrug transporters. These efflux pumps were named the *Candida* drug resistance (CDR) family of transporters (10). Of the ten CDR pumps, only two ABC transporters, encoded by *CDR1* and *CDR2*, have been implicated in azole resistance. *CDR1* is highly homologous to *PDR5*, a multidrug transporter in *S. cerevisiae*, and the protein structures are almost identical (32). *PDR5* confers cycloheximide, chloramphenicol, and fluconazole resistance as a *pdr5Δ* double disruption mutant causes hypersensitivity to cycloheximide, chloramphenicol, and fluconazole (33). *CDR1* was first cloned and characterized for its ability to complement drug resistance in *S. cerevisiae* (29, 32). In *C. albicans* clinical matched sets, *CDR1* was found to be expressed in both susceptible and resistant isolates. In resistant isolates, the expression of *CDR1* increased relative to the MIC of the isolates; the higher the MIC, the more *CDR1* mRNA was detected (29, 34, 35). The *CDR1* gene was also disrupted in a susceptible strain, CAF4, lacking both alleles of *URA3*. In the absence of the ABC transporter, the strain became hypersusceptible to a variety of azoles (36). The overexpression of *CDR1* using an inducible promoter in a *cdr1Δ* double mutant showed resistance to azoles (37).

CDR2, another ABC transporter efflux pump 84% identical to *CDR1*, was also identified by its functional complementation of azole resistance in *S. cerevisiae*. In matched isolates, *CDR2* gene expression could not be detected in susceptible isolates. In resistant isolates, the gene is expressed relative to the MIC (34, 35). The expression of *CDR2* in a resistant isolate is generally several fold higher than the expression of *CDR1* in the same isolates. Disruption of *CDR2* in an auxotrophic strain of *C. albicans* showed little change in susceptibility in response to various drugs. A double disruption construct, *cdr1Δcdr2Δ*, showed hypersusceptibility to azoles.

However, the lack of baseline expression levels of *CDR2* in a susceptible isolate could explain the lack of hypersusceptibility to drugs (38).

The second class of efflux pumps found in *Candida* is the major facilitator superfamily (MFS) transporters. MFS transporters are energy dependent transporters that rely on the proton motive force generated by the proton gradient across membranes rather than ATP for energy. Like ABC transporters, MFS transporters can be symporters, antiporters, or uniporters, and they can be found in all cells of all organisms (39). The MFS transporters have 12 to 14 transmembrane domains and no NBD (10, 40).

In *C. albicans*, the main MFS transporter contributing to fluconazole resistance is an antiporter multidrug resistance 1 (*MDR1*), formerly known as *BEN1*. The *Candida MDR1* is not to be confused with human *MDR1*, which is an ABC transporter. *MDR1* was first cloned for its ability to confer resistance in *S. cerevisiae* to benomyl and methotrexate (41). Overexpression of *MDR1* in *S. cerevisiae* under the control of an *ADHI* promoter showed resistance to fluconazole but no ketoconazole or itraconazole (29). Unlike the ABC transporters, fluconazole and voriconazole seems to be the only azole substrate for *MDR1*. Disruption of *MDR1* in wild-type isolates showed increased susceptibility to methotrexate, cycloheximide, and 4-nitroquinoline-*N*-oxide. The disruption mutants showed no changes in susceptibility to benomyl or fluconazole (20, 36, 42). In clinical matched isolates, *MDR1* mRNA is only detected in resistant isolates; there seems to be no baseline expression of *MDR1* in susceptible isolates (34, 43). The wild-type disruption mutants not being hypersusceptible to fluconazole are most likely due to the lack of baseline expression of *MDR1*.

Changes in lanosterol demethylase, the target of azole antifungals, are another mechanism by which resistance develops. Mutations in *ERG11* translate into amino acid substitutions that change the three-dimensional structure of the enzyme or have decreased affinity for the azole. Several point mutations have been identified in resistant isolates (27, 44, 45). One such point mutation causes an arginine to lysine substitution at position 467. The R467K is highly conserved among lanosterol demethylase found in fungi and mammals and is located in the heme binding region of the enzyme (44, 46). Using site directed mutagenesis, the R467K mutation was introduced into *S. cerevisiae*. In the presence of the mutated Erg11p, fluconazole bound to the enzyme with a lower affinity (46). Thus far, there have been thirteen additional point mutations that lead to amino acid substitutions and increased azole MIC have been identified (27, 44).

Overexpression of *ERG11* can also lead to resistance due to the increased production of lanosterol demethylase overwhelming the azole concentration in the cell. In *S. cerevisiae*, the overexpression of *ERG11* alone is sufficient to confer azole resistance. The *ERG11* gene, under the control of an inducible *GAL1* promoter, could grow on galactose plates with 128µg/ml fluconazole (47). In *C. albicans*, the up-regulation of *ERG11* is generally found in conjunction with other resistance mechanisms. Twenty-five percent of resistant isolates recovered from patients overexpressed *ERG11* and one of the efflux pumps (27). In response to azole treatment, *ERG11* and several other *ERG* genes were up-regulated (48, 49).

TRANSCRIPTIONAL REGULATORS

The overexpression of *CDR1*, *CDR2*, *MDR1*, and *ERG11* can be due to three mechanisms: gene amplification, increased half-life of mRNA, or increased transcription due to promoter mutation or changes in transcriptional activators. Gene amplification occurs when there are more than two copies of a certain gene due to chromosomal duplication. While some isolates have been found to contain trisomies and isochromosomes, many clinical matched sets show overexpression with no chromosomal anomalies (50).

The stability of mRNA also makes an impact on overexpression of resistance genes. A recent study showed that resistant isolates had a slower turnover rate of *CDR1* mRNA than susceptible isolates (51). However, mRNA stability alone does not result in the increased mRNA levels in resistant isolates. Nuclear run-on assays performed on susceptible and resistant isolates show an increased level of transcription in the resistant isolates (34, 51). In the absence of chromosomal anomalies, the increase in transcription can be attributed to interactions between transcription factors and the promoter.

Transcription Factor Classes

There are two major classes of transcription factors involved in drug resistance in yeast. The bZip transcription factor class is found exclusively in eukaryotic cells, and the first bZip proteins were purified in mammalian cells in the late 1980's (52). These transcription factors are defined by a highly conserved basic leucine zipper (bZip) motif. The bZip motif is a 60 to 80 amino acid long bipartite α -helix that binds the major groove of DNA. The N-terminal portion of the bZip motif contains the DNA binding domain (DBD) consisting of two basic amino acid clusters that are responsible for the binding of DNA. The C-terminal domain is the leucine zipper with leucine residues every seven amino acids (52). It is through the leucine zipper that dimerization occurs (52, 53). The activator protein-1 (AP-1) transcription factor family falls into the bZip class of transcription factors. AP-1 transcription factors can be heterodimers consisting of one Fos family and one Jun family of bZip protein or homodimers containing two Jun family proteins. The dimers bind DNA at a specific AP-1 recognition element (ARE), TGA(C/G)TCA (53). This ARE motif is a ubiquitous regulatory element found in promoter and enhancer regions of many genes regulating cell proliferation, differentiation, apoptosis, and oncogenesis.

AP-1 transcription factors have been identified in several fungal species (54, 55). These transcription factors are referred to as yeast AP-1 (yAP1) transcription factors. The first yAP-1 transcription factor cloned and characterized was the *S. cerevisiae* *YAP1* (54, 56). Yap1p was identified for its ability to bind an ARE derived from the Simian virus 40 (SV40) enhancer promoter known to be bound by mammalian AP-1 (56). This yeast promoter motif is called the *YAP1* response element (YRE). *YAP1* homologs were also found in *Schizosaccharomyces pombe* (*PAP1*), *Kluveromyces lactis* (*KAP1*), and *C. albicans* (*CAP1*) (57). The yAP-1 transcription factors have bZip DBDs homologous to that of mammalian AP-1 transcription factors. In addition, yAP-1 transcription factors share two cysteine rich domains (CRD) 3' of the bZip domain. The amino terminal CRD (n-CRD) and the carboxyl terminal CRD (c-CRD) are found in only the yeast bZip transcription factors and play a role in the localization and

activation of the yAP-1 (58). The yAP-1 transcription factors regulate and mediate the response to oxidative stress and multidrug resistance.

In *S. cerevisiae*, the c-CRD has been implicated as the oxidative stress sensor and the nuclear localization of Yap1p. The c-CRD contains three conserved cysteine residues and a region rich in hydrophobic amino acids that resemble a nuclear export signal (58, 59). Studies using a green fluorescent protein (GFP) tagged Yap1p showed that under normal conditions, GFP-Yap1p localizes to the cytoplasm. Nuclear localization occurs when the cells are exposed to oxidative stress such as H₂O₂ or diamide. The oxidative stress sensor function was elucidated when the c-CRD domain was fused to a *GAL4* DBD and GFP. This fused protein was found in the cytoplasm under unstressed conditions, but under oxidative stress conditions, the GFP was localized to the nucleus (60). This shows that the c-CRD alone has the ability to localize the fused transcription factor to the nucleus in the presence of a stressor. Mutations in or deletion of the c-CRD trapped the GFP-Yap1p in the nucleus independent of oxidative stress (58, 59, 60). Nuclear localization occurs either through the inhibition of nuclear export or increases in nuclear import. In yeast, Crm1p has been identified as the nuclear export factor that interacts with yAP-1 to export the proteins out of the nucleus (61). Disulfide bonds form between the cysteine residues in the presence of H₂O₂ or diamide and prevents the interaction between Crm1p and Yap1p (62). Mutations in the c-CRD also prevent the Crm1p-Yap1p interaction. The n-CRD on the other hand determines the response to specific oxidative stresses. In *S. cerevisiae*, disruption of the n-CRD conferred hypersusceptibility to H₂O₂ but resistance to diamide (63). This points to the differences in disulfide bond formation between the cysteine residues in response to oxidative stress. Under diamide stress, a disulfide bond forms between two cysteine residues in the c-CRD. In the presence of H₂O₂, a disulfide bond forms between a cysteine residue in the c-CRD and a cysteine residue in the n-CRD. This disulfide bridge does not occur under diamide stress (59).

The zinc finger protein family is the second major class of transcription factors responsible for drug resistance in yeast and are the largest family of transcriptional regulators in eukaryotes (64). The first zinc finger protein was identified in *Xenopus* in the 1980's (65). This family of transcription factors is characterized as having the common zinc finger motif, consisting of one α helix and a pair of antiparallel β strands. It also contains one or more zinc atoms bound by cysteines or histidines stabilizing the domain and contributing to the structure and function of the protein. The majority of zinc finger proteins bind DNA as transcription factors, but some act as mediators of protein/protein interactions, chromatin remodeling, protein chaperoning, lipid binding, stress response, pleiotropic drug resistance (PDR), and zinc sensing (64).

There are three classes of zinc finger proteins. The Cys₂His₂ class is the classical zinc finger protein that binds DNA as monomers. These proteins have the zinc finger structure Cys-X₂₋₄-Cys-X₁₂-His-X₃₋₅-His. The second class of zinc finger proteins is the Cys₄ (Cys-X₂-Cys-X_n-Cys-X₂-Cys-X_n-Cys-X₂-Cys) class of proteins. These zinc finger proteins bind DNA as homodimers or heterodimers. The homodimers bind inverted repeats while the heterodimers bind direct repeats (64). Cys₆, the third class of zinc finger proteins, have six cysteines (Cys-X₂-Cys-X₆-Cys-X₅₋₁₆-Cys-X₂-Cys-X₆₋₈-Cys) bound by two zinc atoms and are exclusive to fungi. These proteins bind DNA as monomers, homodimers, and heterodimers.

This third class of zinc finger proteins is often referred to as zinc cluster proteins or zinc binuclear proteins (64, 66, 67).

Zinc cluster transcription factors can be found in both the cytoplasm and the nucleus. The proteins found in the cytoplasm require a nuclear localization signal and importin β receptors (64). More commonly, zinc cluster transcription factors remain in the nucleus and are often constitutively bound to the promoter. Zinc cluster proteins contain a DBD, a regulatory domain, and an acidic domain. The DBD consists of the zinc finger motif, the linker, and the dimerization domain (64). The zinc finger motif binds as dimers to CGG nucleotide triplets through major groove interactions. The CGG motifs can be everted, inverted, or direct repeats (64, 66). The linker domain of the DBD has no homology between zinc cluster proteins, but this contributes to the specificity of DNA binding (68). A previous study swapped the linker domains of three different zinc cluster transcription factors, and showed that it was the linker that determined the target DNA. The dimerization domain is highly conserved with heptad repeats and resembles the leucine zipper in bZip proteins (64). The regulatory domain, also known as the middle homology domain, spans 80 amino acids and is only found in zinc cluster transcription factors (69). Deletions of this domain render the transcription factor constitutively active. Partial and complete deletions of the regulatory domain in the *S. cerevisiae* transcription factor Leu3p renders it constitutively active (70). The regulatory domain is not highly conserved among zinc cluster proteins. Differences in the regulatory domain may determine the spacing of the CGG motif that the transcription factor binds (69). The acidic domain acts as the activation domain of the protein. Activation of zinc cluster transcription factors occur through phosphorylation or dephosphorylation events (64). There is little homology between the acidic domains of different zinc cluster proteins and the function of the domain differs between proteins (64). In *S. cerevisiae*, deletion of the acidic domain of Leu3p rendered the transcription factor inactive (70). In the *S. cerevisiae* transcription factor Pdr3p, mutations in this domain results in a gain-of-function of the transcription factor (71).

Transcription Factors and Resistance

Increased transcription of azole resistance genes in *C. albicans* could be due to mutations in the promoter region that results in deregulation of the gene (*cis*-regulation) or mutations in a transcription factor that controls the expression of the gene (*trans*-regulation). The promoter regions of clinical matched isolates known to overexpress *CDR1/CDR2* or *MDR1* were sequenced (72, 73). In the clinical isolates DSY294 (susceptible parent) and DSY296 (resistant), there were no mutations in the promoter regions of the *CDR* genes (73). In the *MDR1* overexpressing isolates, F and G, polymorphisms were found between the alleles of the two isolates. However, the same polymorphisms were found in the susceptible and resistant allele of each isolate pair (72). The lack of mutation suggests that the overexpression of the two efflux mechanisms do not occur through a *cis*-acting mechanism.

In order to determine if a *trans*-regulatory element was responsible for the up-regulation of the efflux pumps, the wild-type promoter regions were fused to a reporter gene to determine transcriptional activation. The promoters of *CDR1* and *CDR2* from genome strain SC5314 were fused to *Renilla* luciferase (*RLUC*), and the constructs were transformed into a resistant strain

(2090) derived from the resistant isolate DSY296 (73). A high level of luciferase activity was detected in the cells transformed with the *CDR1-RLUC* and the *CDR2-RLUC* constructs even though the promoters were derived from a susceptible isolate. Similarly, to examine the activation of *MDR1*, the promoter region of *MDR1* from wild-type strain CAI4 was fused to GFP. The *MDR1-GFP* construct was transformed into two resistant isolates, F5 and G5, and their susceptible parent isolates, F2 and G2. There was no fluorescence detected in the susceptible isolates, whereas fluorescence was detected in the resistant isolates (72). The ability for the *CDR* and *MDR* promoters from susceptible isolates to be activated when transformed into resistant strains suggests that mutations in transcriptional regulators are responsible for the overexpression of resistance mechanisms.

Regulatory networks and drug resistance have been better characterized in the model organism *S. cerevisiae*. In *S. cerevisiae*, two networks of genes, YAP and pleiotropic drug resistance (PDR), are responsible for multidrug resistance. The YAP network of genes is predominantly responsible for the oxidative stress response. Yap1p, a bZip transcription factor described above, binds the YRE motif T(T/G)A(C/G)TAA. Not only has Yap1p been shown to sense hydrogen peroxide and diamide stress and confer resistance to oxidative stress (62), but it has also been shown to protect against a variety of antimicrobial drugs through the overexpression of ABC transporters (*YCF1*) and MFS transporters (*FLR1*). The overexpression of *FLR1* specifically confers resistance to cycloheximide, 4-nitroquinoline *N*-oxide, benomyl, and fluconazole (55). The deletion of *YAP1* abolished the ability for induction of *FLR1* by antimicrobial agents (74). Three YRE motifs have been identified in the promoter region of *FLR1*, and increased activation of Yap1p increases the transcription of *FLR1* (55, 74). Yap1p has also been shown to bind directly to the three YRE motifs, and the deletion of the YREs abolished the induction of *FLR1* by fluconazole (74).

The PDR network is characterized by the overexpression of ABC transporters and MFS transporters. The overexpression of the ABC transporter gene, *PDR5*, confers resistance to several antimicrobial agents including fluconazole. The PDR target genes are controlled by two zinc cluster transcription factors, Pdr1p and Pdr3p. Pdr1p and Pdr3p bind as homodimers or heterodimers to the pleiotropic drug response element (PDRE) motif, TCCGCGGA, with an everted CGG (64, 75). The dimers can also bind degenerate PDRE motifs suggesting different specificities for homodimers versus heterodimers (76). At least one PDRE motif has been found in all target genes of Pdr1p and Pdr3p (77). Two PDRE motifs have been found in the promoter region of *PDR3*, suggesting autoregulation by Pdr3p and regulation by Pdr1p. Hyperactive *PDR1* and *PDR3* mutants increase the expression of *PDR5* and cause resistance to various drugs (78, 79). The up-regulation of *PDR1* and *PDR3* also increases the expression of *PDR3* (71). Deletion of *PDR1* and *PDR3* genes results in hypersusceptibility to drugs and decreased expression of *PDR5* (79, 80).

In addition to the efflux mechanisms, the ergosterol biosynthesis pathway is also better characterized in *S. cerevisiae*. In *S. cerevisiae*, ergosterol biosynthesis genes are regulated by two zinc cluster transcription factors Upc2p and Ecm22p. These transcription factors bind a sterol response element (SRE) motif, CTCGTATAAGC (81). The up-regulation of *UPC2* and *ECM22* causes up-regulation of *ERG2* and *ERG3*. The SRE motif is present in the promoter regions of *ERG2*, *ERG3*, and other ergosterol biosynthesis genes. Deletion of *UPC2* causes

hypersusceptibility to AmB and ketoconazole while deletion of *ECM22* causes hypersusceptibility to cycloheximide and calcofluor white (81, 82). The disruption mutants also abolish the expression of the *ERG* genes (81).

***C. albicans* Transcription Factors**

In *C. albicans*, *CAP1* has been identified as a yAP-1 bZip transcription factor closely related to the *S. cerevisiae* *YAP1*. Functionally, the overexpression of *CAP1* in *S. cerevisiae* restores *FLR1* expression and resistance to fluconazole (55). The C-terminal region of Cap1p and Yap1p, including the c-CRD, are the most highly conserved. As in *S. cerevisiae*, the activation and nuclear localization occurs through the c-CRD in response to oxidative stress (55, 83). The disruption of the c-CRD of Cap1p causes the protein to be constitutively active and remain localized to the nucleus (83, 84). To examine the function of Cap1p in *C. albicans*, the *CAP1* gene was deleted in the susceptible wild-type strain CAI4, and a wild-type or a constitutively active *CAP1* gene was reintroduced into the knockout strain. In the strains with or without *CAP1*, the cells were unable to respond to fluconazole. However, with the constitutively active Cap1p, the strain was resistant to fluconazole, and increased expression of *MDR1* was detected (84). *FLR1* is also homologous to *C. albicans* *MDR1*, a MFS transporter gene responsible for fluconazole resistance (55). A YRE motif was found in the promoter region of *MDR1* (85). The promoter region of *MDR1* was examined in a fluconazole resistant clinical strain disrupted for *URA3* (F5U4). In the absence of the YRE, *MDR1* expression due to benomyl exposure decreased. However, in the absence of the YRE in a resistant isolate, *MDR1* is still expressed (86). In another study, Cap1p regulation of *MDR1* is examined in strains with and without *CAP1*. In the absence of Cap1p, *MDR1* gene expression still occurs in response to benomyl (87). These studies indicate that in the absence of both the YRE motif and Cap1p, *MDR1* gene expression still occurs in response to benomyl and in resistant isolates. This suggests that while Cap1p regulates *MDR1* expression, another regulator may also function in azole resistance.

The ABC transporters *CDR1* and *CDR2* were identified for their ability to complement the *PDR5* phenotype in *S. cerevisiae*. However, there are no *PDR1/PDR3* homologues in *C. albicans*. The promoter regions of *CDR1/CDR2* were examined, and a putative drug responsive element (DRE) motif, ACGG(A/T)TATCGGATA, was identified (73). Genes with the DRE in their promoter region were shown to respond in response to oestradiol including *CDR1/CDR2*. In the absence of this motif, the expression of the genes could not be induced by oestradiol, amorolfine, and terbinafine. In response to oestradiol, band shift assays showed specific protein interaction with the DRE (73). Unlike the PDRE, the DRE has two CGG direct repeats separated by four nucleotides. The two CGG motifs in the promoter region points to a zinc cluster transcriptional regulator. *TAC1* (transcriptional activator of *CDR* genes), a gene encoding a zinc cluster protein, was identified near the mating type locus (88). The disruption of this gene abolished the expression of *CDR1/CDR2* in response to fluphenazine. The disruption strains also showed a hypersusceptibility to fluconazole and terbinafine. When a *TAC1* allele from a resistant isolate was reintroduced into the disruption strain, resistance to fluconazole was conferred. The specific DRE previously identified was shown to be specifically bound by Tac1p, and the PDRE motif bound by Pdr1p/Pdr3p in *S. cerevisiae* was not bound by Tac1p (88).

Sequencing of the *TAC1* alleles in susceptible and resistant clinical isolates showed an N977D mutation in the C-terminal activation domain (89). While this mutation was found in one allele of *TAC1* in the susceptible isolate, the resistant isolate carried two copies of this mutated allele. Because zinc cluster transcription factors function as dimers, the hyperactive allele and the wild-type allele are codominant. It is the loss of heterozygosity that causes overexpression of ABC transporters and increased azole resistance (89).

Ergosterol biosynthesis in *S. cerevisiae* is controlled by two zinc cluster transcription factors Upc2p and Ecm22p. In *C. albicans*, Upc2p is the only homolog of ScUpc2p and ScEcm22p. Disruption of *UPC2* in a wild-type strain conferred hypersusceptibility to sterol biosynthesis inhibitors (SBI), including fluconazole, itraconazole, ketoconazole, terbinafine, and fenpropimorph (82, 90). In the absence of *UPC2*, there was also no transcription of *ERG* genes. The reintroduction of wild-type *UPC2* restores baseline susceptibility in wild-type strains (90). The overexpression of *UPC2* conferred resistance to ketoconazole and fluphenazine (82). The promoter region of the *ERG* genes in *C. albicans* was examined for the SRE motif found in *S. cerevisiae*, and an SRE motif was found in all known *ERG* genes and in *UPC2* itself (82, 90). Using the SRE motif found in *ScERG2* and *CaERG2*, Upc2p was found to bind to both (82).

CENTRAL HYPOTHESIS AND RESEARCH OBJECTIVES

In *S. cerevisiae*, genes and their function have been better defined and characterized than in *C. albicans*. Through the use of *S. cerevisiae* as a model organism, drug efflux mechanisms and ergosterol biosynthesis genes have been identified in *C. albicans* through homology and complementation. Increased expression of *ERG11* in *C. albicans* increases lanosterol demethylase concentrations in the cell, increasing the amount of enzyme not bound by azole antifungals. Decreased accumulation of azoles in *C. albicans* due to increased expression of drug efflux pumps also decreases the efficacy of azole treatment. In *C. albicans*, drug resistance genes have been shown to be controlled by *trans*-regulatory elements rather than *cis*-regulatory elements. Several transcriptional regulators have been identified in *S. cerevisiae* to control drug efflux and sterol biosynthesis. The transcription factors in *S. cerevisiae* have been shown to be co-regulated with the expression of resistance genes; many are also autoregulated. Importantly, the transcriptional regulators in *S. cerevisiae* do not have homologs in *C. albicans*.

I hypothesized that overexpression of resistance genes in *C. albicans* is due to gain-of-function mutations in transcriptional regulators. With the completion of the *C. albicans* genome sequencing project, microarray technology can now be used to study drug resistance. By studying matched clinical isolates that overexpress either *CDR1/CDR2*, *MDR1*, or *ERG11*, genes that are co-regulated with these resistance genes can be identified. Among these co-regulated genes, we have identified previously described as well as novel transcriptional regulators.

Tac1p, a zinc cluster transcription factor, has been previously identified as the transcriptional regulator of the ABC transporters *CDR1* and *CDR2*. Gain-of-function mutations in *TAC1* and loss of heterozygosity contribute to azole resistance. Tac1p has also been shown to regulate the expression of genes co-regulated with *CDR1* and *CDR2*. Our first research objective (Chapter 2) was to examine matched clinical isolates known to overexpress *CDR1/CDR2* by

gene expression and location profiling in an effort to identify additional genes potentially involved in azole resistance. I hypothesized that a gain-of-function mutation in a transcription factor, now known to be Tac1p (88), regulates the expression of *CDR1*, *CDR2*, and co-regulated genes. Our studies show that like many zinc cluster transcription factors in *S. cerevisiae*, *TAC1* is autoregulated and targets a subset of genes involved in multidrug resistance in *C. albicans*. Additionally, we identified the Tac1p regulon as it pertains to known mutations in *TAC1* in clinical matched isolates.

In research objective 2 (Chapter 3), we examined clinical matched isolates known to overexpress *MDR1*. At the time, the transcriptional regulator responsible for *MDR1* expression had yet to be identified. I hypothesized that gain-of-function mutations in a transcription factor regulate the expression of *MDR1* and *MDR1* co-regulated genes. Using expression profiling, we discovered and characterized a previously unidentified zinc cluster transcription factor *MRR1* that regulates the expression of *MDR1* in clinical resistant isolates. We also identified a core group of genes regulated specifically by the gain-of-function mutation in *MRR1*.

In addition to the isolates that overexpress the *CDR* and/or *MDR1* efflux pumps, several resistant isolates have been identified with unknown resistance mechanisms. In research objective 3 (Chapter 4), we characterized a clinical matched isolate pair with no previously identified resistance mechanism by gene expression profiling. I hypothesized that a gain-of-function mutation in *UPC2* is responsible for the regulation of the *ERG* genes in azole resistant clinical isolates. In the resistant isolate, several *ERG* genes were found to be up-regulated. Additionally, *UPC2* was also overexpressed. We identified a gain-of-function mutation in *UPC2* responsible for the increased expression of *ERG* genes. Using expression profiling, we defined a set of genes whose expression depended on the mutation found in *UPC2*.

CHAPTER 2: IDENTIFICATION OF THE TAC1P REGULON*

INTRODUCTION

Several mechanisms of resistance to the azole antifungal agents have been described in *C. albicans* including increased expression of genes encoding multi-drug efflux pumps (91, 92, 93, 94, 95, 96, 97). These include the gene encoding a transporter of the MFS (*MDR1*) and genes encoding two ABC transporters (*CDR1* and *CDR2*) (91, 92, 94, 95, 96). Over-expression of these efflux pumps is presumed to prevent accumulation of effective concentrations of the azole antifungal agents within the fungal cell. Among studies examining multiple matched azole-susceptible and -resistant sets of isolates, some isolates only over-express *MDR1*, whereas others only over-express *CDR1* and *CDR2* (91, 98). These observations suggest that two distinct transcriptional pathways are involved in regulating these efflux pumps.

Previous studies have shown that a wild-type *CDR1* promoter fused to a luciferase reporter construct becomes activated when placed in an azole-resistant isolate that over-expresses *CDR1* and *CDR2* (99). Using this system, it was shown that a conserved DNA sequence element in the *CDR1* and *CDR2* promoters, named drug response element (DRE; 5'-CGGAA/TATCGG), was necessary for *CDR1* constitutive, as well as drug-inducible, transcriptional activation (99). These results suggested that a gain-of-function mutation in a transcription factor was the cause of *CDR1* and *CDR2* constitutive overexpression in azole-resistant isolates (99). The zinc cluster transcription factor Tac1p was recently identified and shown to be responsible for *CDR1* and *CDR2* transcriptional activation (89, 100). An amino acid change from an asparagine to aspartic acid at position 977 (N977D) in Tac1p was able to confer increased expression of *CDR1* and *CDR2* accompanied by decreased azole susceptibility (89). It was also shown that the DBD of Tac1p fused to the glutathione *S*-transferase (GST) protein binds *in vitro* to the *CDR1* and *CDR2* DRE (100). Interestingly, increased azole resistance is observed when a gain-of-function mutation is present in both *TAC1* alleles (89). The *TAC1* locus is on chromosome 5. Homozygosity at the *TAC1* locus occurs either through mitotic recombination between copies of chromosome 5, or through the presence of extra copies of chromosome 5 harboring a gain-of-function *TAC1* allele and loss of chromosome 5 with the wild-type *TAC1* allele (89). Selmecki *et al.* demonstrated that a specific segmental aneuploidy, consisting of an isochromosome composed of the two left arms of chromosome 5, is associated with azole resistance. Increases and decreases in azole resistance were found to be strongly associated with gain and loss of this isochromosome (50). Recent work by Coste *et al.* showed that the effect of specific mutations in azole resistance genes located on chromosome 5 (*TAC1*, *ERG11*) can be further enhanced by loss-of-heterozygosity and/or addition of extra copies of chromosome 5 (101).

In addition to *CDR1* and *CDR2*, other targets of Tac1p (*RTA3*, *HSP12*, and *IFU5*) have been identified, all of which contain a putative DRE in their promoter region (100). In a separate study, it was shown that *PDR16*, encoding a putative phosphatidylinositol transfer protein contributing to clinical azole resistance, is also a target of Tac1p (102, 103). We used functional

*Liu, T. T., S. Znaidi, K. S. Barker, L. Xu, R. Homayouni, S. Saidane, J. Morschhauser, A. Nantel, M. Raymond and P. D. Rogers. 2007. Genome-wide expression and location analyses of the *Candida albicans* Tac1p regulon. *Eukaryot Cell*. 6: 2122-38. Adapted by permission of publisher.

genomic approaches, namely genome-wide expression and location profiling, to identify the Tac1p regulon. In collaboration with our group, the laboratory of Dr. Martine Raymond at Université de Montréal constructed the chromatin immunoprecipitation (ChIP) microarrays used in the study as well as the triple-HA-tagged Tac1p. Our results provide a more comprehensive picture of the molecular effects of Tac1p mutations in azole-resistant clinical isolates and suggest other important cellular functions for Tac1p in *C. albicans*.

MATERIALS AND METHODS

Strains and Growth Media

The *C. albicans* strains used in this study are listed in Table 2-1. The matched clinical isolate sets and strain SZY31 were grown in yeast peptone dextrose (YPD) broth (Sigma-Aldrich, St. Louis, MO) at 30°C. The CAI4 strain and its epitope-tagged derivatives were grown at 30°C in YPD or, under selective conditions, in synthetic complete (SC) medium lacking uracil (104) supplemented or not with 100 µg.ml⁻¹ of uridine and 1 mg.ml⁻¹ of 5-fluoroorotic acid (5-FOA, Toronto Research Chemicals, Inc., Toronto, ON). The *Escherichia coli* DH5α bacterial strain was used for DNA cloning and maintenance of the plasmid constructs.

Construction of *C. albicans* Expression Microarrays

The nucleotide sequences corresponding to 6,165 open reading frames (ORFs) for *C. albicans* were downloaded from the Galar Fungail European Consortium (Assembly 6, http://www.pasteur.fr/Galar_Fungail/CandidaDB/). We set out to design two non-overlapping probesets targeting the 3'- 600 bp region of each ORF. Each probeset consisted of 13 'perfect match' 25-bp probes and their corresponding 'mismatch' control probes containing a single mismatch in the center of the oligonucleotide. For ORFs less than 600 bp in length the sequence was divided in two equal segments for subsequent design procedures. Optimum probesets were selected by the Affymetrix design team based on their model which, among other things, considers probe hybridization quality and cross-hybridization potential. Consequently, in some cases, only one probeset was selected for a given ORF. For quality control and normalization purposes, we made 2-3 additional probe sets spanning the entire sequence of the *C. albicans* 18S rRNA (GenBank Accession M60302), genes encoding GAPDH, actin and Mdr1p (Bmr1p) in addition to the standard Affymetrix controls (BioB, C, D, cre, DAP, PHE, LYS, THR). In the end, the GeneChip contained 10,736 probe sets including 9 controls, 6,123 unique ORFs and duplicate probe sets for 4,604 ORFs. The duplicate probe sets are made to distinct regions of each particular ORF, thereby allowing 2 independent measurements of the mRNA level for that particular gene. The *C. albicans* custom Affymetrix NimbleExpress Arrays (CAN04a530004N) were manufactured by NimbleGen Systems (105) per our specification.

Table 2-1. Strains used in this study.

Strain	Parental strain	Genotype or Description	Fluconazole MIC (µg/mL)	Reference
C43 (DSY294)		Azole-susceptible clinical isolate	0.25 ^a	(97)
C56 (DSY296)	C43	Azole-resistant clinical isolate	128 ^a	(97)
Gu2		Azole-susceptible clinical isolate	6.25 ^b	(95)
Gu5	Gu2	Azole-resistant clinical isolate	> 100 ^b	(95)
3	2	Azole-susceptible clinical isolate	8 ^c	(106)
17	16	Azole-resistant clinical isolate	> 64 ^c	(106)
5457		Azole-susceptible clinical isolate	≤ 0.5 ^d	(102)
5674	5457	Azole-resistant clinical isolate	32.0 ^d	(102)
SZY31	5674	<i>tac1Δ::FRT/tac1Δ::FRT</i>	0.5 ^e	(103)
CAI4		<i>ura3Δ::imm434/ura3Δ::imm434</i>	2.0 ^e	(18)
SZY51	CAI4	<i>TAC1/TAC1::HA₃-URA3-HA₃</i>	ND	This study
SZY63	SZY51	<i>TAC1/TAC1::HA₃</i>	2.0 ^e	This study
SZY103	SZY63	<i>TAC1::HA₃</i>	2.0 ^e	This study
SZY106	SZY63	<i>TAC1::HA₃</i>	2.0 ^e	This study
SZY91	SC5314	<i>tac1Δ/TAC1^{N972D}-MPA^R-FLP</i>	16.0 ^e	(103)

^a Fluconazole concentration yielding at least 90% growth inhibition after 24 h of growth compared with the growth of the control (96).

^b Lowest Fluconazole concentration in which little or no growth was visually detected after 48 h of growth.

^c MIC₈₀ according to the CLSI macrodilution reference method or a microdilution modification of the CLSI method (106, 107).

^d MIC₅₀ values, determined as the first concentration of the azole drug able to reduce growth by 50% as compared with control cells grown in the absence of drug.

^e MIC₇₀ values.

RNA Preparation for Microarrays

The matched clinical isolate sets and strain SZY31 were grown in YPD broth at 30°C in a shaking incubator to mid-log phase as described previously (108). The cell pellets were frozen and stored at -80°C prior to RNA preparation. Experiments were repeated independently three times. Total RNA was isolated using the hot SDS-phenol method (109). Frozen cell pellets were suspended in 12 ml of 50 mM sodium acetate (pH 5.2), 10 mM EDTA at room temperature, after which 1 ml of 20% sodium dodecyl sulfate and 12 ml of acid phenol (Fisher Scientific, Waltham, MA) were added. This mixture was incubated 10 min at 65°C with vortexing each minute, cooled on ice for 5 min, and centrifuged for 15 min at 12,000 x g. Supernatants were transferred to new tubes containing 15 ml of chloroform, mixed and centrifuged at 200 x g for 10 min. The aqueous layer was removed to new tubes, RNA was precipitated with 1 volume isopropanol and 0.1 volume 2 M sodium acetate (pH 5.0), and then collected by centrifugation at 17,000 x g for 35 min at 4°C. The RNA pellet was suspended in 10 ml of 70% ethanol, collected again by centrifugation, and suspended in nuclease free water.

cRNA Synthesis and Labeling

Immediately prior to cDNA synthesis, the purity and concentration of RNA samples were determined from A_{260}/A_{280} readings and RNA integrity was determined by capillary electrophoresis using the RNA 6000 Nano Laboratory-on-a-Chip kit and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) as per the manufacturer's instructions. First and second strand cDNA was synthesized from 15 µg of total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) and oligo-dT24-T7 primer (PrOligo: Sigma-Aldrich) according to the manufacturer's instructions. cRNA was synthesized and labeled with biotinylated UTP and CTP by *in vitro* transcription using the T7 promoter-coupled double stranded cDNA as template and the Bioarray HighYield RNA Transcript Labeling Kit (ENZO Diagnostics, New York, NY). Double-stranded cDNA synthesized from the previous steps was washed twice with 70% ethanol and suspended in 22 µl of RNase-free water. The cDNA was incubated as recommended with reaction buffer, biotin-labeled ribonucleotides, dithiothreitol, RNase inhibitor mix and T7 RNA polymerase for 5 h at 37°C. The labeled cRNA was separated from unincorporated ribonucleotides by passing through a CHROMA SPIN-100 column (Clontech, Mountain View, CA) and ethanol precipitated at -20°C overnight.

Oligonucleotide Array Hybridization and Analysis

The cRNA pellet was suspended in 10 µl of RNase-free water and 10 µg was fragmented by ion-mediated hydrolysis at 95°C for 35 min in 200 mM Tris-acetate (pH 8.1), 500 mM potassium acetate, 150 mM magnesium acetate. The fragmented cRNA was hybridized for 16 h at 45°C to the *C. albicans* NimbleExpress GeneChip arrays. Arrays were washed at 25°C with 6 × SSPE, 0.01% Tween 20 followed by a stringent wash at 50°C with 100 mM MES, 0.1 M NaCl, 0.01% Tween 20. Hybridizations and washes employed the Affymetrix Fluidics Station 450 using their standard EukGE-WS2v5 protocol. The arrays were then stained with phycoerythrin-conjugated streptavidin (Molecular Probes: Invitrogen) and the fluorescence intensities were

determined using the GCS 3000 high-resolution confocal laser scanner (Affymetrix, Santa Clara, CA). The scanned images were analyzed using software resident in GeneChip Operating System v2.0 (GCOS; Affymetrix). Sample loading and variations in staining were standardized by scaling the average of the fluorescent intensities of all genes on an array to a constant target intensity. The signal intensity for each gene was calculated as the average intensity difference, represented by $[\Sigma(\text{PM} - \text{MM})/(\text{number of probe pairs})]$, where PM and MM denote perfect-match and mismatch probes.

Expression Microarray Data Analysis

The scaled gene expression values from GCOS software were imported into GeneSpring 7.2 software (Agilent Technologies) for preprocessing and data analysis. Probe sets were deleted from subsequent analysis if they were called absent by the Affymetrix criterion and displayed an absolute value below 20 in all experiments. The expression value of each gene was normalized to the median expression of all genes in each chip as well as the median expression for that gene across all chips in the study. Pairwise comparison of gene expression was performed for each matched experiment. Data files of each scanned chip were submitted to the Gene Expression Omnibus database (GEO; www.ncbi.nlm.nih.gov/geo/). The accession number for the series is GSE8727.

Quantitative Real-Time PCR for Expression Data

An aliquot of the RNA preparations from the samples used in the microarray experiments was saved for quantitative real-time reverse transcription (RT)-PCR follow-up studies. First-strand cDNAs were synthesized from 2 μg of total RNA in a 21- μl reaction volume using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) in accordance with the manufacturer's instructions. Quantitative real-time PCRs were performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems, Inc.: ABI, Foster City, CA). Independent PCRs were performed using the same cDNA for both the gene of interest and the 18S rRNA, using the SYBR Green PCR Master Mix (Applied Biosystems, Inc.). Gene-specific primers were designed for the gene of interest and the 18S rRNA using Primer Express software (Applied Biosystems, Inc.) and the Oligo Analysis & Plotting Tool (QIAGEN, Valencia, CA) and are shown in Table 2-2. The PCR conditions consisted of AmpliTaq Gold activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the 7000 Sequence Detection System. The change in fluorescence of SYBR Green I dye in every cycle was monitored by the system software, and the threshold cycle (C_T) above the background for each reaction was calculated. The C_T value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔC_T value. The ΔC_T value of an arbitrary calibrator (e.g., untreated sample) was subtracted from the ΔC_T value of each sample to obtain a $\Delta\Delta C_T$ value. The gene expression level relative to the calibrator was expressed as $2^{-\Delta\Delta C_T}$. Statistical analysis was performed using R software version 2.5.0 (www.r-project.org). Fold enrichment was compared using Student's t-test. The statistical significance threshold was fixed at $\alpha=0.05$.

Table 2-2. Primers used for quantitative real-time PCR expression analysis.

Gene Name	Primer Pairs^a	Amplicon Size (bp)
<i>18S</i>	F, 5'-CACGACGGAGTTTCACAAGA-3' R, 5'-CGATGGAAGTTTGAGGCAAT-3'	135
<i>CDR1</i>	F, 5'-ATTCTAAGATGTCGTCGCAAGATG-3' R, 5'-AGTTCTGGCTAAATTCTGAATGTTTTTC-3'	140
<i>CDR2</i>	F, 5'-TAGTCCATTCAACGGCAACATT-3' R, 5'-CACCCAGTATTTGGCATTGAAA-3'	76
<i>PDR16</i>	F, 5'-CTGCGGGACAAGATTCATTAGC-3' R, 5'-TTGAGTACCAACAGGATGTGCTTTA-3'	62
<i>TAC1</i>	F, 5'-TGGCAATGTATTTAGCAGATGAGG-3' R, 5'-TGCTTGAAGTGAAGGTGAATTTTG-3'	71

^a **F**: forward, **R**: reverse

Generation of an HA-Tagged Tac1p-Expressing Strain

A DNA fragment overlapping positions -402 to +937 of *C. albicans* *URA3* ORF (*URA3* marker) was PCR-amplified with *Pfu* DNA polymerase (Stratagene, La Jolla, CA) from plasmid pCaEXP (110), using primers 5'-ATCATCTCTAGATAAGGATGGTATAAACGGAAAC (introduces an *Xba*I site, underlined) and 5'-GCCCCGGGAAGGACCACCTTTGATTG (introduces a *Sma*I site, underlined). The resulting fragment (1,362 bp) was digested with *Xba*I and *Sma*I and used to swap the equivalent *Xba*I-*Xho*I fragment of plasmid pMPY-3×HA (1,078 bp, containing the *S. cerevisiae* *URA3* marker) (111), generating plasmid pCaMPY-3×HA. A *TAC1*-tagging cassette was amplified from plasmid pCaMPY-3×HA using primers FWD: 5'-ttgattgataattcagctgtaaattgccatttagtcaatttaataatttaccattttttcttgacaataatttggggattAGGGAACAAAAGCTGG-3' (the lower case sequence corresponds to positions +2860 to +2943 of the *TAC1* ORF) and REV: 5'-

taggaaaaaatatatgaaacaataaatattacaagatatacatcgtttcaccaattacaactctttttaacccCTATAGGGCGAATTGG-3' (the lower case sequence corresponds to positions +2947 to +3030 of the *TAC1* ORF) which anneal specifically to the in-frame pCaMPY-3×HA vector sequences PET-up and PET-down (upper case sequences) as described previously (111). The resulting fragment (1,821 bp), containing the *C. albicans* *URA3* marker flanked by direct repeats of the 3×HA epitope-encoding sequences and 84 bp of sequences homologous to the 3' end of the *TAC1* gene, was used to transform strain CAI4. Counterselection of the *URA3* gene was carried out on plates containing 5-FOA as described previously (112) except that uracil was replaced with uridine.

Candida albicans Transformation

C. albicans transformations were performed as described previously (113) with minor modifications. The cells were grown overnight in 15 ml of YPD medium, diluted to an OD₆₀₀ of 0.1 in 100 ml of fresh YPD and allowed to grow to an OD₆₀₀ of 0.4. The cells were harvested, washed once with 10 ml of sterile water and resuspended in 1 ml of 1X LiAc solution (113). Cells (100 µl) were transferred to a sterile tube containing 6 µg of gel-purified PCR-fragment and 100 µg of denatured salmon sperm DNA as carrier. A LiAc/40% PEG solution (700 µl of 10 mM Tris-HCl pH 7.5, 1 mM EDTA pH 8.0, 100 mM LiAc, 40% (w/v) PEG4000) was added and the cell suspensions were incubated overnight at 30°C with gentle rotation. The cells were heat shocked at 42°C for 15 min and plated on SC-ura plates.

Genomic DNA Isolation and Southern Blot Analysis

C. albicans genomic DNA was prepared as described for *S. cerevisiae* (114). For the Southern hybridization, genomic DNAs (500 ng) were digested to completion with *Hind*III and *Eco*RV, electrophoresed on a 1% agarose gel and transferred to a nylon membrane (Hybond-N; Amersham Biosciences, Piscataway, NJ). Prehybridization, hybridization and washing steps were carried out as previously described (102). The *TAC1* probe used consists of a ³²P-radiolabeled 927-bp PCR-amplified fragment from SC5314 genomic DNA overlapping positions +2020 to +2946 in the *TAC1* ORF. The membrane was exposed to a FUJIFILM Imaging Plate

screen. The signal was quantified using the Multi Gauge program, version 2.3 (FUJIFILM). The membrane was subsequently exposed to a Kodak XAR film at -80°C.

Total Protein Preparation and Western Blotting

Total protein was prepared from 2 O.D. units of strains CAI4 and SZY63 grown overnight, as described for *S. cerevisiae* (114). Extracts were boiled for 5 min, and 35 µl (out of 100 µl total) was separated by electrophoresis on an SDS–8% polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane with a Trans Blot SD Semi-Dry transfer apparatus (Bio-Rad, Hercules, CA) and analyzed with a mouse anti-HA monoclonal antibody (HA-probe (F-7): sc-7392, Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a dilution of 1:2000, using a chemiluminescence detection system under conditions recommended by the manufacturer (Pierce Biotechnology Inc., Rockford, IL).

Chromatin Immunoprecipitation

Three independent cultures (50 ml each) of strains CAI4 and SZY63 were grown in YPD medium to an OD₆₀₀ of 0.8. The cultures were then treated with 1.4 ml of 37% formaldehyde (final concentration 1%) to induce protein-DNA cross-links. Cells were incubated 30 min at room temperature with agitation and the cross-linking stopped by adding 1.2 ml of 2.5 M glycine. Cells were harvested, washed 3 times with 40 ml of ice-cold TBS buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl) and snap frozen in liquid nitrogen. Total cell extracts were prepared by adding 0.7 ml of lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 1 mM PMSF, 1 mM benzamidine, 10 µg/mL aprotinin, 1 µg/mL leupeptin, 1 µg/mL pepstatin), 0.5 ml of acid-washed glass beads (Sigma) and bead beating 4 times for 5 min at highest settings in a mini bead-beater (Biospec Products). Preparation of soluble chromatin fragments was performed by sonicating the extracts 4 times during 20 sec at power 2-3 (output power 8-9 RMS) using Sonic Dismembrator Model 100 sonicator (Fisher Scientific). Immunoprecipitation was performed by incubating the sonicated extracts (from both the tagged SZY63 strain and the untagged parental CAI4 strain) overnight with monoclonal mouse anti-HA antibody (Santa Cruz Biotech) coupled to magnetic beads (Dynabeads Pan Mouse IgG, Dynal Biotech, Brown Deer, WI) at 4°C. Beads were then washed twice with lysis buffer, twice with lysis buffer supplemented with 360 mM NaCl, twice with wash buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP40, 0.5% Na-deoxycholate, 1 mM EDTA) and once with TE buffer (10 mM Tris pH 8.0, 1 mM EDTA). The cross-links were reversed by incubating the washed beads overnight in 50 µl of TE/1% SDS at 65°C. The eluted material was then treated with proteinase K, RNase A, extracted twice with phenol:chloroform:isoamyl-alcohol (25:24:1) and the DNA precipitated with 100% ethanol and resuspended in 50 µl of TE. 40 µl of the immunoprecipitated (IPed) DNA fragments were used for amplification and Cy5/Cy3 dye labeling prior to hybridization to *C. albicans* intergenic DNA microarrays.

DNA Labeling, Hybridization to Intergenic Microarrays and Data Analysis

Labeling was conducted as described by Drouin *et al.* (115). Briefly, the IPed DNA fragments were blunted with T4 DNA polymerase and ligated to unidirectional linkers. The DNA was then amplified by LM-PCR in the presence of aminoallyl-modified dUTP. The labeling was carried out post-PCR using monoreactive Cy-dye NHS esters (CyTM5/CyTM3 Mono-Reactive Dye Packs, Amersham Biosciences) that react specifically with the aminoallyl-modified dUTP (5-(3-aminoallyl)-2' deoxyuridine-5'-triphosphate, Sigma-Aldrich). Both pools of labeled IPed DNA from the tagged SZY63 strain (Cy5) and the untagged parental CAI4 strain (Cy3) were mixed and hybridized to a *C. albicans* intergenic DNA microarray that will be described elsewhere (H. Hogues, H. Lavoie, A. Sellam, M. Mangos, T. Roemer, E. Purisima, A. Nantel and M. Whiteway, submitted for publication). Images of Cy5 and Cy3 fluorescence intensities were generated by scanning arrays using Axon 4000b scanner and were analyzed with GenePix Pro 4.1 software (Molecular Devices, formerly Axon Instruments, Inc, Downington, PA). Normalization of the data was conducted using ArrayPipe 1.7 (116), and replicate slides (n=3) were combined using a weighted average method as described in Ren *et al.* (117).

Quantitative Real-Time PCR Confirmation of the Genome-Wide Location Data

Q-PCR was performed with three independent CAI4 and SZY63 ChIP samples prepared as described above, except that the cell cultures were carried out in the presence of DMSO (0.08%). Quantification of the DNA recovered from the CAI4 and SZY63 ChIPs was performed using the Quant-iTTM PicoGreen® dsDNA Assay Kit (Molecular Probes: Invitrogen). A standard curve was prepared using *C. albicans* gDNA quantified by fluorometry and serially diluted in TE buffer (0, 0.1, 0.01, and 0.001 ng/μl). The CAI4 and SZY63 ChIP samples (1 μl) were resuspended in 49 μl of TE buffer. The samples (50 μl) were transferred, in duplicate, in a black 96-well plate (Costar 3694, Corning Inc., Corning, NY) and 50 μl of the PicoGreen reagent was added. The fluorescence was measured using the Envision luminometer (Perkin-Elmer, Waltham, MA) at an excitation and emission wavelength of 485 and 535 nm, respectively. The DNA concentration of the ChIP samples was an average 500 pg/μl, yielding approximately 25 ng of total immunoprecipitated DNA per ChIP sample (50 μl).

Q-PCR assays were designed using the Universal ProbeLibrary (Roche Applied Science, formerly Exiqon's ProbeLibray, Indianapolis, IN) and the TaqMan (Integrated DNA Technologies, IDT, Coralville, IA) methodologies. The different primers and probe combinations used for Q-PCR are listed in Table 2-3. The *CDR1*, *PDR16*, *TAC1* and *FUR1* promoter sequences were submitted to the Universal ProbeLibrary, using the web-based ProbeFinder software (version 2.34, Roche Applied Sciences) available on the Roche Applied Science website (www.universalprobelibrary.com). The software assigned optimal PCR specific primer sequences to be combined with the corresponding Universal ProbeLibrary probe for each promoter sequence (Universal ProbeLibrary probes, Roche Diagnostics Corp., Basel, Switzerland). We also used the PrimerQuest tool from the IDT website (<http://www.idtdna.com/Scitools/Applications/Primerquest/>) to design a custom TaqMan probe for the *TAC1* promoter (with its corresponding forward and reverse primers) that binds closer to

Table 2-3. Primers used for quantitative real-time PCR binding assays.

Promoter	Primers/Probe Sequences 5'-3'	Amplicon Location ^a
<i>CDR1</i>	F: GGTGCACACACACACAAACACACA R: TTGAGCTCCCCTATCCGATCCCTA P: TaqMan probe ^b : CCGCCCTCACTCTGTTCCATACAAAT	-435 → -341
<i>PDR16</i>	F: GAAAAGAAAAAGAAATGGCAACAT R: TCGACACGTCTTCCATCAC P: Universal ProbeLibrary ^c probe # 77 (cat.no. 04689003001) : GGTGGTGG	-424 → -501
<i>TAC1</i>	F: CACGAAGATAAAAATTGTGGTAGC R: AATTGTGCTGATATTTAATTGTTGGT P: Universal ProbeLibrary probe # 18 (cat.no. 04686918001) : CAGCAGGA	-1024 → -948
<i>FUR1</i>	F: GGTGCTTTTGGGAGAATGAA R: CTCCTCAAAACAAAACCTGCAA P: Universal ProbeLibrary probe # 27(cat.no. 04687582001) : GCTGCCTG	-987 → -913
<i>SPS4^d</i>	F: TACAGTTGCCCCAGTCAACA R: TGTCTTGGAACGGAAACTCA P: Universal ProbeLibrary probe # 15 (cat.no. 04685148001) : TCCTGCTC	-636 → -574

F: forward, R: reverse, P: probe.

^aposition according to the ATG start codon

^bTaqMan probe (42)

^cUniversal ProbeLibrary probe (64)

^d*orf19.7568*

the DRE motif than the Universal ProbeLibrary probe. The *C. albicans* homologue of *S. cerevisiae* *SPS4* (orf19.7568) was used as the reference promoter and *FURI* was used as a control, to perform statistical analyses. The *SPS4* and *FURI* genes were selected because i) they were not modulated in our microarray expression study and ii) their promoters were not enriched in the ChIP-chips experiments. A set of probe and primers was designed for each of the *SPS4* and *FURI* promoters using the ProbeFinder software (Table 2-3).

Q-PCR reaction mixtures were prepared using the TaqMan Universal PCR Master Mix (Applied Biosystems, Inc.) according to the manufacturer's instructions. For reactions using probes from the Universal ProbeLibrary, 200 pg of ChIP DNA, 250 nM of each forward and reverse primer, 100 nM of the probe, 5 µl of the TaqMan Universal PCR Master Mix and water were combined in a final volume of 10 µl. Q-PCR reactions using the TaqMan probe were prepared using the same conditions except that the probe and the primers (forward and reverse) were added to a final concentration of 100 nM and 200 nM, respectively. Reactions were performed in MicroAmp Optical 384-Well Reaction Plate (Applied Biosystems, Inc.) using an ABI 7900 HT Real-Time PCR instrument with 1 cycle at 95°C for 10 min and 40 cycles at (95°C for 15 sec and 60°C for 1 min). Each biological replicate sample (three) was processed in triplicate.

Data analysis was performed using the Sequence Detection System software (SDS 2.2.2; ABI). For each sample, C_T values were determined using the SDS software. The fold enrichments of the targets (*CDRI*, *PDR16* and *TAC1* promoters) were calculated using the relative quantification according to the $2^{-\Delta\Delta C_t}$ method, where $\Delta C_t = C_{T \text{ target}} - C_{T \text{ reference}(SPS4 \text{ promoter})}$ and $\Delta\Delta C_t = \Delta C_{T \text{ test (SZY63 ChIP sample)}} - \Delta C_{t \text{ calibrator (CAI4 ChIP sample)}}$ (118). The *FURI* promoter was used as a negative control to confirm the fold enrichment obtained using the $2^{-\Delta\Delta C_t}$ method. Statistical analysis was performed using the R software (www.r-project.org version 2.5.1) and the $\Delta\Delta C_t$'s were compared using Welch's t-test. The statistical significance threshold was fixed at $\alpha=0.001$.

DRE Motif Analysis

The promoter sequences (1.5 kb upstream of the starting ATG) were retrieved from the Candida Genome Database (<http://www.candidagenome.org>) and analyzed for the presence of potential DRE motifs using the fuzznuc software from EMBOSS (<http://emboss.bioinformatics.nl/>).

RESULTS

Global Gene Expression Profile

As a means to identify genes that are co-regulated with the *CDRI* and *CDR2* multidrug efflux pumps and thus potentially new transcriptional targets of Tac1p, we performed gene expression profiling analyses of four matched sets of azole- susceptible and -resistant *C. albicans*

clinical isolates in which the acquisition of azole resistance is associated with *CDR1* and *CDR2* overexpression (Table 2-1). Three of these azole-resistant isolates possess previously defined gain-of-function mutations in the C-terminus of *TAC1*: a N977D amino acid substitution in isolate C56 (also known as isolate DSY296) (89), a N972D amino acid substitution in isolate 5674 (103), and an A736C amino acid substitution in one allele and L962-N969 deletion in the second allele of *TAC1* in isolate 17 (101). Isolate 17 is the last in a series of 17 clinical isolates taken from a single HIV-infected patient (92, 106). Relative to the parental isolate 2, isolate 3 exhibits increased expression of *MDR1* whereas isolate 17 exhibits increased expression of *CDR1* and *CDR2* as well as *MDR1* (92, 106). We therefore compared isolate 17 to isolate 3 to identify the genes co-regulated with *CDR1* and *CDR2*. The other azole-resistant isolate, Gu5, overexpresses *CDR1* and *CDR2* and contains a G980E amino acid substitution in Tac1p (unpublished data). The eight strains did not show major chromosomal rearrangements or aneuploidies, as determined by comparative genomic hybridization analyses (data not shown and J. Berman, personal communication).

Three independent RNA samples per strain were hybridized to custom designed Affymetrix *C. albicans* microarrays and the data analyzed as described in Materials and Methods. Genes were initially considered to be differentially expressed if 1) their average fold change in expression was ≥ 1.5 for each matched isolate, 2) their expression changed by at least 1.5-fold in at least 2 of the 3 experiments for each matched isolate, and 3) if the average fold change was statistically significant by t-test. Using these criteria, there were 222 genes up-regulated and 150 genes down-regulated in C56 as compared to C43, 104 genes up-regulated and 63 genes down-regulated in Gu5 as compared to Gu2, 126 genes up-regulated and 92 genes down-regulated in 17 as compared to 3, and 327 genes up-regulated and 243 genes down-regulated in 5674 as compared to 5457 (Table A-1 and Table A-2). Genes which met these criteria for all 4 matched isolates and whose average fold change was statistically significant by t-test in at least 2 of the 4 matched isolates were included in a final data set (Table 2-4 and Table 2-5). We found 31 genes to be up-regulated in all four matched clinical isolate sets (Table 2-4) including the known Tac1p targets *CDR1*, *CDR2*, *IFU5*, *HSP12* and *RTA3* (100). Other up-regulated genes included *GPXI* (putative glutathione peroxidase), *CHK1* (histidine kinase), *LCB4* (Sphingoid long-chain base kinase), *NDH2* (NADH dehydrogenase), *SOU1* (sorboside dehydrogenase), orf19.3047 (transcription cofactor), orf19.4531 (ABC transporter) as well as *TAC1* itself, consistent with our previous proposition that *TAC1* transcription is autoactivated, either directly or indirectly, as a consequence of gain-of-function mutations in Tac1p (103). We also found 12 genes to be down-regulated in all four matched clinical isolate sets. These included *FTR1* (iron transporter), *IHD1* (putative GPI-anchored protein of unknown function), and *OPT6* (oligopeptide transporter) all of which encode integral membrane proteins, and *SOD5* (superoxide dismutase) which encodes a cell wall protein (Table 2-5). As judged from the expression data, clinical isolate 5674, which carries the Tac1p N972D amino acid substitution, results in the highest impact on gene expression among the isolates tested, as 38 out of the 43 genes modulated in a *TAC1*-dependent manner had the highest fold modulation in this strain (Table 2-4 and Table 2-5).

In order to determine which of these genes require Tac1p for their constitutive differential expression in these isolates, the gene expression profile of strain SZY31, a *tac1Δ/tac1Δ* derivative of the azole-resistant strain 5674 (Table 2-1), was compared with that of the azole-susceptible parental strain 5457 (85). Genes whose expression was modulated in strain

Table 2-4. Genes up-regulated in all four matched clinical isolate sets.

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 v C43	Gu5 v Gu2	17 v 3	5674 v 5457	SZY31 v 5457
orf19.6000	<i>CDR1</i>	<i>CDR1</i>	Multidrug transporter activity	1.7	3.0	2.4	4.4	-1.0
orf19.5958	<i>CDR2</i>	<i>CDR2</i>	Transporter activity	50.6	14.1	17.1	50.5	-1.3
orf19.86	--	<i>GPX1</i>	Glutathione peroxidase activity	2.3	3.0	2.3	4.0	-1.2
orf19.896	<i>CHK1</i>	<i>HK1</i>	Protein histidine kinase activity	2.5	1.9	1.8	4.5	1.3
orf19.3160	<i>HSP12</i>	<i>HSP12</i>	--	5.3	2.9	3.1	16.7	-1.7
orf19.157	--	<i>IFA24.3</i>	--	1.5	1.7	1.6	2.1	1.1
orf19.2568	<i>IFU5</i>	<i>IFU5</i>	--	3.3	3.0	3.7	5.4	1.5
orf19.5257	--	<i>LCB4</i>	D-erythro-sphingosine kinase activity	2.2	1.7	2.2	4.0	1.1
orf19.278	--	<i>MTR</i>	--	3.1	1.8	1.9	5.2	-1.1
orf19.5713	<i>YMX6</i>	<i>NDH2</i>	NADH dehydrogenase activity	5.3	3.1	2.9	15.3	1.2
orf19.7218	<i>RBE1</i>	<i>PRY2</i>	--	2.1	1.8	1.7	1.7	1.2
orf19.23	<i>RTA3</i>	<i>RTA3</i>	Phospholipid-translocating ATPase activity	40.4	15.9	22.0	41.0	1.0
orf19.2896	<i>SOU1</i>	<i>SOU1</i>	Sorbose dehydrogenase activity	2.0	1.5	1.5	7.0	-2.0
orf19.3104	--	<i>YDC1</i>	--	3.3	1.6	2.1	5.3	1.4
orf19.2726	--	<i>IPF10262</i>	--	2.5	2.8	1.9	6.2	-2.9
orf19.4459	--	<i>IPF11849</i>	--	2.0	4.1	4.3	5.0	-1.5
orf19.2244	--	<i>IPF12897</i>	--	1.8	1.7	1.5	5.4	-1.7
orf19.344	--	<i>IPF1514</i>	--	15.5	4.7	4.0	28.9	1.9
orf19.4907	--	<i>IPF17283</i>	--	3.2	1.6	2.3	2.0	1.1
orf19.5313	--	<i>IPF19810</i>	--	36.0	29.4	18.1	143.1	1.7
orf19.5777	--	<i>IPF19961</i>	--	2.0	1.7	2.2	4.2	-1.3
orf19.6840	--	<i>IPF3535</i>	--	1.8	2.3	1.9	2.3	-1.4
orf19.3047	<i>SIP3</i>	<i>IPF3598</i>	Transcription cofactor activity	1.5	1.5	1.7	3.0	1.3
orf19.2531	<i>CSP37</i>	<i>IPF4991</i>	ER to Golgi vesicle-mediated transport	2.5	1.8	5.5	6.8	-1.3
orf19.7310	--	<i>IPF5981</i>	--	4.9	2.7	2.0	16.8	1.2
orf19.1887	--	<i>IPF6464</i>	--	2.3	1.8	1.7	4.5	1.2

Table 2-4 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 v C43	Gu5 v Gu2	17 v 3	5674 v 5457	SZY31 v 5457
orf19.2790	--	<i>IPF7260</i>	Histone-lysine N-methyltransferase activity	1.8	1.6	1.6	2.8	-1.0
orf19.4531	--	<i>IPF7530</i>	ATPase activity	2.7	3.8	3.5	4.0	-1.6
orf19.4898	--	<i>IPF8950</i>	--	2.4	1.6	1.8	3.4	1.1
orf19.3188	<i>TAC1</i>	<i>IPF9191.3f</i>	--	2.7	2.8	2.8	4.2	-11.7
orf19.3644	--	<i>IPF9683</i>	--	2.5	1.5	2.1	3.4	1.1

^a orf19 nomenclature according to the Assembly 19 version.

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Systematic names in bold designate overlap with ChIP-chip experiments.

Gene expression values are represented as fold changes between resistant isolates and susceptible isolates.

Gene expression values in bold designate statistical significance (p-value < 0.05) in all three experiments.

Table 2-5. Genes down-regulated in all four matched clinical isolate sets.

Systematic Name ^a	CGD Name ^b	Candida DB Name ^c	GO Annotation (Molecular Function) ^d	C56 v C43	Gu5 v Gu2	17 v 3	5674 v 5457	SZY31 v 5457
orf19.7219	<i>FTR1</i>	<i>FTR1</i>	Ferrous iron transporter activity	-2.7	-3.0	-2.1	-4.0	-1.6
orf19.5025	<i>MET3</i>	<i>MET3</i>	Sulfate adenylyltransferase (ATP) activity	-2.0	-7.9	-1.8	-2.5	-1.2
orf19.2947	<i>SNZ1</i>	<i>SNZ1</i>	--	-3.7	-1.8	-1.7	-2.2	1.3
orf19.5760	<i>IHD1</i>	<i>IPF10662</i>	--	-1.9	-2.2	-3.1	-9.0	1.7
orf19.3475	--	<i>IPF11725</i>	--	-3.9	-2.1	-2.7	-5.8	-1.2
orf19.4655	<i>OPT6</i>	<i>IPF12193</i>	Oligopeptide transporter activity	-1.8	-2.0	-2.4	-3.1	-1.4
orf19.2060	<i>SOD5</i>	<i>IPF1222</i>	Copper, zinc superoxide dismutase activity	-2.3	-8.6	-17.8	-26.3	-1.4
orf19.2059	--	<i>IPF1228</i>	--	-2.0	-1.7	-1.7	-2.9	1.2
orf19.4749	--	<i>IPF13921</i>	--	-2.3	-1.8	-2.4	-3.0	-1.4
orf19.670.2	--	<i>IPF20159</i>	--	-2.3	-2.0	-1.7	-4.9	-1.9
orf19.6679	--	<i>IPF2314</i>	--	-1.5	-2.1	-1.8	-2.2	1.1
orf19.7561	<i>DEF1</i>	<i>IPF946</i>	--	-3.4	-1.5	-1.6	-2.8	-1.4

^a orf19 nomenclature according to the Assembly 19 version.

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Gene expression values are represented as fold changes between resistant isolates and susceptible isolates.

Gene expression values in bold designate statistical significance (p-value < 0.05) in all three experiments.

5674 as compared to strain 5457 and whose expression in strain SZY31 was returned to levels similar to those observed in strain 5457 were determined to be dependent upon Tac1p for their up- or down-regulation. As shown in Table 2-4 and Table 2-5, the majority of commonly differentially expressed genes were found to be Tac1p-dependent.

We selected four genes of interest for confirmation of differential expression by real-time RT-PCR. These included *CDR1*, *CDR2*, *TAC1*, and *PDR16*. *PDR16* was selected as it has been shown to be co-regulated with *CDR1* and *CDR2* in association with azole resistance, yet this is one of a limited number of genes not represented on the microarray used in this study (102, 119). As expected, all four genes were confirmed to be up-regulated in all four isolate sets (Figure 2-1). Likewise, up-regulation of all four of these genes in isolate 5674 was ablated in strain SZY31 (Figure 2-1).

Identification of Tac1p-Binding Sites *in vivo*

To determine if the *TAC1*-dependent genes that were differentially expressed in the clinical isolates have their promoter bound *in vivo* by Tac1p and to identify additional direct targets of Tac1p, we conducted genome-wide location analyses (ChIP-chip), a procedure combining ChIP with hybridization to DNA intergenic microarrays (117). For this purpose, Tac1p was tagged at its C-terminus with a triple hemagglutinin (HA) epitope, using homologous recombination at the *TAC1* chromosomal locus (Figure 2-2). First, we adapted the *S. cerevisiae* PCR epitope-tagging vector pMPY-3×HA (111) for its use in *C. albicans*, by substituting the *S. cerevisiae* *URA3* marker with the *C. albicans* *URA3* gene (see Materials and Methods for details). The resulting vector (pCaMPY-3×HA) contains the Ca*URA3* marker flanked by direct repeats of the HA₃ epitope. The *TAC1*-tagging cassette was amplified with 100-bp primers (16 bp of vector sequences and 84 bp from the gene to be tagged), allowing tagging of Tac1p at its C-terminus (Figure 2-2A). After transformation of the CAI4 strain and integration of the cassette by homologous recombination, the marker was excised via recombination between the two directly repeated HA₃ tags and the excision mutants were selected on 5-FOA medium (Figure 2-2A). The resulting pre-excision (strain SZY51) and post-excision (strain SZY63) strains were characterized by Southern hybridization using *Hind*III and *Eco*RV double-digested genomic DNA and the *TAC1* probe (Figure 2-2B). As predicted (Figure 2-2B), the probe detected two fragments, one of 3.3 kb and one of 1.4 kb in the CAI4 strain, corresponding to the wild-type *TAC1-1* (orf19.3188) and *TAC1-2* (orf19.10700) alleles, respectively (Figure 2-2C, lane 1). Integration of the HA₃-tagging cassette occurred at the *TAC1-2* allele in the pre-excision strain SZY51, as the 1.4-kb band was shifted to the 3.1-kb band (Figure 2-2C, lane 2). Excision of the Ca*URA3* marker in strain SZY63 was confirmed by the presence of the 1.6-kb band (Figure 2-2C, lane 3). To detect the Tac1p-HA₃ fusion protein, Western blotting was performed in strains CAI4 and SZY63, using an anti-HA monoclonal antibody (Figure 2-2D). A signal corresponding to the Tac1p-HA₃ fusion protein was detected in the HA-tagged SZY63 strain but not in strain CAI4 (control) (Figure 2-2D). The size of this band was approximately 120 kDa, consistent with the predicted molecular weight of the Tac1p-HA₃ fusion protein (118 kDa). The functionality of the Tac1p-HA₃ protein was examined by the Raymond lab and there was change in Tac1p function.

Figure 2-1. Quantitative real-time RT-PCR analysis of selected genes differentially expressed in the microarray experiments.

(A) Genes differentially expressed in each of the azole resistant clinical isolates as compared to their parent isolates. (B) *CDR2* gene expression in each of the azole resistant clinical isolates. Error bars denote standard error (n=3). Asterisks denote statistical significance by t-test (p-value ≤ 0.05).

Figure 2-2. Chromosomal tagging of Tac1p.

(A) PCR epitope tagging strategy for C-terminally tagging transcription factor Tac1p with the triple HA epitope. Top, 100-nt primers (FWD and REV, see Materials and Methods) are designed such that the 5' 84 bases of the forward (FWD) and reverse primers are homologous to sequences of the *TAC1* gene and the 3' 16 bases are complementary and in-frame to unique sequences (open boxes) in the tagging cassette which contains the *C. albicans URA3* marker (CaURA3, light gray box) flanked by direct repeats of the 3×HA-encoding sequences (HA, black boxes). The Tac1p stop codon is indicated by the asterisk. Middle, PCR amplification results in a fragment whose ends include the primer sequences, allowing integration by homologous recombination of the tagging cassette upstream of the *TAC1* 3' untranslated region (horizontal thick line). The orientation of the *TAC1* ORF (dark gray box) is indicated by the arrow. Bottom, CaURA3 marker excision results in the final product, *TAC1-HA₃*. (B) Schematic representations of the *TAC1-1* (orf19.3188) and *TAC1-2* (orf19.10700) alleles (gray boxes) in strain CAI4 and sizes of the *HindIII*, *EcoRV* double-digested fragments detected by the *TAC1* probe (top, thick line) used for the Southern blot experiment are given for the *TAC1-2* allele following integration of the HA₃-tagging cassette (open box) and excision of the CaURA3 marker through HA₃ recombination. (C) Southern blot of genomic DNA from the CAI4 strain and its *URA3*-pre- (SZY51) and post-excision (SZY63) derivatives, digested with *EcoRV* and *HindIII* and hybridized with the *TAC1* probe shown in (B). Marker sizes are indicated on the left. (D) Western blot analysis of protein extracts from strains CAI4 and the Tac1p-HA₃ integrant SZY63 with an anti-HA monoclonal antibody. Molecular weights markers are indicated on the left.

A **B** **C** **D**

ChIP-chip experiments were conducted using the CAI4 and SZY63 strains and intergenic DNA microarrays containing 70-mer oligonucleotides covering the promoter regions of the *C. albicans* genome. Using a *P*-value < 0.001 (enriched binding ratio ≥ 1.5), we identified 37 promoters bound by Tac1p (Table 2-6). These included the promoters of genes known to be regulated by Tac1p: *CDR1* (10.5), *IFU5* (3.0), *PDR16* (2.1), *RTA3* (1.8) and *CDR2* (1.7) (100, 103), thus validating our ChIP-chip procedure and showing that Tac1p binds *in vivo* to the promoter of these genes to regulate their expression. *HSP12*, also known to be regulated by Tac1p, had a binding ratio of 1.2 (*P*-value=0.09) and thus was not significantly bound by Tac1p. These experiments also identified several new targets, including genes involved in lipid metabolism, oxidative stress response as well as genes of unknown function. On two occurrences, Tac1p binding was found at promoters of two genes located on opposite strands (thus sharing the same probe on the chip): *UGA33* (orf19.7317) and *SUC1* (orf19.7319) as well as *LPE10* (orf19.3455) and *KIC2* (orf19.3456) (Table 2-6). While the expression data did not allow to discriminate which one of the *LPE10* or *KIC2* genes is regulated by Tac1p, we found that *SUC1* was up-regulated in a *TAC1*-dependent manner in 3 out of the 4 clinical isolates tested by expression microarrays Table A-1 while *UGA33* expression was unchanged in 3 out of the 4 clinical isolates, suggesting that *SUC1*, a sucrose uptake zinc cluster regulator (120), is a target of Tac1p. The identification of several promoters bound *in vivo* by Tac1p under non-activating conditions (i.e. in the absence of drugs or gain-of-function mutations) demonstrates that Tac1p is at least constitutively bound to its targets and suggests the possible involvement of post-binding mechanisms for Tac1p-mediated transcriptional activation.

Using quantitative real-time PCR, we confirmed the binding of Tac1p to the *CDR1* and *PDR16* promoters, with enrichment ratios of 29.1 ± 4.0 and 4.1 ± 0.2 , respectively (Figure 2-3). As a control, we investigated binding of Tac1p to the promoter of the *FUR1* gene, which was not enriched in the ChIP-chip experiments nor modulated in the azole-resistant clinical isolates, and found no significant enrichment of that promoter by Q-PCR (1.1 ± 0.2), confirming the validity of the data obtained in the ChIP-chip experiments (Figure 2-3).

Although our results suggested that the expression of the *TAC1* gene is controlled by Tac1p in an autoregulatory loop, the ChIP-chip data showed no enrichment for Tac1p at its own promoter, supporting an indirect auto-regulation mechanism. However, the fact that the *TAC1* promoter contains a DRE-like motif located between positions -1051 and -1060 relative to the ATG translation start codon (Table 2-7; see below) and that the *TAC1* oligo printed on the intergenic chips is located at position -480 may have prevented us from detecting Tac1p binding at the *TAC1* promoter. Therefore, we used for Q-PCR a set of TaqMan probe and primers hybridizing close to this DRE motif (Table 2-2). Under these conditions, we observed a strong enrichment of the *TAC1* promoter (179.6 ± 25.2) (Figure 2-3). These results strongly support a direct autoregulatory loop controlling *TAC1* expression.

Identification of Potential Tac1p-Binding Motifs

Tac1p belongs to the fungal-specific family of zinc cluster transcription factors that contain a DBD formed by six cysteines coordinating two zinc atoms (121). Zinc cluster factors can bind as homodimers to CGG triplets with various orientations (everted, inverted, or direct

Table 2-6. List of Tac1p-enriched promoters (P -value < 0.001).

Systematic Name ^a	CGD/ NRC Name ^b	Candida DB Name ^c	<i>S. cerevisiae</i> Ortholog ^d	GO Terminology ^e	BR ^f	P ^g	E 5674/ 5457 ^h	E SZY31/5457 ⁱ
orf19.6000	<i>CDR1</i>	<i>CDR1</i>	<i>PDR5</i>	Transporter activity	10.5	0.0000	4.4*	-1.0
orf19.6869	<i>AST2</i>	<i>IPF8041</i>	<i>AST2</i>	Peptidase activity	4.1	0.0000	1.6	1.9
orf19.769	<i>IFE1</i>	<i>IFE1</i>	<i>BDH2</i>	--	3.3	0.0000	--	--
orf19.2568	<i>IFU5</i>	<i>IFU5</i>	<i>WWM1</i>	--	3.0	0.0000	5.4*	1.5
orf19.86	<i>GPX2</i>	<i>GPX1</i>	<i>HYR1</i>	Glutathione peroxidase activity	2.8	0.0000	4.0*	-1.2
<u>orf19.3406</u>	--	<i>IPF9898</i>	<i>YHL008c</i>	--	2.5	0.0000	-2.7*	-1.5
orf19.7166	--	<i>IPF2186</i>	<i>YGR110w</i>	--	2.8	0.0000	5.0	1.2
<u>orf19.7042</u>	--	<i>IPF3080</i>	--	--	2.1	0.0000	5.5*	1.3
orf19.93	--	<i>IPF14895</i>	<i>MIC17</i>	--	2.1	0.0000	1.5	-1.1
orf19.1027	<i>PDR16</i>	<i>PDR17</i>	<i>PDR16</i>	Phosphatidylinositol transporter activity	2.1	0.0000	20.0 ^j	1.2 ^j
orf19.3395	--	<i>IPF9483</i>	<i>YHR048w</i>	--	2.0	0.0000	--	--
<u>orf19.5877</u>	<i>ATF1</i>	<i>IPF1837</i>	<i>ATF1</i>	Alcohol O-acetyltransferase activity	2.0	0.0000	2.8*	1.2
<u>orf19.6627</u>	--	<i>IPF2557</i>	--	--	1.9	0.0000	2.6*	-1.0
orf19.23	<i>RTA3</i>	<i>RTA3</i>	<i>RSB1</i>	Phospholipid-translocating ATPase activity	1.8	0.0000	41.0*	1.0
<u>orf19.6501</u>	--	<i>IPF3931</i>	<i>YJU3</i>	--	1.8	0.0000	4.6*	1.1
orf19.5037	--	<i>IPF17054</i>	--	--	1.8	0.0000	7.7	2.1
orf19.4898	--	<i>IPF8950</i>	<i>FMP52</i>	--	1.8	0.0001	3.4	1.1
orf19.1267	<i>CAJ1</i>	<i>IPF10278</i>	<i>CAJ1</i>	Chaperone regulator activity	1.7	0.0001	2.5	-1.2
orf19.1887	--	<i>IPF6464</i>	<i>YEH1</i>	--	1.7	0.0001	4.5	1.2

Table 2-6 (continued).

Systematic Name ^a	CGD/ NRC Name ^b	Candida DB Name ^c	<i>S. cerevisiae</i> Ortholog ^d	GO Terminology ^e	BR ^f	P ^g	E 5674/ 5457 ^h	E SZY31/5457 ⁱ
orf19.691	<i>GPD2</i>	<i>GPD2</i>	<i>GPD1</i>	Glycerol-3-phosphate dehydrogenase (NAD ⁺) activity	1.7	0.0001	1.1	1.1
orf19.2175	--	<i>IPF19998</i>	<i>AIF1</i>	Oxoreductase activity	1.7	0.0001	3.3	1.3
orf19.1444	<i>ENT2</i>	<i>IPF17555.3</i>	<i>ENT2</i>	Cytoskeletal adaptor activity	1.7	0.0002	1.5	1.1
orf19.5958	<i>CDR2</i>	<i>CDR2</i>	<i>PDR5</i>	Transporter activity	1.7	0.0003	50.5*	-1.3
orf19.3455 ^k	<i>LPE10^k</i>	<i>IPF9782^k</i>	<i>LPE10</i>	Magnesium ion transporter activity	1.6	0.0003	1.7	-1.7
<u>orf19.1089</u>	<i>PEX11</i>	<i>PEX11</i>	<i>PEX11</i>	Peroxisome organization and biogenesis	1.6	0.0003	1.8*	-2.4
orf19.1665	<i>MNT1</i>	<i>MNT1</i>	<i>KTR1</i>	Alpha-1,2-mannosyltransferase activity	1.6	0.0003	--	--
orf19.7306	--	<i>IPF5987</i>	<i>YPR127_w</i>	--	1.7	0.0003	2.1	1.1
<u>orf19.7319^k</u>	<i>SUC1^k</i>	<i>SUC1^k</i>	<i>MAL13</i>	Transcription factor activity	1.6	0.0003	5.1*	1.2
orf19.1718	<i>ZCF8</i>	<i>IPF19769</i>	--	--	1.7	0.0004	-1.6	-1.8
orf19.406	<i>ERG1</i>	<i>ERG1</i>	<i>ERG1</i>	Squalene monooxygenase activity	1.7	0.0005	-1.6	1.2
orf19.7603	--	<i>IPF660</i>	<i>YMR244c-A</i>	--	1.6	0.0005	--	--
<u>orf19.5005</u>	<i>OSM2</i>	<i>OSM2</i>	<i>OSM1</i>	Fumarate reductase (NADH) activity	1.6	0.0005	2.0*	1.2
orf19.577	--	<i>IPF7353</i>	<i>YDL057c</i>	--	1.6	0.0006	2.3	1.5
orf19.5257	<i>LCB4</i>	<i>LCB4</i>	<i>LCB4</i>	D-erythro-sphingosine kinase activity	1.6	0.0007	4.0*	1.1
<u>orf19.5525</u>	--	<i>IPF4328</i>	<i>YMR315_w</i>	--	1.5	0.0008	2.3*	1.1

Table 2-6 (continued).

Systematic Name ^a	CGD/ NRC Name ^b	Candida DB Name ^c	<i>S. cerevisiae</i> Ortholog ^d	GO Terminology ^e	BR ^f	P ^g	E 5674/ 5457 ^h	E SZY31/5457 ⁱ
orf19.4476	<i>IFD6</i>	<i>IFD6</i>	<i>YPL088w</i>	Aryl-alcohol dehydrogenase activity	1.6	0.0009	2.2	1.2
orf19.951	--	<i>IPF1548</i>	--	--	1.6	0.0009	-1.5	1.1

^a orf19 nomenclature according to the Assembly 19 version; orfs in bold are modulated in the four sets of isolates whereas underlined orfs are modulated at least in strain 5674.

^b Gene name according to CGD (<http://www.candidagenome.org/>) or the NRC *Candida albicans* database (<http://candida.bri.nrc.ca/candida/index.cfm?page=CaGeneSearch>).

^c Gene name according to CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^d *S. cerevisiae* ortholog or best hit according to CGD (<http://www.candidagenome.org/>).

^e Gene ontology (GO) terminology according to CGD.

^f Binding ratio. Probes are spotted in duplicate on the ChIP-chip arrays (H. Hogues, H. Lavoie, A. Sellam, M. Mangos, T. Roemer, E. Purisima, A. Nantel and M. Whiteway, submitted for publication). Binding ratio with the most significant *P*-value is shown.

^g *P*-value of the corresponding binding ratio.

^h Fold expression of the gene in strain 5674 relative to strain 5457. An asterisk (*) indicates that fold expression is statistically significant (Table A-1).

ⁱ Fold expression of the gene in strain SZY31 relative to strain 5457.

^j Value obtained by quantitative real-time RT-PCR.

^k The intergenic oligonucleotide probe corresponds to a common promoter of two adjacent genes. The orf19.3455 probe is shared by *LPE10* and *KIC2*; the orf19.3719 probe is shared by *SUC1* and *UGA33*.

Figure 2-3. *In vivo* enrichment of Tac1p binding at the *CDR1*, *PDR16* and *TAC1* promoters using Q-PCR.

The CAI4 and SZY63 strains were submitted to ChIP (3 biological replicates) and the recovered DNA samples were analyzed by Q-PCR, using Universal ProbeLibrary probes (64) for the *PDR16*, *TAC1*, *SPS4* and *FUR1* promoters or a TaqMan probe (42) for the *CDR1* promoter. Fold enrichments are presented in log scale: 3.8 for the *PDR16* promoter (95% confidence interval: 4.0, 5.0), 28.8 for the *CDR1* promoter (95% confidence interval: 21.4, 38.9), 189.3 for the *TAC1* promoter (95% confidence interval: 128.6, 278.6) and 1.1 for the *FUR1* promoter (95% confidence interval: 0.9, 1.3), which was used as a negative control.

Table 2-7. DRE motifs found in the Tac1p target promoters.

SYSTEMATIC NAME ^a	CGD NAME ^b	MOTIFS ^c	STRAND ^d	POSITION ^e
CGGN ₄ CGG				
orf19.6000	<i>CDR1</i>	<u>CGGATATCGG</u>	S	-457
orf19.6869	<i>AST2</i>	<u>CGGCTAACGG</u>	A	-262
orf19.2568	<i>IFU5</i>	<u>CGGAAATCGG</u>	A	-235
orf19.7166	--	<u>CGGGTAACGG</u>	S	-292
		<u>CGGAGAACGG</u>	S	-285
orf19.7042	--	<u>CGGATAACGG</u>	S	-731
		<u>CGGTTACCGG</u>	A	-632
		<u>CGGATTACGG</u>	A	-639
orf19.5877	<i>ATF1</i>	<u>CGGATATCGG</u>	A	-206
orf19.6627	--	<u>CGGATATCGG</u>	A	-176
orf19.23	<i>RTA3</i>	<u>CGGAACTCGG</u>	S	-595
orf19.4898	--	<u>CGGGTGACGG</u>	S	-232
orf19.5958	<i>CDR2</i>	<u>CGGAAATCGG</u>	S	-220
orf19.1089	<i>PEX11</i>	<u>CGGGGAACGG</u>	A	-340
orf19.4476	<i>IFD6</i>	<u>CGGTTGTCTGG</u>	A	-339
orf19.10700	<i>TAC1</i>	<u>CGGAGCACGG</u>	A	-1,054 ^f
Consensus ^g		C G G A T A A C G G 1.0 1.0 1.0 0.6 0.6 0.7 0.5 1.0 1.0 1.0		

Table 2-7 (continued).

SYSTEMATIC NAME ^a	CGD NAME ^b	MOTIFS ^c	STRAND ^d	POSITION ^e
CGGN ₃ CGG				
orf19.3406	--	CGGCAACGG	A	-332
orf19.1027	<i>PDR16</i>	CGGATTCGG	S	-558
orf19.5037	--	CGGTTCGG	S	-358
		CGGAACCGG	A	-351
		CGGGGGCGG	A	-250
orf19.2175	--	CGGAAGCGG	A	-167
orf19.3455	<i>LPE10</i>	CGGCTGCGG	S	-246
orf19.1718	<i>ZCF8</i>	CGGAATCGG	S	-797
orf19.6869	<i>AST2</i>	CGGGCGCGG	A	-1,161
orf19.10700 ^h	<i>TAC1</i>	CGGAAACCGG	A	-1,040
Consensus ^g		C G G A A G C G G		
		1.0 1.0 1.0 0.5 0.5 0.4 1.0 1.0 1.0		

^a orf19 nomenclature according to the Assembly 19 version.

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c The underlined motifs have been previously described (16)

^d S denotes sense strand and A denotes antisense strand.

^e Nucleotide position from the ATG translation start site.

^f Position in contig19.20170, upstream of orf19.10700; position in contig.10170, upstream of orf19.3188 is -1,051 due to sequence polymorphisms.

^g The consensus sequence was obtained using the AlignX program (component of Vector NTI Advance 10.1.1, Invitrogen).

Nucleotide frequency at each position is given below.

^h Present only in contig.20170 due to a sequence polymorphism (**CCGAAACGG**).

repeats) and spacing (121). The *CDR1* and *CDR2* DRE consists of a direct CGG repeat with 4 intervening nucleotides (CGGAA/TATCGG), a configuration also found in the promoter of the other Tac1p-regulated genes *RTA3* and *IFU5* (99, 100). We thus looked for a DRE motif in the promoter of the 37 genes identified by the ChIP-chip experiments, using 1.5 kb of promoter sequence and the motif definition CGGN₄CGG which allows for complete degeneracy for the 4 nucleotides between the CGG repeats. In addition to *CDR1*, *CDR2*, *RTA3* and *IFU5*, this analysis identified 8 new genes containing the CGGN₄CGG sequence: *AST2*, *ATF1*, *PEX11*, *IFD6* and 4 ORFs of unknown function (orf19.7166, orf19.7042, orf19.6627 and orf19.4898), for a total of 15 matches in 12 genes (Table 2-7). As a control, we searched the CGGN₄CGG motif in 1.5 kb of promoter sequence from 6,068 orfs and found an average of 1.5 match per 37 promoters, yielding a 10-fold enrichment for the presence of the CGGN₄CGG sequence in the Tac1p-bound promoters. We also searched the 37 enriched promoters for the motif CGGN₃CGG since our unpublished data indicated that a CGGATTCGG sequence in the *PDR16* promoter is involved in its transcriptional activation by Tac1p. This analysis identified 7 genes (including *PDR16*), one of them with three CGGN₃CGG motifs (Table 2-7). Searching the 6,068 promoter sequences yielded an average of 2.2 genes per 37 promoters, resulting in a 3.2-fold enrichment for that sequence in the Tac1p-bound promoters.

Finally, we examined whether the CGGN₃₋₄CGG motifs appear in the promoter region of the genes whose expression was modulated in all 4 resistant isolates (31 up-regulated and 12 down-regulated) compared to their matched parental strains. We found 7 up-regulated genes containing the CGGN₄CGG motif, 5 of which also identified by the ChIP-chip experiments (*CDR1*, *CDR2*, *IFU5*, *RTA3*, orf19.4898; the two other ones being *TAC1* and orf19.3047) as well as two up-regulated genes with the CGGN₃CGG motif (*TAC1* and orf19.344) (Table 2-7). Finally, searching the down-regulated genes identified one gene with the CGGN₄CGG motif (*SOD5*) and one gene with the CGGN₃CGG motif (*OPT6*).

Expression and Location Data Mining

When merging the expression and location data, we identified 8 genes whose promoters were bound by Tac1p *in vivo* and which were up-regulated in the four azole-resistant clinical isolates in a *TAC1*-dependent manner, thus qualifying these genes as *bona fide* Tac1p targets. These genes were *CDR1* and *CDR2*, *GPX1* (putative glutathione peroxidase), *LCB4* (putative sphingosine kinase), *RTA3* (putative phospholipid flippase) as well as three genes of unknown function: *IFU5*, orf19.1887, and orf19.4898 (Table 2-6, in bold, and Table A-1). We also identified 9 genes whose promoters were bound by Tac1p *in vivo* and that were significantly modulated in a *TAC1*-dependent manner in at least strain 5674, the isolate displaying the strongest fold change in gene expression. These genes were orf19.5877 (*ATF1*, alcohol acetyltransferase), orf19.1089 (*PEX11*, fatty acid oxidation), orf19.5005 (*OSM2*, fumarate reductase), orf19.7319 (*SUC1*, sucrose metabolism) as well as 5 ORFs of unknown function (orf19.6627, orf19.7042, orf19.5525, orf19.3406 and orf19.6501) (Table 2-6, underlined, and Table A-1). These results

suggest that, in addition to its function in azole drug resistance, Tac1p regulates other cellular functions such as lipid metabolism and oxidative stress response.

DISCUSSION

To identify the Tac1p regulon, we combined genome-wide expression and location analyses, two technologies with complementary strengths and limitations. Expression profiling is extremely valuable since it can identify all of the changes in transcript abundance associated with the perturbation of a specific transcriptional regulator, however it cannot distinguish between direct or indirect effects at individual target promoters. Location profiling is a very powerful tool to identify all the direct targets of a specific transcription factor, however it does not allow the determination of whether the bound factor acts as an activator or a repressor of these targets. Each method also gives rise to false positives and false negatives. Merging the results from the two approaches thus generates data that is most complete and cross-validated.

While merging the expression and location data sets for Tac1p, we found that, out of 37 genes whose promoter was bound by Tac1p, 20 (64%) did not have their expression modulated (Table 2-6). Possible technical explanations for this limited overlap are that some of these genes did not pass the stringent criteria applied to the expression microarray data or that they represent false positives. However, a biological explanation could be that the expression of the bound promoters is not modulated in response to gain-of-function mutations in Tac1p but rather in response to other potential Tac1p-activating signals. We also found that, out of 43 modulated genes, 35 (81%) were not identified in the location profiling experiments, suggesting that these genes are indirect targets of Tac1p (i.e. controlled by Tac1p-regulated transcription factors and/or other transcriptional regulators) or that they are not bound by Tac1p under non-activating conditions (see below). It is also possible that the detection of Tac1p binding to some of these targets may have been prevented by the fact that their Tac1p-binding site is too far from the oligonucleotide sequence printed on the intergenic chip, as we found for *TAC1*. Lastly, some of these genes could have been missed by using a P -value < 0.001 as the cut-off in the location array experiments. This seems to be the case for the *SIP3* gene whose expression was up-regulated in the four azole-resistant isolates in a *TAC1*-dependent manner and which had a binding ratio of 1.6 but a P -value of 0.0014. Taken together, combining the expression and location profiling data allowed us to identify many new genes which unambiguously belong to the Tac1p regulon and thereby gain new insights into the biological functions of Tac1p.

Previously, we examined the gene expression profiles of matched isolates 2, 3, 15, and 17 using an earlier generation microarray (108). In that analysis we identified 5 genes as being co-regulated with *CDR1* and *CDR2*. Two of these, *GPX1* and *RTA3*, were found in the present study to be up-regulated in all four matched isolate sets studied, whereas a third, *ERG2*, was found to be up-regulated in three of these isolate sets. Likewise, of the 14 genes we previously found to be down-regulated in association with azole resistance in isolate 17, orf19.3475, orf19.2060, and orf19.5760 were found to be

down-regulated in all four isolate sets, and *FET34* was found to be down-regulated in three of the four in this study. Using the same early generation arrays, Coste *et al* examined the gene expression profiles of isolate C56 as well as strain DSY2926 (isolate C43 expressing a *TAC1* allele carrying a gain-of-function mutation) compared to that of their parent isolate C43 (100). They identified 7 genes to be up-regulated in common between these two experimental conditions. In addition to *CDR1* and *CDR2*, these included *RTA3* and *HSP12*, both of which were found to be up-regulated in all four matched isolate sets examined in the present study. In a separate study by the same group, Karababa *et al* examined the gene expression profiles of isolate C56 compared to parent isolate C43 as well as strain CAF2-1 exposed to the *CDR1*- and *CDR2*-inducing agent fluphenazine compared to this strain grown in the absence of drug (122). In addition to *CDR1* and *CDR2*, they observed 7 genes to be commonly up-regulated between these two conditions. These included *HSP12*, *GPX1*, *RTA3*, orf19.344, *IFU5*, orf19.1862 and orf19.7284, all of which were found in the present study to be up-regulated in either three or all of the four clinical isolates examined. In this report, we used a more recent generation microarray together with well-characterized sets of azole resistant clinical isolates and a *tac1Δ/tac1Δ* mutant, which allowed us to identify 36 additional genes whose expression is modulated in azole-resistant strains, dependent upon Tac1p.

Known Tac1p targets such as *CDR1*, *CDR2*, *IFU5* and *RTA3*, are induced in a *TAC1*-dependent manner upon exposure of the cells to fluphenazine or estradiol or upon expression of a gain-of-function *TAC1* allele in a *tac1Δ/tac1Δ* background (99, 100). This indicates that the activation of Tac1p by such inducers or by gain-of-function mutations is required for Tac1p-mediated transcriptional regulation. Our ChIP-chip experiments were done with cells grown in rich media, under uninduced conditions (123). We found that, under these conditions, Tac1p binds to its target promoters, indicating that this binding is constitutive or at least partially constitutive since it cannot be excluded that Tac1p binding increases in the presence of an inducer or an activating mutation. Functional characterization of the epitope-tagged Tac1p strain showed that Tac1p binding to its targets is not due to an activating effect of introducing the HA tag at the C-terminus of Tac1p. It was recently reported that the *S. cerevisiae* zinc cluster regulator Pdr1p, which controls the expression of the multidrug transporters *PDR5*, *SNQ2* and *YOR1* (124), also binds constitutively to its target promoters *in vivo* (125). Likewise, the *S. cerevisiae* zinc cluster transcription factor War1p, which controls the expression of the ABC transporter Pdr12p in response to weak acid stress, has been shown to constitutively bind to the *PDR12* promoter *in vivo* (126). Thus, the mechanisms by which Tac1p activates transcription in response to drugs or to gain-of-function mutations appear to be similar to those already documented for zinc cluster factors in *S. cerevisiae* and most likely involve post-binding mechanisms such as loss of interaction with a repressor protein, as proposed for Upc2p and Ecm22p (127) or recruitment of coactivator complexes (SAGA, Mediator, SWI/SNF) as shown for Pdr1p (128).

Our analyses of the Tac1p-bound promoters for the presence of a DRE-like motif CGGN₄CGG allowed us to identify 8 new Tac1p targets, in addition to *CDR1*, *CDR2*, *RTA3* and *IFU5*, containing this sequence (Table 2-7). The fact that 25 of the Tac1p-

bound promoters do not contain this motif suggests that Tac1p may recognize additional configurations of the CGG triplets, including monomeric CGG triplets or CGG triplets with different spacing and/or orientations (121). The latest proposition is supported by our unpublished data that a CGGATTCGG sequence in the *PDR16* promoter is involved in its transcriptional activation by Tac1p and the enrichment of the CGGN₃CGG motif in the Tac1p-bound promoters (Table 2-7). Alternatively, Tac1p may bind upstream or downstream of the DNA sequences analyzed (1.5 kb of upstream sequences). Finally, it is also possible that Tac1p binds to some of its targets indirectly, through its association with other DNA-binding proteins. For instance, the *S. cerevisiae* zinc cluster proteins Rsc30p and Rsc3p, which are part of the chromatin remodelling complex, have been shown to bind indirectly to DNA (129). Whether or not the DRE motifs identified in the Tac1p-bound promoters are functional as well as the sequences/factors mediating Tac1p binding to its target promoters in the absence of a classical DRE remain to be experimentally determined.

We previously showed that strain 5674 overexpresses the *TAC1* gene and that introduction of a *TAC1* allele carrying the N972D gain-of-function mutation in a *tac1Δ/tac1Δ* strain leads to the constitutive upregulation of the *TAC1* transcript, which suggested that Tac1p is positively autoregulated, directly or indirectly (103). We show in the present study that i) *TAC1* is upregulated in three additional azole-resistant isolates in which the Tac1p pathway is activated (Table 2-4); ii) the *TAC1* promoter contains a DRE motif, and iii) Tac1p binds *in vivo* to its own promoter (Figure 2-3). Taken together, our findings support the proposition that *TAC1* expression is controlled by a direct positive autoregulatory loop. This situation is similar to *S. cerevisiae* Pdr3p, which binds *in vivo* to two PDRE located in the *PDR3* promoter to transactivate its own expression (94). It appears that direct self-regulatory loops are a common feature among zinc cluster transcription factors in yeast, including Pdr3p and Yrr1p (pleiotropic drug resistance), Hap1p (respiration) and Stb5p (pentose phosphate pathway) (121). Studies in budding yeast have shown that transcription factor autoregulation is necessary to respond to environmental stresses. As examples, autoregulation of *PDR3* in a *pdr1Δ* background is crucial for growth on a medium containing cycloheximide (94) and autoregulation of the basic leucine zipper transcription factor Hac1p, which controls the unfolded protein response, is required to protect the cells from prolonged endoplasmic reticulum stress (130). Similarly, autoregulation of *C. glabrata* *AMT1*, a copper-sensing transcription factor, is necessary to protect the cells upon exposure to high environmental copper levels (131). Thus, one possible outcome of Tac1p autoregulation would be an amplifiable production of the Tac1p protein necessary for rapid and sustained response to drugs and yet unknown activating signals (123).

Three previously identified Tac1p targets (*CDR1*, *CDR2*, *PDR16*) have been shown to play a role in azole drug resistance (38, 102, 132). Thus Tac1p confers azole resistance by activating different effectors, each contributing to some extent to the overall azole resistance of the cells. Additional Tac1p targets identified by our studies and co-regulated with *CDR1*, *CDR2* and *PDR16* may also play a protective role against toxic injuries. For instance, orf19.4531 is found among the group of genes differentially expressed under the gene ontology (GO) molecular function term “ATPase activity

coupled to movement of substances”, including *CDR1*, *CDR2* and *RTA3* (Table 2-4). orf19.4531 encodes a putative ABC transporter of the PDR subfamily (to which *CDR1* and *CDR2* belong). Whether this transporter impacts azole resistance, alone or in conjunction with Cdr1p and Cdr2p, remains to be determined. This efflux pump may also protect the cell from other toxic compounds. As another example, orf19.86 (*GPX1*), which encodes a putative glutathione peroxidase, is an integral component of the glutathione and glutathione-dependent enzyme system which has been implicated in the resistance of tumor cells to anticancer agents (133, 134). Increased activity of this enzyme system is often observed in conjunction with increased activity of the ABC transporter P-glycoprotein in drug-resistant human cancer cells (135).

Although not found among the 37 promoters observed to be bound by Tac1p, *CHK1* was among the 31 genes that were consistently co-regulated with *CDR1* and *CDR2* in all four matched isolate sets. Its up-regulation in these isolates is at least influenced by Tac1p as deletion of *TAC1* in isolate 5674 reduced its expression to normal levels. Chk1p is a histidine kinase involved in a two-component signaling pathway, along with the response regulator Ssk1p, that regulates cell wall biosynthesis (136, 137). Interestingly it was recently shown that strains of *C. albicans* lacking either of these signal transduction proteins are hypersensitive to fluconazole (138). Up-regulation of *CHK1* in association with *CDR1*- and *CDR2*-mediated azole resistance suggests that, in addition to its requirement for baseline azole tolerance in azole-susceptible cells, this protein may contribute to azole resistance in clinical isolates. In the related fungal species *C. glabrata*, ATPase activity of the ABC transporter Cdr1p and the drug efflux activity of the ABC transporter Pdh1p (Cdr2p) are regulated by phosphorylation (139, 140). Likewise, phosphorylation has been shown to modulate transcriptional activity of the transcription factor Gal4p (141), and has been suggested to regulate the activity of the transcriptional regulator Pdr3p (142). It is therefore tempting to speculate that Chk1p is involved in the phosphorylation and activity of the efflux pumps Cdr1p and Cdr2p, or possibly Tac1p itself. Further investigation into the contribution of this signaling pathway to azole resistance will be required to address this question.

Our genome-wide location experiments revealed that Tac1p binds to the promoter of a group of genes involved (or predicted to be involved) in lipid metabolism. These genes are *CDR1*, *CDR2*, *PDR16*, *RTA3*, *ATF1*, *ERG1*, *LCB4*, orf19.6501, orf19.7166, and orf19.1887. With the exception of *ERG1*, all these genes were significantly up-regulated in a *TAC1*-dependent manner at least in one out of the four clinical isolates tested by expression microarrays, strongly suggesting i) that these genes are direct transcriptional targets of Tac1p and ii) a role for Tac1p in lipid metabolism. It has been shown that Rsb1p, the *S. cerevisiae* orthologue of *C. albicans* Rta3p, plays an essential role in the translocation of long-chain bases across the plasma membrane (143), suggesting a role for Rsb1p, and thus potentially Rta3p, in regulating the sphingolipid composition of the plasma membrane. Interestingly, *S. cerevisiae* *LCB4* encodes a major sphingolipid long-chain base kinase required for synthesis of long-chain base phosphates and for the rapid incorporation of long chain bases from the culture medium into sphingolipids (144). We also found that orf19.3104, the orthologue of *S. cerevisiae* *YDC1* encoding an alkaline dihydroceramidase, is up-regulated in a *TAC1*-dependent

manner in all four sets of clinical isolates (Table 2-4), although binding of Tac1p to the promoter of this gene was not detected. Ydc1p hydrolyzes dihydroceramide to free fatty acid and dihydrosphingosine, the substrate of *LCB4* (145). Taken together, these findings suggest a role for Tac1p in the synthesis and translocation of sphingolipids into the plasma membrane.

Likewise, *C. albicans* Cdr1p and Cdr2p have been shown to function as plasma membrane energy-dependent translocators of phospholipids, mediating their in-to-out movement (floppases) (146). Thus, Cdr1p and Cdr2p would act in concert with Rta3p to establish the asymmetry of membrane lipids in the plasma membrane. Interestingly, Tac1p binds to the promoter of the *S. cerevisiae* *AST1* homologue, orf19.6869 (*AST2*). *S. cerevisiae* Ast1p was shown to be required for the raft association and restoration of surface delivery of a mutant of the major plasma membrane proton ATPase, Pma1p, that was mistargeted to the vacuole (147). Elsewhere, it has been shown that Cdr1p is a lipid raft associated protein (148). An attractive model would be that Tac1p contributes to the raft association and proper routing of its target gene-products Cdr1p, Cdr2p and Rta3p to the plasma membrane by regulating *AST2* expression. Recent studies have demonstrated that both sphingolipids and sterols are important determinants of surface localization of Cdr1p, as reduced membrane localization of Cdr1p was associated to increased susceptibility of *C. albicans* to ketoconazole in mutants defective in ergosterol biosynthesis (149). Interestingly, we found that Tac1p binding was enriched at the *ERG1* (1.7 fold, *P*-value 0.0005) and *ERG2* (1.6 fold, *P*-value 0.0011) promoters. Moreover, *ERG2* gene expression was significantly up-regulated in a *TAC1*-dependent manner in isolates C56, Gu5 and 17 (Table A-1), suggesting a role of Tac1p in ergosterol biosynthesis. *PDR16*, another target of Tac1p, is the functional homologue of the *S. cerevisiae* *PDR16*, encoding a lipid transfer protein of the Sec14 family (102, 150). *S. cerevisiae* Pdr16p was shown to localize to lipid particles and to transport phosphatidylinositol (151). There is strong evidence showing that lipid transfer proteins exchange lipid molecules between organelle membranes and plasma membrane at membrane contact sites (152). Thus, by controlling the expression of the *PDR16* gene, Tac1p would act as a regulator of phosphatidylinositol transfer within membrane contact sites, further supporting the role of Tac1p in membrane lipid traffic.

Consistent with the potential role of Tac1p in membrane lipid metabolism, we found that the Tac1p targets orf19.7166, orf19.6501 and orf19.1887 encode three putative sterol ester hydrolases or triglycerides lipases as i) a Pfam analysis detected an α/β hydrolase fold in their primary sequences (Pfam Entries PF04083.6 for orf19.7166 and PF00561 for orf19.1887 and orf19.6501), ii) their primary sequences contain the lipase consensus sequence motif GX SXG (153) and iii) Kyte-Doolittle hydrophathy plots revealed hydrophobic regions in the primary sequences suggesting that these proteins are potentially membrane-anchored to lipid particles (data not shown). Steryl esters and triacylglycerols are neutral lipids stored in lipid particles and serve as an energy source as well as a rapid fatty acid source needed upon lipid depletion conditions (154). The orf19.1887 protein is highly homologous to *S. cerevisiae* Yeh1p, a sterol ester hydrolase localized to lipid particles (155, 156), whereas the orf19.7166 and orf19.6501 proteins display moderate homology to the products of the *S. cerevisiae* YGR110W and *YJU3*

genes. Yju3p was shown to be localized in lipid particles as well (157, 158). Another Tac1p target whose product was shown in *S. cerevisiae* to be localized in lipid particles is *ATF1*, encoding an alcohol acetyl transferase (159). This gene was significantly up-regulated in a *TAC1*-dependent manner in strain 5674 (Table A-1). The fact that Atf1p localizes to lipid particles and functions in esterification process suggests that it is involved in the metabolism of lipids probably by transferring acetyl groups to free hydroxyl groups of fatty acids. Thus, in addition to be a potential regulator of membrane lipid traffic, Tac1p appears to be an important regulator of lipid mobilization in *C. albicans*, a function needed for rapid restoration of membrane lipids upon lipid depletion conditions (154).

Our studies identified two Tac1p target genes, *GPX1* and *SOD5*, which have been shown to be involved in the oxidative stress response. We provide strong evidence that Tac1p directly regulates the expression of *GPX1*, which is implicated in response to oxidative stress (160). Interestingly, although the *C. albicans* genome encodes two other putative glutathione peroxidases (encoded by orf19.85 and orf19.87) with high homology to Gpx1p, only Gpx1p was found to be overproduced upon treatments with diamide or hydrogen peroxide (160), suggesting that, among the three putative glutathione peroxidases, *GPX1* plays an important role in oxidative stress response. It was also shown that *S. cerevisiae* *GPX1* encodes a phospholipid hydroperoxide glutathione peroxidase which protects the cell against phospholipid hydroperoxides during oxidative stress (161).

SOD5, a copper, zinc superoxide dismutase found in the cell wall of *C. albicans*, has been shown to be important in protection against osmotic and oxidative stress (162). Since *SOD5* seems to play an important role in this process, it is interesting that the gene is in fact down-regulated in all four azole resistant clinical isolates studied. Martchenko, *et al.* showed that while deleting *SOD5* does not decrease cell viability, susceptibility to hydrogen peroxide under nutrient poor conditions is increased (162). Indeed, we have previously shown that azole resistant isolate 5674, while resistant to diamide, is hypersusceptible to hydrogen peroxide (163). While this hypersusceptibility to hydrogen peroxide could be explained by the down-regulation of *SOD5*, resistance to diamide could be attributed to the up-regulation of the *CDR2* gene by Tac1p since *CDR2* expression was shown to confer diamide resistance in *S. cerevisiae* (163). Taken together, these observations indicate that *TAC1* differentially protects the cell against different oxidative stresses.

CHAPTER 3: GAIN-OF-FUNCTION MUTATIONS IN *MRR1* REGULATE THE EXPRESSION OF *MDR1*[†]

INTRODUCTION

Long term treatment of *C. albicans* with azoles results in the development of resistance over time (27, 29, 35, 43, 91, 95). One mechanism of resistance occurs due to changes in *ERG11*, the gene that encodes the azole drug target lanosterol demethylase. Mutations that occur in *ERG11* result in amino acid substitutions in Erg11p that decrease the binding affinity of the azoles (45). Up-regulation of *ERG11* confers resistance by increasing the amount of enzyme in the cell to bind azoles, requiring more drugs to be as efficacious (27, 91). Another mechanism of resistance is the up-regulation of genes encoding efflux pumps. *CDR1* and *CDR2* encode two ABC transporters that are responsible for resistance to azoles (28, 32, 35, 37, 38, 73). Tac1p, a zinc cluster transcription factor, has been recently identified as the transcriptional regulator of *CDR1* and *CDR2* (88, 89). The up-regulation of *MDR1* is also responsible for azole resistance.

MDR1 encodes a MFS transporter that has been implicated in fluconazole resistance in *C. albicans* (35, 39, 43, 72). *MDR1* was first cloned for its ability to confer resistance to benomyl and methotrexate (41). In clinical matched isolates, *MDR1* expression can only be detected in resistant isolates (34, 43). Disruption of *MDR1* in wild-type isolates shows no hypersusceptibility to fluconazole, most likely due to the lack of *MDR1* expression in susceptible isolates (20, 36, 42). Disruption of *MDR1* in a resistant isolate decreased resistance to fluconazole (21). The promoter region of *MDR1* from a susceptible clinical isolate fused to the reporter GFP was transformed into a resistant isolate, and fluorescence was detected pointing to a *trans*-regulatory element as the regulator of the efflux pump (72).

The bZip transcription factor Cap1p has been implicated in the overexpression of *MDR1* in response to benomyl (84). The promoter region of *MDR1* has been shown to have a YRE motif (85). Sequential disruption of the promoter region of *MDR1* in a resistant isolate showed that the absence of the YRE motif decreased the expression of *MDR1*, but the expression was not completely abolished (86). The disruption of *CAP1* did not abolish *MDR1* expression in response to benomyl (87). This indicates that while Cap1p does bind to the promoter of *MDR1*, the bZip transcription factor is not responsible for azole resistance in clinical isolates.

In this study, we examined three clinical matched isolates known to overexpress *MDR1*. Using genome-wide expression profiling, we identified genes co-regulated with *MDR1*, including a zinc cluster transcription factor encoded by *MRR1* as the regulator of *MDR1* mediated azole resistance. In collaboration with our group, the lab of Joachim Morschhäuser at the Universität Würzburg sequenced *MRR1* and constructed the

[†] Morschhäuser, J., K. S. Barker, T. T. Liu, B. W. J. Bla, R. Homayouni and P. D. Rogers. 2007. The transcription factor Mrr1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. PLoS Pathog. 3: e164. Adapted by permission of authors.

disruption and reintegrated mutants used in this study. In addition, the Morschhäuser lab also performed the drug susceptibility tests that showed *MRR1* as the regulator of drug induced *MDR1* gene expression.

MATERIALS AND METHODS

Strains and Growth Conditions

C. albicans strains used in this study are listed in Table 3-1. All strains were stored as frozen stocks with 15% glycerol at -80°C. The strains were routinely grown in YPD medium (10 g yeast extract, 20 g peptone, 20 g glucose per liter) at 30°C.

DNA Microarray Analysis

The nucleotide sequences corresponding to 6,165 open reading frames (ORFs) for *C. albicans* were downloaded from the Galar Fungail European Consortium (Assembly 6, http://www.pasteur.fr/Galar_Fungail/CandidaDB/). We set out to design two non-overlapping probesets targeting the 3'-600 bp region of each ORF. Each probeset consisted of 13 'perfect match' 25-bp probes and their corresponding 'mismatch' control probes containing a single mismatch in the center of the oligonucleotide. For ORFs less than 600 bp in length the sequence was divided in two equal segments for subsequent design procedures. Optimum probesets were selected by the Affymetrix design team based on their model which, among other things, considers probe hybridization quality and cross-hybridization potential. Consequently, in some cases, only one probeset was selected for a given ORF. For quality control and normalization purposes, we made 2-3 additional probe sets spanning the entire sequence of the *C. albicans* 18S rRNA (GenBank Accession M60302), genes encoding GAPDH, actin and Mdr1p (Bmr1p) in addition to the standard Affymetrix controls (BioB, C, D, cre, DAP, PHE, LYS, THR). In the end, the GeneChip contained 10,736 probe sets including 9 controls, 6,123 unique ORFs and duplicate probe sets for 4,604 ORFs. The duplicate probe sets are made to distinct regions of the ORF, thereby allowing 2 independent measurements of the mRNA level for that particular gene. The *C. albicans* custom Affymetrix NimbleExpress Arrays (CAN04a530004N) were manufactured by NimbleGen Systems (105) per our specification.

RNA Preparation for Microarrays

The matched clinical isolate sets and strain SZY31 were grown in YPD broth at 30°C in a shaking incubator to mid-log phase as described previously (108). The cell pellets were frozen and stored at -80°C prior to RNA preparation. Experiments were repeated independently three times. Total RNA was isolated using the hot SDS-phenol

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

Strain	Parent	Relevant Characteristics or Genotype	Reference
SC5314		Wild-type <i>C. albicans</i> model strain	(164)
CAI4	SC5314	<i>ura3Δ::imm434/ura3Δ::imm434</i>	(18)
F1		Fluconazole-susceptible	(43)
F2		Fluconazole-susceptible	(43)
F5		Fluconazole-resistant	(43)
G1		Fluconazole-susceptible	(43)
G2		Fluconazole-susceptible	(43)
G5		Fluconazole-resistant	(43)
5833		Fluconazole-susceptible	(102)
6692		Fluconazole-resistant	(102)
SCMRR1M4A	SC5314	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
SCMRR1M4B	SC5314	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
F5MRR1M4A	F5	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
F5MRR1M4B	F5	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
G5MRR1M4A	G5	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
G5MRR1M4B	G5	<i>mrr1Δ::FRT/mrr1Δ::FRT</i>	this study
SCMRR1M4K2A	SCMRR1M4A	<i>mrr1Δ /MRR1F2-1-caSAT1</i>	this study
SCMRR1M4K2B	SCMRR1M4B	<i>mrr1Δ /MRR1F2-1-caSAT1</i>	this study
SCMRR1M4K3A	SCMRR1M4A	<i>mrr1Δ /MRR1F5-caSAT1</i>	this study
SCMRR1M4K3B	SCMRR1M4B	<i>mrr1Δ /MRR1F5-caSAT1</i>	this study
SCMRR1M4K4A	SCMRR1M4A	<i>mrr1Δ/MRR1G2-2-caSAT1</i>	this study
SCMRR1M4K4B	SCMRR1M4B	<i>mrr1Δ/MRR1G2-2-caSAT1</i>	this study
SCMRR1M4K5A	SCMRR1M4A	<i>mrr1Δ/MRR1G5-caSAT1</i>	this study
SCMRR1M4K5B	SCMRR1M4B	<i>mrr1Δ/MRR1G5-caSAT1</i>	this study

method (109). Frozen cell pellets were suspended in 12 ml of 50 mM sodium acetate (pH 5.2), 10 mM EDTA at room temperature, after which 1 ml of 20% sodium dodecyl sulfate and 12 ml of acid phenol (Fisher Scientific, Waltham, MA) were added. This mixture was incubated 10 min at 65°C with vortexing each minute, cooled on ice for 5 min, and centrifuged for 15 min at 12,000 x g. Supernatants were transferred to new tubes containing 15 ml of chloroform, mixed and centrifuged at 200 x g for 10 min. The aqueous layer was removed to new tubes, RNA was precipitated with 1 volume isopropanol and 0.1 volume 2 M sodium acetate (pH 5.0), and then collected by centrifugation at 17,000 x g for 35 min at 4°C. The RNA pellet was suspended in 10 ml of 70% ethanol, collected again by centrifugation, and suspended in nuclease free water.

cRNA Synthesis and Labeling

Immediately prior to cDNA synthesis, the purity and concentration of RNA samples were determined from A₂₆₀/A₂₈₀ readings and RNA integrity was determined by capillary electrophoresis using the RNA 6000 Nano Laboratory-on-a-Chip kit and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) as per the manufacturer's instructions. First and second strand cDNA was synthesized from 15 µg of total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) and oligo-dT24-T7 primer (PrOligo: Sigma-Aldrich) according to the manufacturer's instructions. cRNA was synthesized and labeled with biotinylated UTP and CTP by *in vitro* transcription using the T7 promoter-coupled double stranded cDNA as template and the Bioarray HighYield RNA Transcript Labeling Kit (ENZO Diagnostics, New York, NY). Double-stranded cDNA synthesized from the previous steps was washed twice with 70% ethanol and suspended in 22 µl of RNase-free water. The cDNA was incubated as recommended with reaction buffer, biotin-labeled ribonucleotides, dithiothreitol, RNase inhibitor mix and T7 RNA polymerase for 5 h at 37°C. The labeled cRNA was separated from unincorporated ribonucleotides by passing through a CHROMA SPIN-100 column (Clontech, Mountain View, CA) and ethanol precipitated at -20°C overnight.

Oligonucleotide Array Hybridization and Analysis

The cRNA pellet was suspended in 10 µl of RNase-free water and 10 µg was fragmented by ion-mediated hydrolysis at 95°C for 35 min in 200 mM Tris-acetate (pH 8.1), 500 mM potassium acetate, 150 mM magnesium acetate. The fragmented cRNA was hybridized for 16 h at 45°C to the *C. albicans* NimbleExpress GeneChip arrays. Arrays were washed at 25°C with 6 × SSPE, 0.01% Tween 20 followed by a stringent wash at 50°C with 100 mM MES, 0.1 M NaCl, 0.01% Tween 20. Hybridizations and washes employed the Affymetrix Fluidics Station 450 using their standard EukGE-WS2v5 protocol. The arrays were then stained with phycoerythrin-conjugated streptavidin (Molecular Probes: Invitrogen) and the fluorescence intensities were determined using the GCS 3000 high-resolution confocal laser scanner (Affymetrix, Santa Clara, CA). The scanned images were analyzed using software resident in GeneChip Operating System v2.0 (GCOS; Affymetrix). Sample loading and variations

in staining were standardized by scaling the average of the fluorescent intensities of all genes on an array to a constant target intensity. The signal intensity for each gene was calculated as the average intensity difference, represented by $[\Sigma(\text{PM} - \text{MM})/(\text{number of probe pairs})]$, where PM and MM denote perfect-match and mismatch probes.

Microarray Data Analysis

The scaled gene expression values from GCOS software were imported into GeneSpring 7.2 software (Agilent Technologies) for preprocessing and data analysis. Probe sets were deleted from subsequent analysis if they were called absent by the Affymetrix criterion and displayed an absolute value below 20 in all experiments. The expression value of each gene was normalized to the median expression of all genes in each chip as well as the median expression for that gene across all chips in the study. Pairwise comparison of gene expression was performed for each matched experiment. Among direct comparisons between matched clinical isolates genes were considered to be differentially expressed if their change in expression was ≥ 1.5 -fold in three independent experiments.

Quantitative Real-Time RT-PCR

An aliquot of the RNA preparations from the samples used in the microarray experiments was saved for quantitative real-time reverse transcription (RT)-PCR follow-up studies. First-strand cDNAs were synthesized from 2 μg of total RNA in a 21- μl reaction volume using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) in accordance with the manufacturer's instructions. Quantitative real-time PCRs were performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems, Inc.: ABI, Foster City, CA). Independent PCRs were performed using the same cDNA for both the gene of interest and the 18S rRNA, using the SYBR Green PCR Master Mix (Applied Biosystems, Inc.). Gene-specific primers were designed for the gene of interest and the 18S rRNA using Primer Express software (Applied Biosystems, Inc.) and the Oligo Analysis & Plotting Tool (QIAGEN, Valencia, CA) and are shown in Table 3-2. The PCR conditions consisted of AmpliTaq Gold activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the 7000 Sequence Detection System. The change in fluorescence of SYBR Green I dye in every cycle was monitored by the system software, and the threshold cycle (C_T) above the background for each reaction was calculated. The C_T value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔC_T value. The ΔC_T value of an arbitrary calibrator (e.g., untreated sample) was subtracted from the ΔC_T value of each sample to obtain a $\Delta\Delta C_T$ value. The gene expression level relative to the calibrator was expressed as $2^{-\Delta\Delta C_T}$.

Table 3-2. Primers used for quantitative real-time RT-PCR.

Gene Name	Primer Pairs^a	Amplicon Size (bp)
<i>18S</i>	F, 5'-CACGACGGAGTTTCACAAGA-3' R, 5'-CGATGGAAGTTTGAGGCAAT-3'	135
<i>MDR1</i>	F, 5'-ACATAAATACTTTGCCCATCCAGAA-3' R, 5'-AAGAGTTGGTTTGTAATCGGCTAAA-3'	82
<i>MRR1</i>	F, 5'-AGTGAAGAAGCCCAACAGCAAA-3' R, 5'-GACTGCGAACTTGTAACGGC-3'	59

^a **F**: forward, **R**: reverse

RESULTS

Transcription Factor *MRR1* Is Coordinately Regulated with *MDR1* in Resistant Clinical *C. albicans* Isolates

In order to identify the genes coordinately regulated with *MDR1*, we examined three clinical matched isolates using genome-wide expression profiling (Table 3-1). Previous studies have shown that F1, G1, and 5833 (azole susceptible isolates) do not express baseline *MDR1*; F5, G5, and 6692 (azole resistant isolates) have been shown to overexpress *MDR1* (43, 102). Three independent RNA samples were hybridized to custom *C. albicans* Affymetrix microarrays. Genes were considered to be differentially expressed if genes changes 1.5-fold in all three independent experiments. Using these criteria, there were 111 genes up-regulated and 90 genes down-regulated in F5 compared to F1 (Table B-1 and Table B-2). In G5 compared to G1, there were 194 genes up-regulated and 184 genes down-regulated. In 6692 compared to 5833, 66 genes were up-regulated and 84 genes were down-regulated. The core genes considered to be co-regulated with *MDR1* were the genes in common among all three clinical isolates. There were the 21 genes up-regulated including the previously identified genes *GRP2*, *IFD1*, *IFD4*, *IFD5*, *IFD7*, and *MDR1* (Figure 3-1). There were also 7 genes that were down-regulated among all three clinical matched isolates (Figure 3-1). Interestingly, one gene encoding a zinc cluster transcription factor, orf19.7372 or IPF1266, was up-regulated in all three resistant clinical isolates. This zinc cluster transcription factor gene was named multidrug resistance regulator, *MRR1*.

This transcription factor gene was disrupted in the resistant isolates F5 and G5 by the Morschhäuser lab, and genome-wide expression profiling performed on the disruption mutants. In the *mrr1Δ* mutant in F5, 123 genes were down-regulated in the absence of *MRR1*; in the *G5mrr1Δ* mutant, 94 genes were down-regulated (Table B-3). There were 28 genes commonly down-regulated in the absence of *MRR1*. Of these genes, *MDR1* was down-regulated as well as 13 other genes that were up-regulated in all three resistant clinical isolates (Table 3-3). Unlike the disruption of *CAP1*, the disruption of *MRR1* abolished the expression of *MDR1*.

Identification of Mrr1p Target Genes

The Morschhäuser lab identified a P683S mutation in one allele of *MRR1* in F5 and a G997V mutation in one allele in G5. In order to identify the genes directly under the control of the mutated Mrr1p, the wild-type *MRR1* allele (*MRR1*^{F2-1} or *MRR1*^{G2-1}) or the constitutively active mutant allele (*MRR1*^{F5} and *MRR1*^{G5}) was reintegrated into SC5314 with *MRR1* disrupted. In the *MRR1*^{F5} allele compared to *MRR1*^{F2-1} allele in SC5314, 20 genes were up-regulated specifically due to the P683S (Table 3-4). Of these 20 genes, 16 genes were also up-regulated in the clinical matched isolates. Of the genes that were up-regulated in resistant isolate F5 and up-regulated in SC5314 with *MRR1*^{F5},

Figure 3-1. Identification of genes that are differentially expressed in *MDR1* overexpressing clinical *C. albicans* isolates.

(A, B) Venn diagrams showing the number of genes that are up-regulated (A) or down-regulated (B) in the *MDR1* overexpressing, drug-resistant isolates F5, G5, and 6692 as compared with their matched drug-susceptible isolates F1, G1, and 5833, respectively. (C) Heat map showing genes that are up-regulated (106) or down-regulated (105) in all three *MDR1* overexpressing isolates. Results are from three independent experiments (Exp. 1 to Exp. 3) for each comparison. The genes are ordered according to their relative degree of up- and down-regulation, as indicated by the brightness of the red and green fields.

C.

Table 3-3. Genes commonly down-regulated by *MRR1* deletion in F5 and G5.

Gene Name ^a	orf19 Number ^b	GO Annotation (Molecular Function) ^c	F5Δ <i>mrr1</i>	G5Δ <i>mrr1</i>
<i>IPF9538</i>	orf19.271	2,4-dienoyl-CoA reductase (NADPH) activity	-282.4	-17.7
<i>MDR1</i>	orf19.5604	Multidrug transporter activity	-46.9	-126.3
<i>MRR1</i>	orf19.7372	RNA polymerase II transcription factor activity	-50.7	-44.7
<i>IFD6</i>	orf19.4476	Aryl-alcohol dehydrogenase activity	-30.3	-31.4
<i>IPF5987</i>	orf19.7306	--	-7.4	-9.8
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	-13.2	-4.0
<i>IPF17186</i>	orf19.251	--	-9.2	-7.0
<i>IPF7863</i>	orf19.1449	--	-11.8	-3.7
<i>IFD7</i>	orf19.629	Aryl-alcohol dehydrogenase activity	-6.7	-8.1
<i>HGT11</i>	orf19.4527	Glucose transporter activity	-5.1	-5.9
<i>HGT12</i>	orf19.3668	Glucose transporter activity	-3.3	-7.2
<i>IPF525</i>	orf19.7085	--	-6.2	-2.4
<i>IPF2186</i>	orf19.7166	--	-3.4	-4.9
<i>IFK3</i>	orf19.857	--	-4.6	-3.0
<i>IPF19538</i>	orf19.6957.3	--	-2.0	-5.4
<i>IPF7817</i>	orf19.3131	NADPH dehydrogenase activity	-4.4	-3.0
<i>IFD1</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	-2.6	-4.6
<i>IFD4</i>	orf19.4477	Aryl-alcohol dehydrogenase activity	-2.9	-3.7
<i>AAF1</i>	orf19.7436	--	-3.1	-3.4
<i>FRE30.53</i>	orf19.6140	Ferric-chelate reductase activity	-3.5	-2.8
<i>FRE30.3</i>	orf19.6139	Ferric-chelate reductase activity	-3.2	-2.9
<i>IFD2</i>	orf19.771	Aryl-alcohol dehydrogenase activity	-3.1	-2.8
<i>IPF1634</i>	orf19.6578	Inorganic phosphate transporter activity	-2.4	-3.3
<i>GRP2</i>	orf19.4309	Oxidoreductase activity	-1.9	-3.4
<i>ARD8</i>	orf19.6322	2,4-dienoyl-CoA reductase (NADPH) activity	-2.3	-2.3
<i>FRE7</i>	orf19.7077	Ferric-chelate reductase activity	-2.2	-2.2
<i>GAP6</i>	orf19.6659	Amino acid permease activity	-2.1	-1.7
<i>AKL1</i>	orf19.5357	Protein serine/threonine kinase activity	-1.7	-1.7

^a Gene name according to CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b orf19 nomenclature according to the Assembly 19 version.

^c Gene ontology (GO) terminology according to CGD (<http://www.candidagenome.org/>).

Gene expression values are represented as fold changes between disruption strain and resistant parent isolate.

Genes in bold are genes found to be up-regulated in all three clinical matched isolates.

Table 3-4. Identification of Mrr1p target genes in F isolates by DNA microarray analysis.

Genes that were up-regulated by the P683S mutation in the SC5314 genetic background ($MRR1^{F5}$ vs. $MRR1^{F2-1}$). The genes are ordered according to their average degree of up-regulation in four repeat experiments performed with two independent transformants (strains A and B). The fold changes in the expression level of these genes in the drug-resistant clinical isolate F5 as compared with a matched susceptible isolate (F5 vs. F1) and in two independently constructed *mrr1Δ* mutants (strains A and B) as compared with their wild-type progenitor F5 ($F5mrr1Δ$ vs. F5) are shown for comparison. Up-regulated genes are highlighted in red, down-regulated genes are highlighted in green, and genes that were not differentially expressed are not highlighted in all pairwise comparisons.

Gene Name	orf19 Number	GO Annotation (Molecular Function)	$MRR1^{F5}$ vs. $MRR1^{F2-1}$				F5 vs. F1			$F5mrr1Δ$ vs. F5			
			Strain A		Strain B					Strain A		Strain B	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>IFD6</i>	orf19.4476	Aryl-alcohol dehydrogenase activity	256.4	1121.8	284.7	1147.1	965.7	1290.0	3064.8	-31.2	-41.8	-27.0	-21.3
<i>IFD1</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	130.7	179.8	100.4	72.1	76.2	200.7	141.5	-3.3	-2.1	-2.6	-2.6
<i>BMR1</i>	orf19.5604	Multidrug transporter activity	114.8	114.4	118.2	39.7	47.8	152.1	112.2	-21.4	-47.5	-35.0	-83.7
<i>IFD7</i>	orf19.629	Aryl-alcohol dehydrogenase activity	56.3	75.9	184.2	35.3	113.1	338.2	332.4	-10.5	-4.3	-5.8	-6.0
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	64.8	116.6	84.5	50.4	140.8	361.9	144.3	-15.0	-12.2	-15.8	-9.6
<i>IFD4</i>	orf19.4477	Aryl-alcohol dehydrogenase activity	15.4	13.6	21.4	56.6	21.3	37.9	75.5	-2.7	-4.6	-2.4	-1.9
<i>IPF5987</i>	orf19.7306	--	19.6	19.3	28.3	27.6	48.6	49.5	63.3	-7.4	-6.4	-9.0	-6.9
<i>IPF9538</i>	orf19.271	2,4-dienoyl-CoA reductase (NADPH) activity	16.3	24.6	5.2	6.4	108.9	279.5	131.7	-164.3	-548.1	-280.0	-137.2
<i>IPF17186</i>	orf19.251	--	10.4	11.9	10.2	11.2	20.7	38.9	39.7	-10.9	-11.0	-8.7	-6.2
<i>GRP2</i>	orf19.4309	Oxidoreductase activity	4.2	6.9	7.0	6.5	3.0	5.3	3.4	-2.1	-1.9	-1.8	-1.8
<i>IPF7863</i>	orf19.1449	--	3.5	3.8	3.6	3.4	6.1	9.4	9.1	-16.9	-6.9	-15.5	-8.0
<i>ALS10</i>	orf19.2355	Cell adhesion molecule binding	2.0	3.7	2.7	1.9	1.2	1.7	1.1	-14.0	-27.2	-1.6	-1.2
<i>IPF19538</i>	orf19.6957.3	--	3.2	2.5	1.8	2.6	2.2	1.7	6.0	-1.5	-2.0	-2.1	-2.1

Table 3-4 (continued).

Gene Name	orf19 Number	GO Annotation (Molecular Function)	<i>MRR1</i> ^{F5} vs. <i>MRR1</i> ^{F2-1}				F5 vs. F1			F5 <i>mrr1</i> Δ vs. F5			
			Strain A		Strain B		Exp. 1	Exp. 2	Exp. 3	Strain A		Strain B	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2				Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>ALS3.5eoc</i>	orf19.1816	Cell adhesion molecule binding	2.2	3.6	2.1	1.5	1.4	1.8	1.2	-43.4	-22.9	-1.6	-1.2
<i>IPF2130</i>	orf19.7204	--	2.0	2.7	2.4	1.6	1.8	1.8	-1.4	-2.8	1.2	-2.4	-1.5
<i>IPF7940</i>	orf19.6608	--	1.9	2.2	2.1	2.0	5.4	4.3	10.3	-2.2	-1.9	1.1	1.0
<i>IPF9377</i>	orf19.1428	--	2.2	1.6	1.5	2.8	1.2	1.5	-1.1	1.1	1.6	1.1	-4.0
<i>IPF7817</i>	orf19.3131	NADPH dehydrogenase activity	2.2	2.0	2.1	1.7	9.4	13.7	13.4	-3.8	-4.7	-5.5	-3.6
<i>DUR1,2</i>	orf19.780	Allophanate hydrolase activity	2.0	1.5	1.6	2.4	1.7	1.9	2.2	-2.2	1.8	-2.0	1.5
<i>IPF2186</i>	orf19.7166	--	1.5	2.0	1.9	2.0	3.0	6.0	7.3	-2.9	-3.5	-3.7	-3.4

14 genes were down-regulated in the absence of both alleles of *MRR1*. In the *MRR1*^{G5} allele compare to *MRR1*^{G2-1} allele in SC5314, 27 genes were up-regulated specifically due to the G997V mutation (Table 3-5). Of these 27 genes, 20 genes were up-regulated in resistant isolate G5. Of these 20 genes, 15 genes were down-regulated in the absence of both *MRR1* alleles. *MDR1* and *MRR1* gene expression was confirmed with real-time RT-PCR (Figure 3-2). Interestingly, 14 genes were up-regulated in all three clinical resistant isolate, up-regulated in both *MRR1*^{F5} and *MRR1*^{G5}, and down-regulated in the *Δmrr1* disruption mutants. These genes are considered the core genes regulated by Mrr1p.

DISCUSSION

Increased drug efflux due to the overexpression of efflux pumps is a major mechanism of resistance in *C. albicans*. The up-regulation of *CDR1* and *CDR2* results in resistance to azole antifungal agents. Gain-of-function mutations in the gene encoding the zinc cluster transcription factor Tac1p was recently identified as the cause of *CDR1/CDR2* overexpression (88, 89). The expression of the MFS transporter *MDR1* is another resistance mechanism found in azole resistant clinical isolates. While the regulator of *MDR1* was previously unknown, studies have shown that a *trans*-regulatory element was responsible for the up-regulation of *MDR1* (72). Previously, the bZip transcription factor Cap1p was identified as the possible transcriptional regulator of *MDR1* (84). The promoter region of genes co-regulated with *MDR1* as well as the promoter region of *MDR1* contained a YRE motif. However, promoter deletion studies performed in azole resistant isolates showed that the deletion of the YRE motif did not abolish the up-regulation of *MDR1* (21, 86). This suggested that the transcriptional regulator of azole resistance was not limited to *CAP1*.

Genome-wide expression profiling of clinical matched isolates overexpressing *CDR1/CDR2* identified *TAC1* as a gene co-regulated with the azole resistance genes. Using this technology, we examined three clinical matched isolates that overexpressed *MDR1*. Of the genes found to be differentially expressed in all three resistant isolates, a gene encoding the zinc cluster transcription factor *MRR1* was moderately up-regulated. The degree by which *MRR1* contributes to azole resistance was studied by looking at gene expression profiles of *MRR1* disruption mutants. Unlike the *CAP1* disruption mutants, the resistant strains without *MRR1* could no longer express *MDR1* along with the *MDR1* co-regulated genes.

Additionally, in collaboration with our group the Morschhäuser lab demonstrated that the *MRR1* deletion mutants in resistant isolates were more susceptible to fluconazole, cerulenin, brefeldin A, and diamide. They also showed that the gain-of-function mutation in *MRR1* was required for constitutive *MDR1* expression and resistance to fluconazole, cerulenin, brefeldin A, and diamide. *MRR1* was also required for the induction of *MDR1* by benomyl and hydrogen peroxide. In the absence of *CAP1*, benomyl could still induce *MDR1* expression; without *MRR1* there was no induction by benomyl. This suggests that while Cap1p may play a role in *MDR1* gene expression,

Table 3-5. Identification of Mrr1p target genes in G isolates by DNA microarray analysis.

Genes that were up-regulated by the G997V mutation in the SC5314 genetic background ($MRR1^{G5}$ vs. $MRR1^{G2-1}$). The genes are ordered according to their average degree of up-regulation in four repeat experiments performed with two independent transformants (strains A and B). The fold changes in the expression level of these genes in the drug-resistant clinical isolate G5 as compared with a matched susceptible isolate (G5 vs. G1) and in two independently constructed *mrr1Δ* mutants (strains A and B) as compared with their wild-type progenitor G5 ($G5mrr1Δ$ vs. G5) are shown for comparison. Up-regulated genes are highlighted in red, down-regulated genes are highlighted in green, and genes that were not differentially expressed are not highlighted in all pairwise comparisons.

Gene Name	orf19 Number	GO Annotation (Molecular Function)	<i>MRR1</i> ^{G5} vs. <i>MRR1</i> ^{G2-1}				G5 vs. G1			G5 <i>mrr1</i> Δ vs. G5			
			Strain A		Strain B		Exp. 1	Exp. 2	Exp. 3	Strain A		Strain B	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2				Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>IFD6</i>	orf19.4476	Aryl-alcohol dehydrogenase activity	179.0	617.6	1559.4	1735.9	565.4	818.4	1916.6	-12.8	-22.1	-12.6	-78.1
<i>IPF9538</i>		2,4-dienoyl-CoA reductase (NADPH) activity	125.7	99.9	128.9	219.6	7.8	24.5	18.1	-15.3	-16.6	-25.5	-13.3
<i>IFD1</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	135.0	123.5	175.1	139.4	151.6	195.9	299.8	-6.1	-5.2	-4.5	-2.9
<i>IFD7</i>	orf19.629	Aryl-alcohol dehydrogenase activity	112.0	134.5	131.9	91.9	55.2	66.8	139.0	-8.0	-8.2	-9.6	-6.5
<i>BMR1</i>	orf19.5604	Multidrug transporter activity	103.8	73.8	135.4	68.3	116.2	181.2	210.3	-168.9	-149.4	-143.0	-43.9
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	59.6	82.9	92.6	141.5	137.7	155.8	201.1	-4.9	-4.2	-4.1	-2.7
<i>IFD4</i>	orf19.4477	Aryl-alcohol dehydrogenase activity	29.3	44.7	21.2	31.1	81.0	115.9	149.7	-4.0	-4.4	-3.4	-2.9
<i>IPF5987</i>	orf19.7306	--	30.9	24.3	22.0	32.7	41.5	50.1	81.4	-8.6	-12.5	-7.4	-10.5
<i>IPF17186</i>	orf19.251	--	15.4	12.8	14.4	13.1	9.2	14.8	14.6	-5.5	-9.5	-5.6	-7.4
<i>GRP2</i>	orf19.4309	Oxidoreductase activity	6.1	5.7	5.1	7.4	4.9	6.1	5.5	-3.7	-3.7	-3.5	-2.9
<i>IPF7863</i>	orf19.1449	--	4.1	4.6	7.7	7.8	7.2	7.2	5.7	-2.9	-4.5	-3.0	-4.3
<i>IPF11694</i>	orf19.4355	Nicotinamide mononucleotide permease activity	2.0	4.9	3.7	10.2	-1.5	-1.1	1.1	-2.0	-3.2	1.7	1.4

Table 3-5 (continued).

Gene Name	orf19 Number	GO Annotation (Molecular Function)	<i>MRR1</i> ^{G5} vs. <i>MRR1</i> ^{G2-1}				G5 vs. G1			<i>G5mrr1</i> Δ vs. G5			
			Strain A		Strain B		Exp. 1	Exp. 2	Exp. 3	Strain A		Strain B	
			Exp. 1	Exp. 2	Exp. 1	Exp. 2				Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>IPF19538</i>	orf19.6957.3	--	3.4	4.6	5.1	5.2	8.5	4.4	2.3	-7.6	-6.8	-4.1	-3.1
<i>IPF2130</i>	orf19.7204	--	3.1	3.0	2.2	7.9	17.2	37.0	15.2	-2.1	-4.4	-1.5	-3.6
<i>IPF7940</i>	orf19.6608	--	5.5	2.3	1.6	3.9	4.3	5.2	20.2	-1.2	-1.9	-1.2	-4.9
<i>HST2</i>	orf19.2580	NAD-dependent histone deacetylase activity	1.7	2.0	2.8	3.7	1.4	1.1	1.5	-2.0	-1.2	-2.1	1.1
<i>DUR1,2</i>	orf19.780	Allophanate hydrolase activity	2.2	3.4	1.6	3.0	2.1	4.0	2.6	1.5	-1.2	1.9	-1.0
<i>ALS10</i>	orf19.2355	Cell adhesion molecule binding	2.1	3.4	1.9	2.5	-1.6	-2.0	-2.0	-1.5	-1.8	-1.6	1.7
<i>IPF2965</i>	orf19.4287	(R,R)-butanediol dehydrogenase activity	2.4	2.1	1.6	3.4	-1.0	2.1	1.7	4.4	1.1	3.5	1.5
<i>IPF2186</i>	orf19.7166	--	1.9	1.8	3.0	2.9	14.0	15.8	11.7	-7.0	-5.1	-4.8	-2.7
<i>IPF8976</i>	orf19.1369	--	2.2	1.6	1.5	4.1	6.2	7.3	4.0	1.3	-1.2	-1.4	-1.8
<i>ALS3.3eoc</i>	orf19.2355	Cell adhesion molecule binding	2.1	2.9	1.8	2.3	-1.4	-1.5	-1.6	-1.7	-2.0	-1.6	1.6
<i>IPF7817</i>	orf19.3131	NADPH dehydrogenase activity	2.1	1.6	3.4	1.7	7.7	11.0	8.2	-3.6	-2.8	-3.3	-2.1
<i>ALS3.5eoc</i>	orf19.1816	Cell adhesion molecule binding	2.0	2.8	2.0	2.0	-1.1	-1.3	-4.9	-2.0	-1.0	-2.0	2.1
<i>GBA1</i>	orf19.4015	GTPase activity	1.6	1.6	1.5	3.5	41.8	21.8	20.5	-1.1	-1.2	-1.1	1.4
<i>PDC12</i>	orf19.4608	Pyruvate decarboxylase activity	1.6	1.6	1.8	2.3	1.6	-1.5	1.2	-1.7	-1.4	-4.8	-2.4
<i>IPF7456</i>	orf19.2047	--	1.8	1.7	1.5	2.1	1.6	2.1	1.7	1.6	-1.3	2.4	1.1

Figure 3-2. Quantitative real-time PCR analysis of *MDR1* and *MRR1*.

(A) Expression of *MDR1* in the clinical resistant isolates F5, G5, and 6922. (B) Expression of *MRR1* in clinical resistant isolates F5, F5, and 6922. (C) Expression of *MDR1* in resistant isolate F5 and G5 as well as SC5314 with either P683S or G997V mutant alleles. (D) Expression of *MRR1* in resistant isolate F5 and G5 as well as SC5314 with either P683S or G997V mutant alleles. Error bars represent standard errors (n=3).

A.

B.

C.

D.

MRR1 is more essential to the regulation of *MDR1* and azole resistance.

Examination of the gene expression profiles of the resistant isolates, the disruption mutants, and the *MRR1* gain-of-function alleles reintegrated in a wild-type isolates revealed a core group of 14 genes whose expression depend on *MRR1*. Of the 14 co-regulated genes, six genes have been implicated in the catalysis of an oxidation-reduction (redox) reaction. *IPF7817* has been shown to be important in the response to oxidative stress. This gene has been shown to be up-regulated in response to hydrogen peroxide in a *CAP1* dependent manner (164). *GRP2* is another gene that seems to play a role in oxidative stress response. The expression of *GRP2* has been shown to be induced by hydrogen peroxide and benomyl (122, 165). A YRE motif has also been identified in the promoter region of *GRP2* (122). In *S. cerevisiae*, *GRP2* is homologous to *ScGRE2* a gene that encodes a stress-induced NADH-dependent methylglyoxal reductase. In *S. cerevisiae*, *ScGRE2* is regulated by the zinc cluster transcription factor Pdr1p. The *IFD* family of genes has also been implicated in oxidative stress. In the presence of hydrogen peroxide or benomyl, the *IFD* family of genes is up-regulated (122, 165). The *S. cerevisiae* homolog *YPL088w* is regulated by the two zinc cluster transcription factors Yrr1p and Yrm1p (166). Because a large portion of the *MRR1* core genes respond to oxidative stress, *MRR1* may play a role in oxidative stress response. Because these oxidoreductase genes also have YRE motifs in their promoter regions, it is possible that *MRR1* and *CAP1* function in conjunction to respond to oxidative stresses.

CHAPTER 4: GAIN-OF-FUNCTION MUTATIONS IN *UPC2* REGULATE THE EXPRESSION OF ERGOSTEROL BIOSYNTHESIS GENES AND IMPART FLUCONAZOLE RESISTANCE IN A CLINICAL ISOLATE OF *CANDIDA ALBICANS*

INTRODUCTION

The azole antifungals bind to and inhibit the activity of lanosterol demethylase (Erg11p), a key enzyme in the ergosterol biosynthesis pathway (167). Mechanisms of resistance to the azole antifungal agents that have been described for *C. albicans* include increased expression of the gene encoding the MFS transporter Mdr1p and genes encoding two ABC transporters, Cdr1p and Cdr2p (29, 35, 43, 95, 168). Other mechanisms of resistance involve the *ERG11* gene itself. Mutations in *ERG11* that interfere with the ability of the azole to bind to its target can confer resistance. Furthermore, overexpression of *ERG11* leads to increased production of lanosterol demethylase, which can also result in azole resistance (27, 35, 43, 169, 170, 171).

Constitutive overexpression of *CDR1* and *CDR2* in azole-resistant clinical isolates has been shown to be due to gain-of-function mutations in the zinc cluster transcription factor Tac1p and loss of heterozygosity at the *TAC1* locus (88, 89). Recently, similar mutations and loss of heterozygosity in the zinc cluster transcription factor Mrr1p was demonstrated to cause constitutive overexpression of *MDR1* in fluconazole resistant clinical isolates (172). The comparison of gene expression in matched fluconazole-susceptible and -resistant isolates has proved a powerful tool to identify resistance mechanisms of clinical *C. albicans* isolates. Such studies initially pointed to the involvement of efflux pump overexpression as well as *ERG11* overexpression in fluconazole-resistant strains (29, 35). More recently, genome-wide transcriptional profiling experiments using DNA microarrays have revealed additional alterations that might be involved in the development of drug resistance (48, 173, 174). This approach has led to the identification of the transcription factor Mrr1p, which controls expression of the *MDR1* efflux pump (172).

In the present study we performed genome-wide gene expression profiling of a matched pair of azole-susceptible and -resistant isolates from a series in which no overexpression of *CDR1/CDR2* or *MDR1* in resistant isolates was detected by Northern hybridization in a previous study (95). We observed up-regulation of the gene encoding Upc2p as well as known target genes of this transcription factor. In collaboration with our group, the Morschhäuser lab sequenced the *UPC2* genes and described a gain-of-function mutation in *UPC2*. They also created the disruption and reintegrated *UPC2* mutants used in the study. In addition, we show for the first time that a gain-of-function mutation in *UPC2* leads to increased expression of *ERG11*.

MATERIALS AND METHODS

Strains and Growth Conditions

C. albicans strains used in this study are listed in Table 4-1. All strains were stored as frozen stocks with 15% glycerol at -80°C and subcultured on YPD agar plates (10 g yeast extract, 20 g peptone, 20 g glucose, 15 g agar per liter) at 30°C. For routine growth of the strains YPD liquid medium was used.

Construction of *C. albicans* Affymetrix® Expression Arrays

We developed a new Affymetrix custom expression array (CAN07, 49-5241 array format with 11 micron features) for *C. Albicans*. The nucleotide sequences corresponding to 6,165 open reading frames (ORFs) for *C. albicans* were downloaded from the Galar Fungail European Consortium (Assembly 6, http://www.pasteur.fr/Galar_Fungail/CandidaDB). We planned to design two separate probe sets for each ORF, each consisting of 11 perfect match and 11 mismatch overlapping 25 bp oligonucleotides, to the 3' 600 bp region. For ORFs less than 600 bp in length the sequence was divided in two equal segments for subsequent design procedures. For quality control and normalization purposes, we made additional control probesets corresponding to GAPDH (1 probeset), actin (1 probeset) and the 5' and middle region of Mdr1p (Bmr1p) transcript in addition to the standard Affymetrix controls (BioB, C, D, cre, DAP, PHE, LYS, THR). The probe selection was performed by the Chip Design group at Affymetrix, Inc. using their proprietary algorithm to calculate probe set scores, which includes a probe quality metric, cross-hybridization penalty and gap penalty. The probe sets were then examined for cross-hybridization against all other sequences in the *C. albicans* genome as well as a number of constitutively expressed genes and rRNA from other common organisms. Consequently, for some target regions we were not able to design high quality probe sets. In the end, the GeneChip contained 10,014 probesets, corresponding to 5,806 unique ORFs. Two probes were represented for 4,208 ORFs, allowing 2 independent measurements of the mRNA level for that particular gene.

RNA Preparation for Microarrays

Total RNA was isolated using the hot SDS-phenol method (175). Frozen cells were suspended in 12 ml of 50 mM sodium acetate (pH 5.2), 10 mM EDTA at room temperature, after which 1 ml of 20% sodium dodecyl sulphate and 12 ml of acid phenol (Fisher Scientific) were added. This mixture was incubated 10 min at 65 °C with mixing each minute, cooled on ice for 5 min, and centrifuged for 15 min at 12,000g. Supernatants were transferred to new tubes containing 15 ml of chloroform, mixed, and centrifuged at 200g for 10 min. The aqueous layer was removed to new tubes, RNA was precipitated with 1 volume isopropanol and 0.1 volume 2 M sodium acetate (pH 5.0), and then collected by centrifugation at 17,000g for 35 min at 4 °C. The RNA pellet was suspended in 10 ml of 70% ethanol, collected again by centrifugation, and suspended in nuclease-free water.

Table 4-1. *C. albicans* strains used in this study.

Strain	Parent	Relevant characteristics or genotype	Reference
S1		Susceptible isolate from patient S	(95)
S2		Resistant isolate from patient S	(95)
SC5314		Wild-type <i>C. albicans</i> model strain	(176)
UPC2M1A	SC5314	<i>upc2-1Δ::SAT1-FLIP^a/UPC2-2</i>	this study
UPC2M1B	SC5314	<i>UPC2-1/upc2-2Δ::SAT1-FLIP</i>	this study
UPC2M2A	UPC2M1A	<i>upc2-1Δ::FRT/UPC2-2</i>	this study
UPC2M2B	UPC2M1B	<i>UPC2-1/upc2-2Δ::FRT</i>	this study
UPC2M3A	UPC2M2A	<i>upc2-1Δ::FRT/upc2-2Δ::SAT1-FLIP</i>	this study
UPC2M3B	UPC2M2B	<i>upc2-1Δ::SAT1-FLIP/ upc2-2Δ::FRT</i>	this study
UPC2M4A	UPC2M3A	<i>upc2-1Δ::FRT/upc2-2Δ::FRT</i>	this study
UPC2M4B	UPC2M3B	<i>upc2-1Δ::FRT/ upc2-2Δ::FRT</i>	this study
UPC2M2K21A	UPC2M2A	<i>upc2-1Δ::FRT/UPC2^{S1-1}-caSAT1</i>	this study
UPC2M2K21B	UPC2M2B	<i>UPC2-1^{S1-1}-caSAT1/upc2-2Δ::FRT</i>	this study
UPC2M2K22A	UPC2M2A	<i>UPC2-1^{S1-1}-caSAT1/UPC2-2</i>	this study
UPC2M2K22B	UPC2M2B	<i>UPC2-1/UPC2^{S1-1}-caSAT1</i>	this study
UPC2M2K31A	UPC2M2A	<i>upc2-1Δ::FRT/UPC2^{S2-1}-caSAT1</i>	this study
UPC2M2K31B	UPC2M2B	<i>UPC2-1^{S2-1}-caSAT1/upc2-2Δ::FRT</i>	this study
UPC2M2K32A	UPC2M2A	<i>UPC2-1^{S2-1}-caSAT1/UPC2-2</i>	this study
UPC2M2K32B	UPC2M2B	<i>UPC2-1/UPC2^{S2-1}-caSAT1</i>	this study

^a *SAT1-FLIP* denotes the *SAT1* flipper cassette.

cRNA Synthesis and Labeling

Immediately prior to cDNA synthesis, the purity and concentration of RNA samples were determined from A260/A280 readings, and RNA integrity was determined by capillary electrophoresis using the RNA 6000 Nano Laboratory-on-a-Chip kit and Bioanalyzer 2100 (Agilent Technologies) as per the manufacturer's instructions. First and second strand cDNA was synthesized from 15 µg total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen) and oligo-dT24-T7 primer (PrOligo) according to the manufacturer's instructions. cRNA was synthesized and labeled with biotinylated UTP and CTP by in vitro transcription using the T7 promoter-coupled double-stranded cDNA as template and the Bioarray HighYield RNA Transcript Labeling Kit (ENZO Diagnostics). Double-stranded cDNA synthesized from the previous steps was washed twice with 70% ethanol and suspended in 22 µl of RNase-free water. The cDNA was incubated as recommended with reaction buffer, biotin-labeled ribonucleotides, dithiothreitol, RNase inhibitor mix, and T7 RNA polymerase for 5 h at 37 °C. The labeled cRNA was separated from unincorporated ribonucleotides by passing through a CHROMA SPIN-100 column (Clontech) and ethanol precipitated at -20 °C overnight.

Oligonucleotide Array Hybridization and Analysis

The cRNA pellet was suspended in 10 µl of RNase-free water and 10 µg was fragmented by ion-mediated hydrolysis at 95 °C for 35 min in 200 mM Tris-acetate (pH 8.1), 500 mM potassium acetate, 150 mM magnesium acetate. The fragmented cRNA was hybridized for 16 h at 45 °C to the *C. albicans* NimbleExpress GeneChip arrays. Arrays were washed at 25 °C with 6 × SSPE, 0.01% Tween 20 followed by a stringent wash at 50 °C with 100 mM MES, 0.1 M NaCl, 0.01% Tween 20. Hybridizations and washes employed the Affymetrix Fluidics Station 450 using their standard EukGE-WS2v5 protocol. The arrays were then stained with phycoerythrin-conjugated streptavidin (Molecular Probes) and the fluorescence intensities were determined using the GCS 3000 high-resolution confocal laser scanner (Affymetrix). The scanned images were analyzed using software resident in GeneChip Operating System v2.0 (Affymetrix). Sample loading and variations in staining were standardized by scaling the average of the fluorescent intensities of all genes on an array to a constant target intensity (250). The signal intensity for each gene was calculated as the average intensity difference, represented by $[\Sigma(\text{PM} - \text{MM})/(\text{number of probe pairs})]$, where PM and MM denote perfect-match and mismatch probes.

Microarray Data Analysis

The scaled gene expression values from GeneChip Operating System v2.0 software were generated using the MAS5.0 algorithm. The value of each probe set is generated using a Tukey Biweight computation. The algorithm considers the contribution of each match and mismatch probe corrected for the background of the average of the probe set. A weighted mean is then calculated for each probe pair. The raw signal values are then $\log_2$ transformed and the array normalized to this value. Probe sets were deleted from subsequent analysis if they were called absent by the Affymetrix criterion. Pairwise comparison of gene expression was performed for

each matched experiment. Among direct comparisons between matched clinical isolates, genes were considered to be differentially expressed if their change in expression was 1.5-fold in three independent experiments.

Quantitative Real-Time PCR

An aliquot of the RNA preparations from the samples used in the microarray experiments was saved for quantitative real-time RT-PCR follow-up studies. First-strand cDNAs were synthesized from 2 µg of total RNA in a 21-µl reaction volume using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) in accordance with the manufacturer's instructions. Quantitative real-time PCRs were performed in triplicate using the 7000 Sequence Detection System (Applied Biosystems). Independent PCRs were performed using the same cDNA for both the gene of interest and the 18S rRNA, using the SYBR Green PCR Master Mix (Applied Biosystems). Gene-specific primers were designed for the gene of interest and the 18S rRNA using Primer Express software (Applied Biosystems) and the Oligo Analysis & Plotting Tool (QIAGEN) and are shown in Table 4-2. The PCR conditions consisted of AmpliTaq Gold activation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the 7000 Sequence Detection System. The change in fluorescence of SYBR Green I dye in every cycle was monitored by the system software, and the threshold cycle (C_T) above the background for each reaction was calculated. The C_T value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔC_T value. The ΔC_T value of an arbitrary calibrator (e.g., untreated sample) was subtracted from the ΔC_T value of each sample to obtain a $\Delta\Delta C_T$ value. The gene expression level relative to the calibrator was expressed as $2^{-\Delta\Delta C_T}$.

Bioinformatics Analysis

The promoter sequences (1kb upstream of the start codon) were downloaded from the Candida Genome Database (<http://www.candidagenome.org>). Using the fuzznuc application from EMBOSS (<http://emboss.bioinformatics.nl>), the promoter sequences were examined for putative SRE motifs.

RESULTS

Expression of the Transcription Factor *UPC2* Is Coordinately Up-Regulated with *ERG11* in Azole Resistant *C. albicans* Clinical Isolate S2

The *C. albicans* isolates S1 (fluconazole-susceptible) and S2 (fluconazole-resistant) were recovered from an AIDS patient who failed fluconazole therapy for treatment of oropharyngeal candidiasis and are the first two isolates of a series in which no overexpression of the transporter genes *CDR1/CDR2* and *MDR1* was detected by Northern hybridization (95). In order to identify

Table 4-2. Primers used for quantitative real-time PCR analysis.

Gene Name	Primer Pair^a	Amplicon Size
<i>18S</i>	F, 5'-CACGACGGAGTTTCACAAGA-3' R, 5'-CGATGGAAGTTTGAGGCAAT-3'	135
<i>CDR1</i>	F, 5'-ATTCTAAGATGTCGTCGCAAGATG-3' R, 5'-AGTTCTGGCTAAATTCTGAATGTTTTTC-3'	140
<i>CDR2</i>	F, 5'-TAGTCCATTCAACGGCAACATT-3' R, 5'-CACCCAGTATTTGGCATTGAAA-3'	76
<i>MDR1</i>	F, 5'-ACATAAATACTTTGCCCATCCAGAA-3' R, 5'-AAGAGTTGGTTTGTAATCGGCTAAA-3'	82
<i>ERG11</i>	F, 5'-TTTAGTTTCTCCAGGTTATGCTCAT-3' R, 5'-ATTAGCTTTGGCAGCAGCAGTA-3'	100
<i>UPC2</i>	F, 5'-TCCATCCTTGACCCCTAGTCCT-3' R, 5'-CGGCTGAGTTTTGATGTCTTGA-3'	52

^a F: forward, R: reverse

genes that are differentially expressed between these two isolates we compared their transcriptional profiles using DNA microarrays. As can be seen in Table C-1 and Table C-2, 138 genes were up-regulated and 173 genes were down-regulated in isolate S2 as compared with S1. Interestingly, among the up-regulated genes were several that are involved in ergosterol biosynthesis (*ERG1*, *ERG3*, *ERG4*, *ERG7*, *ERG24*, *ERG26*, *ERG27*, *POT14*, and *NCPI*) as well as *UPC2*, which encodes a transcription factor that controls expression of ergosterol biosynthesis genes and induces their expression in the presence of azoles (82, 90, 177). *ERG11*, the gene encoding the target of fluconazole, was not found to be differentially expressed between these isolates by microarray analysis. However, real-time RT-PCR analysis revealed increased expression of *ERG11* in isolate S2 (Figure 4-1).

Resistant Isolate S2 Contains a Gain-of-Function Mutation in *UPC2*

In collaboration with our group, the Morschhäuser lab sequenced the *UPC2* alleles in both the resistant isolate S2 and the susceptible isolate S1. Two different alleles were found in isolate S1. Allele one was found to be similar to the *UPC2* allele in the wild-type genome strain SC5314; allele two had several mutations, four of which resulted in amino acid substitutions (R68K, I142S, S190N, S228N). Isolate S2 contained the same alleles as S1, with the exception of a G1943A mutation in allele one. This mutation resulted in a G648D amino acid substitution in Upc2p. *UPC2* was then disrupted in the wild-type isolate SC5314, and one copy of either wild-type allele one from S1 or gain-of-function mutant allele one from S2 was reintegrated into the disruption strain.

These mutants in the wild-type background were then exposed to the ergosterol biosynthesis inhibitors fluconazole and terbinafine. In the absence of one or both alleles *UPC2*, the strains were hypersusceptible to fluconazole and terbinafine (Figure 4-2). In the presence of the gain-of-function allele, the strain became resistant to fluconazole and terbinafine. When the wild-type allele was reintegrated into the *upc2Δ* strain, the strain exhibited hypersusceptibility to fluconazole similar to the *UPC2* heterozygous disruption strain. In the strain with the wild-type allele reintegrated into a heterozygous *UPC2* disruption strain, fluconazole susceptibility returned to the levels found in SC5314.

Identification of *UPC2* Target Genes

In order to identify target genes of Upc2p, we compared the gene expression profiles of strains expressing either a wild-type *UPC2* allele (*UPC2*^{S1-1}) or its constitutively active, mutated counterpart (*UPC2*^{S2-1}) in the SC5314 background. As shown in Table C-1, 93 genes were consistently up-regulated in the transformants expressing the *UPC2*^{S2-1} allele. Thirty two of these were also up-regulated in azole-resistant isolate S2 as compared to isolate S1 (Table 4-3). Sixty-six genes were consistently downregulated in the transformants expressing the *UPC2*^{S2-1} allele. Of these, only eleven were also downregulated in isolate S2 (Table 4-4). These represent the core set of Upc2p target genes. Among these, 18 genes contained at least one SRE motif within their promoters (Table 4-5).

Figure 4-1. Quantitative real-time PCR for genes of interest.

Genes differentially expressed in clinical matched isolate (S2 vs. S1) and two strains with the $UPC2^{S2-1}$ or $UPC2^{S1-1}$ allele in SC5314. Asterisks denote statistical significance by t-test (p-value ≤ 0.05). Error bars represent standard error (n=3).

Figure 4-2. Susceptibilities to fluconazole and terbinafine of wild-type and gain-of-function mutant *UPC2* strains by spot assay.

All strains were in the SC5314 background. Serial 10-fold dilutions of YPD overnight cultures of the strains were spotted on YPD plates without or with $2 \mu\text{g ml}^{-1}$ fluconazole or $2 \mu\text{g ml}^{-1}$ terbinafine and incubated for 2 days at 30°C .

Table 4-3. Genes up-regulated by the G648D mutation in Upc2p.

CandidaDB Name ^a	Common Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	<i>UPC2</i> ^{S2-1} vs. <i>UPC2</i> ^{S1-1}					
				S2 vs. S1		Strain A		Strain B	
				Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>BMR1</i>	<i>MDR1</i>	orf19.5604	Multidrug transporter activity	2.1	3.7	5.4	3.5	2.5	5.1
<i>DDR48</i>	<i>DDR48</i>	orf19.4082	--	7.1	8.0	2.4	3.4	2.4	3.8
<i>ECM3</i>	<i>ECM3</i>	orf19.1563	--	1.6	1.5	1.7	4.1	2.9	3.2
<i>ERG1</i>	<i>ERG1</i>	orf19.406	Squalene monooxygenase activity	2.4	1.6	1.5	1.7	1.6	1.8
<i>ERG24</i>	<i>ERG24</i>	orf19.1598	Delta 14-sterol reductase activity	2.3	2.0	3.2	2.9	3.5	2.9
<i>ERG26</i>	<i>ERG26</i>	orf19.2909	C-3 sterol dehydrogenase (C-4 sterol decarboxylase) activity	2.0	1.6	2.0	2.0	1.9	2.0
<i>ERG27</i>	<i>ERG27</i>	orf19.3240	3-keto sterol reductase activity	2.3	2.1	2.2	3.6	3.0	2.8
<i>FEN2</i>	--	orf19.5535	Pantothenate transporter activity	2.2	2.2	2.3	2.7	2.7	2.8
<i>FTH1</i>	<i>FTH1</i>	orf19.4802	--	1.5	1.5	1.8	4.3	2.4	3.7
<i>IDP2</i>	<i>IDP2</i>	orf19.3733	Isocitrate dehydrogenase (NADP+) activity	3.6	2.1	1.8	1.6	1.8	1.7
<i>IFA24.3</i>	<i>FGR51</i>	orf19.156	--	1.8	1.9	1.7	1.7	1.6	1.7
<i>NCP1</i>	<i>NCP1</i>	orf19.2672	NADPH-hemoprotein reductase activity	2.5	1.5	1.9	2.2	1.7	1.8
<i>QRI8</i>	--	orf19.7329	Ubiquitin-protein ligase activity	1.5	2.0	1.6	1.7	1.5	1.7
<i>TEF41</i>	<i>TEF4</i>	orf19.2652	Translation elongation factor activity	4.1	3.4	18.8	28.9	19.0	14.9
<i>IPF12101</i>	<i>PGA7</i>	orf19.5635	--	1.6	2.7	5.4	8.8	6.0	9.0
<i>IPF1218</i>	<i>SOD4</i>	orf19.2062	Copper, zinc superoxide dismutase activity	2.3	2.3	2.2	1.7	1.8	1.6
<i>IPF14119</i>	<i>DAG7</i>	orf19.4688	--	1.8	1.6	1.7	1.6	1.7	1.8
<i>IPF14652</i>	--	orf19.2653	--	4.3	1.6	1.7	2.8	4.3	1.9
<i>IPF18080</i>	--	orf19.7357	--	1.9	1.6	1.5	1.8	1.6	1.6
<i>IPF20010</i>	--	orf19.5799	--	2.0	2.2	2.2	2.8	1.6	2.0
<i>IPF20169</i>	<i>PGA10</i>	orf19.5674	Heme binding	4.1	10.8	5.6	13.0	7.0	11.1
<i>IPF2903</i>	--	orf19.7456	--	1.8	2.3	2.0	1.7	2.2	1.6
<i>IPF3352</i>	--	orf19.4013	--	2.3	2.3	2.0	2.5	1.9	2.0
<i>IPF4059</i>	--	orf19.1865	--	1.6	2.1	2.7	2.5	1.9	2.4
<i>IPF4721</i>	--	orf19.3737	--	1.5	1.6	2.3	1.5	1.8	1.6
<i>IPF6298</i>	--	orf19.1964	--	3.0	1.6	1.7	1.7	2.3	1.7
<i>IPF6518</i>	--	orf19.1691	--	3.3	5.3	1.7	2.3	2.0	2.7
<i>IPF7289</i>	<i>UPC2</i>	orf19.391	Transcription factor activity	1.6	2.4	2.1	2.5	2.4	2.5
<i>IPF7397</i>	--	orf19.1800	--	2.9	2.6	1.8	1.7	1.6	2.2

Table 4-3 (continued).

CandidaDB Name ^a	Common Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	<i>UPC2</i> ^{S2-1} vs. <i>UPC2</i> ^{S1-1}					
				S2 vs. S1		Strain A		Strain B	
				Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>IPF7432</i>	--	orf19.6219	--	2.0	2.3	1.9	2.4	2.1	2.2
<i>IPF867</i>	<i>SET3</i>	orf19.7221	NAD-dependent histone deacetylase activity	2.1	1.7	3.6	2.9	3.2	2.4
<i>IPF8970</i>	--	orf19.496	--	1.9	2.5	2.2	1.9	1.6	1.8

^a Gene name from CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name from CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to Assembly 19.

^d Gene Ontology (33) annotation found at CGD (<http://www.candidagenome.org/>).

Gene expression values are represented as fold changes between resistant isolates and susceptible isolates.

Table 4-4. Genes down-regulated by the G648D mutation in Upc2p.

CandidaDB Name ^a	Common Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}					
				S2 vs. S1		Strain A		Strain B	
				Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>COQ1</i>	--	orf19.7478	Trans-hexaprenyltranstransferase activity	-2.8	-2.9	-1.5	-2.5	-2.4	-3.8
<i>MAS2</i>	--	orf19.6295	Mitochondrial processing peptidase activity	-1.6	-1.7	-1.6	-2.3	-2.7	-2.9
<i>MET14</i>	<i>MET14</i>	orf19.946	Adenylylsulfate kinase activity	-5.6	-6.9	-2.3	-2.9	-3.5	-3.7
<i>MET3</i>	<i>MET3</i>	orf19.5025	Sulfate adenylyltransferase (ATP) activity	-4.2	-3.8	-3.3	-4.0	-3.5	-5.9
<i>PET127</i>	--	orf19.2309	RNA binding	-1.7	-1.8	-2.0	-4.4	-7.7	-5.9
<i>IPF11801</i>	--	orf19.2798	Helicase activity	-1.6	-1.7	-2.3	-2.2	-2.0	-1.8
<i>IPF14872</i>	--	orf19.4133	--	-2.0	-1.8	-1.8	-3.5	-2.3	-4.0
<i>IPF20118</i>	--	orf19.3846	--	-3.8	-4.4	-3.6	-3.1	-1.7	-1.6
<i>IPF525</i>	--	orf19.7085	--	-2.4	-2.2	-1.7	-3.1	-1.7	-1.7
<i>IPF6504</i>	--	orf19.1544	--	-2.1	-2.0	-1.8	-1.5	-1.7	-1.6
<i>IPF7334</i>	--	orf19.3724	--	-2.3	-2.2	-1.6	-3.1	-1.8	-3.4

^a Gene name from CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name from CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to Assembly 19.

^d Gene Ontology (33) annotation found at CGD (<http://www.candidagenome.org/>).

Gene expression values are represented as fold changes between resistant isolates and susceptible isolates.

Table 4-5. Genes up-regulated in both isolate S2 and transformants expressing the *UPC2*^{S2-1} allele with SRE motifs in their promoter.

CandidaDB Name^a	Common Name^b	orf19 Number^c	SRE Motifs	Coordinates
<i>DDR48</i>	<i>DDR48</i>	orf19.4082	TGTT TCGTATA ACT	-661 to -649
<i>ECM3</i>	<i>ECM3</i>	orf19.1563	ATGT TATACGATGA	-312 to -300
<i>ERG1</i>	<i>ERG1</i>	orf19.406	TCAT TCGTATATTT	-376 to -364
<i>ERG11</i>	<i>ERG11</i>	orf19.922	ATCT TATACGACGA	-489 to -477
			ATGT TCGTATATTC	-235 to -223
<i>ERG24</i>	<i>ERG24</i>	orf19.1598	CGTT TATACGACCG	-142 to -130
<i>ERG26</i>	<i>ERG26</i>	orf19.2909	CTCT TATACGACAA	-283 to -271
<i>ERG27</i>	<i>ERG27</i>	orf19.3240	GTTT TATACGATCG	-154 to -142
<i>QRI8</i>	--	orf19.7329	ATTT TCGTATATCT	-367 to -355
<i>TEF41</i>	<i>TEF4</i>	orf19.2652	TGCT TATACGATGG	-308 to -296
<i>IPF18080</i>	--	orf19.7357	CCCT TCGTATAGCG	-910 to -898
<i>IPF2903</i>	--	orf19.7456	ATTT TATACGACAT	-278 to -266
<i>IPF4059</i>	--	orf19.1865	CGAT TCGTATATAC	-215 to -203
<i>IPF6518</i>	--	orf19.1691	TTCT TATACGACAG	-716 to -704
<i>IPF7289</i>	<i>UPC2</i>	orf19.391	AGCT TATACGACTT	-440 to -428
<i>IPF7397</i>	--	orf19.1800	TGAT TCGTATAGAT	-566 to -554
<i>IPF7432</i>	--	orf19.6219	CACT TATACGACGA	-353 to -341
<i>IPF867</i>	<i>SET3</i>	orf19.7221	TCGT TCGTATAGAC	-544 to -532
			TGAT TCGTATAGAG	-685 to -673
			GTCT TATACGACCA	-721 to -709
<i>IPF8970</i>	--	orf19.496	CAGT TCGTATACTG	-348 to -336
			TATT TATACGATGG	-211 to -199

^a Gene name from CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name from CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to Assembly 19.

The SRE is in bold.

Disruption of *UPC2* in wild-type strain SC5314 also provided the opportunity to identify genes whose base-line expression requires Upc2p under the growth conditions used in our study. We therefore compared the gene expression profile of strain SC5314 to that of the two independently constructed homozygous *upc2Δ* mutants UPC2M4A and UPC2M4B. Surprisingly only 5 genes were expressed at higher levels in strain SC5314 as compared to the disruption mutants (Table C-3), suggesting that Upc2p is not required for base-line expression of the core target genes listed in Table 4-3 and Table 4-4. Previous studies have shown that disruption of *UPC2* in *C. albicans* results in no baseline changes in expression of *ERG2*, *ERG7*, *ERG11*, and *ERG25* (82, 90). Interestingly disruption of *UPC2* in strain SC5314 resulted in up-regulation of 41 genes (Table C-4). This raises the possibility that Upc2p may also have a role as a transcriptional repressor. Alternatively, this may simply reflect compensatory changes in gene expression.

DISCUSSION

Zinc cluster transcription factors constitute a large family of transcription factors that is specific to fungi. In *S. cerevisiae* mutations in the zinc cluster transcription factor Pdr1p result in constitutive up-regulation of its target genes, including the ABC transporter Pdr5p, and cause the pleiotropic drug resistance phenotype. Azole-resistant clinical *C. albicans* isolates that overexpress the ABC transporters Cdr1p and Cdr2p have acquired mutations in the zinc cluster transcription factor Tac1p and become homozygous for the mutated *TAC1* alleles (88, 89, 178). Likewise, fluconazole resistant clinical isolates that constitutively overexpress the major facilitator superfamily transporter Mdr1p contain mutations in the zinc cluster transcription factor Mrr1p and have also become homozygous for the mutated *MRR1* alleles (172). In addition to decreased drug accumulation via efflux pumps, resistance to azoles can occur due to changes in the ergosterol biosynthesis pathway. Point mutations in the *ERG11* gene, encoding the drug target lanosterol demethylase, results in amino acid substitutions that decrease the binding affinity of the enzyme to azoles (27, 35, 44).

Increased transcription of ergosterol biosynthesis genes has also been associated with resistance. Overexpression of *ERG11* has been observed in azole-resistant isolates. In *S. cerevisiae*, overexpression of *ERG11* alone is sufficient to confer azole resistance. A strain containing the *ERG11* gene placed under the control of an inducible *GALI* promoter could grow on galactose plates at fluconazole concentrations of 128μg/ml (47). The overexpression of *ERG11* in serially collected clinical *C. albicans* isolates coincided with increases in the MIC (27, 168). In a series of isolates described by Lopez-Ribot et al, resistant isolate 2307 (MIC >128 μg/ml) expressed more *ERG11* than both parent isolate 412 (MIC=0.5 μg/ml) and isolate 1907 (MIC=8 μg/ml). There was increased expression of *CDR1* and *CDR2* in isolate 1907 compared to parent isolate 412, but the expression of the ABC transporters were not present in resistant isolate 2307 even though the MIC increases (168). In another study, a clinical resistant *C. albicans* isolate 3795 (MIC>64 μg/ml) overexpressed *ERG11* as compared to the parent isolate 1002 (MIC=0.25 μg/ml). There was no evidence of any other known resistance mechanisms, and there were no point mutations in *ERG11* (27).

Although *ERG11* alone can cause azole resistance in *C. albicans*, the up-regulation of *ERG11* is often found in conjunction with other resistance mechanisms. In the study by Perea et al., of the twelve patients from which clinical isolates were collected, eleven isolates had point mutations in *ERG11* in conjunction with another resistance mechanism. Seven isolates exhibited up-regulation of *CDR1* and *CDR2*, seven isolates exhibited up-regulation of *MDR1*, and three isolates exhibited up-regulation of both classes of efflux pumps (27).

In this study, we characterized the gene expression profile of the matched clinical isolate pair S1 and S2. The gene expression profile of S2 showed a number of ergosterol biosynthesis genes up-regulated as compared to susceptible parent S1. Transcriptional profiling and real-time RT-PCR showed that 8 *ERG* genes were up-regulated in S2. In addition to the *ERG* genes, *UPC2* was also coordinately regulated. Sequence analysis of the *UPC2* alleles identified a G1943A mutation in allele 1 of S2 that was not present in S1. This resulted in a G648D substitution in Upc2p in S2. Transcriptional profiling of the mutated *UPC2*^{S2-1} allele expressed in SC5314 as compared to the wild-type *UPC2*^{S1-1} allele showed 9 genes up-regulated. *UPC2* was also up-regulated in the isolate expressing the *UPC2*^{S2-1} allele. In total, 12 *ERG* genes were differentially expressed in either S2 or *UPC2*^{S2-1} expressed in SC5314 with four *ERG* genes up-regulated in both cases.

C. albicans UPC2 is homologous to two zinc cluster transcription factor genes *UPC2* and *ECM22* in *S. cerevisiae*. Upc2p and Ecm22p both play a role in the regulation of sterol biosynthesis and uptake of sterols from the environment (81, 82, 90, 179, 180). Deletion of *ScUPC2* causes hypersusceptibility to AmB and ketoconazole while deletion of *ScECM22* causes hypersusceptibility to cycloheximide and calcofluor white (81, 82). In addition to drug resistance, *ScUPC2* and *ScECM22* are also involved in sterol uptake. Under aerobic conditions, ergosterol is synthesized and no exogenous sterols are imported. Under anaerobic conditions, the heme in lanosterol demethylase cannot be activated and sterol biosynthesis is inhibited. However, in the absence of oxygen, *S. cerevisiae* has the ability to import exogenous sterols. The mutant hyperactive allele, *upc2-1*, has a G888D mutation that causes exogenous sterol uptake under aerobic conditions. Strains that carry this allele have the ability to take up ten times more exogenous sterols than the wild-type parental strain (181). A G790D mutation was introduced into a wild-type *ECM22* allele that duplicated the mutation present in hyperactive allele *upc2-1* (180). This mutation resulted in aerobic sterol uptake similar to that of *upc2-1*. Deletion of *ScUPC2* and *ScECM22* did not change aerobic sterol uptake, but it decreased the amount of sterol imported under anaerobic condition (179). Sequence alignment showed that the G648D mutation that we identified in one of the *UPC2* alleles of *C. albicans* isolate S2 corresponds exactly to the G888D and G790D mutations that result in hyperactive *UPC2* and *ECM22* alleles, respectively, in *S. cerevisiae*. It is striking that the same mutation has been selected for in a clinical *C. albicans* isolate during fluconazole therapy.

In response to azole antifungals (fluconazole, itraconazole, ketoconazole), wild-type *C. albicans* strains overexpress *ERG11*, *UPC2*, and other *ERG* genes as well as *NCPI* and *DDR48* (48, 174, 182). Forced overexpression of *UPC2* using a *MET3* promoter confers resistance to azoles, whereas disruption of *UPC2* in wild-type isolates causes hypersusceptibility to the azoles and other sterol biosynthesis inhibitors such as terbinafine and fenpropimorph (82, 90). In response to sterol biosynthesis inhibitors, *UPC2* disruption strains show no induction of *ERG* genes (90). The disruption strain accumulates lower levels of exogenous sterols than the wild-type and the reconstituted strains (90), demonstrating the role of *UPC2* in the uptake of exogenous sterols. Strains without *UPC2* also show a growth defect under anaerobic conditions (82), presumably due to the inability to take up sterols in the absence of *UPC2*.

S. cerevisiae Upc2p and Ecm22p were first identified for their ability to regulate *ScERG2* and *ScERG3*. An 11-bp SRE was identified in the promoters of *ScERG2* and *ScERG3* and was found to be bound directly by Upc2p or Ecm22p. Analysis of the promoter region of other ergosterol biosynthesis genes identified the same SRE motifs (81). Mammalian cells contain sterol regulatory element binding proteins (SREBP) to regulate cholesterol synthesis. There are no homologous SREBPs in yeast, but *ScUPC2* and *ScECM22* regulate ergosterol biosynthesis. Promoter analysis on *C. albicans* *ERG* genes was performed with known *S. cerevisiae* SRE (TCGTATA) (81). Several *ERG* genes contained the putative SRE motif in their promoters. The DBD of *C. albicans* Upc2p was purified and used to test direct binding to the SRE found in the promoter region of *S. cerevisiae* and *C. albicans* *ERG2*. Upc2p could bind the SREs of both *S. cerevisiae* and *C. albicans*, and no binding occurred when the SRE was mutated (82). Of the 12 *ERG* genes up-regulated in S2 or by *UCP2*^{S2-1} in SC5314, nine (*ERG1*, *ERG2*, *ERG5*, *ERG6*, *ERG10*, *ERG11*, *ERG24*, *ERG26*, and *ERG27*) have been previously identified to have the *S. cerevisiae* putative SRE core motif, TCGTATA (82, 90). In addition, *ERG7* and *UPC2* were previously identified as having a putative SRE (82). While our analyses did not find an SRE motif in the promoter of *ERG7*, we did find the TCGTATA motif in *UPC2* itself, suggesting this gene may be directly or indirectly autoregulated.

In addition to the *ERG* genes, *UPC2* regulates a host of other genes. One gene of particular interest that was found to be up-regulated by the G648D mutation in Upc2p is *MDR1*. Constitutive overexpression of this efflux pump leads to fluconazole resistance and represents a major mechanism of resistance in clinical isolates (27, 28, 29, 35, 43, 95, 168). Previous Northern hybridization analyses showed no *MDR1* up-regulation in isolates of the S series, although isolate S2 itself was not tested in that study (95). However, in the present study, microarray analysis revealed *MDR1* to be up-regulated in isolate S2 by between 2- and 4-fold. This observation was confirmed by real-time RT-PCR. This is modest in comparison to isolates in which *MDR1* has been shown to contribute to fluconazole resistance. Such isolates exhibit increased expression of *MDR1* on the order of 100-fold. It is therefore unlikely that enhanced expression of *MDR1* contributes significantly to fluconazole resistance in isolate S2. Moreover, as *MDR1*-mediated resistance appears to be specific for fluconazole, but not other ergosterol biosynthesis inhibitors, it is unlikely that it contributes to the *UPC2*-dependent changes in

susceptibility to terbinafine observed in the present study. Although we were unable to identify an SRE motif in the promoter region of *MDR1*, these data suggest that Upc2p may play a role in the regulation of *MDR1* expression.

Another gene that was highly up-regulated by the G648D mutation in Upc2p is *PGA10*, a gene required for hemoglobin-iron utilization (183). *FTH1*, a gene homologous to *ScFTH1* that encodes a putative high affinity iron transporter, was also overexpressed. Several other iron permease genes were overexpressed 1.5- to 40-fold in either isolate S2 or strains expressing the *UPC2*^{S2-1} allele, including *FET34*, *FRE5*, *FRE10*, and *FTR1*. *FRE10* and *FTR1* were up-regulated in S2 whereas *FRP1* was up-regulated in transformants expressing the *UPC2*^{S2-1} allele. *FET34* appears to be up-regulated in both cases, because *FET34* and *FET5* are two alleles of *FET34* according to the Candida Genome Database (CGD; <http://www.candidagenome.org>). A previous study determined that under iron deprivation conditions, *C. albicans* down-regulates *ERG11* and is more susceptible to fluconazole, cycloheximide, nystatin, and anisomycin. The down-regulation of *ERG11* decreases the amount of membrane ergosterol, causing decreased membrane fluidity and increased drug susceptibility (184). In the present study, the expression of *ERG11* seems to be coordinately regulated with the genes that transport iron into cells, even though the iron uptake genes do not contain an SRE in the promoter. The *ERG11* gene product, lanosterol demethylase, has a heme molecule in the active site that is required in order for it to bind molecular oxygen. Because iron is central to this heme, up-regulation of *ERG11* may necessitate an increase of iron uptake. It is tempting to speculate that differential expression of these iron metabolism genes may be a direct result of altered ergosterol production, and not directly regulated by Upc2p.

In the azole resistant isolate S2, the most highly up-regulated gene was *DDR48* which was also found to have a putative SRE in its promoter region. Previous studies have shown *DDR48*, a gene associated with cell stress response, to be differentially expressed in resistant isolates overexpressing *CDR1*, *CDR2*, and *MDR1* (172, 178). It has also been found to be overexpressed in response to fluconazole, itraconazole, ketoconazole, AmB, and caspofungin treatment (48, 174, 182). *DDR48* was also found to be up-regulated under hypoxic conditions (185). Interestingly, *DDR48* was also one of the 41 genes up-regulated in the SC5314 *upc2Δ/upc2Δ* disruption mutants. While the biological role of *DDR48* requires further study, our data suggest it may be both positively and negatively regulated by Upc2p.

As mentioned above, *ScUPC2* has been implicated in the uptake of exogenous sterols. Sterol uptake is mediated by an ABC transporter, *ScaUS1* (186). In *C. albicans*, *ScaUS1* is homologous to a number of ABC transporters including *CDR1* and *CDR2*. However, the actual mechanism of sterol uptake in *C. albicans* is unknown. By microarray analysis we found genes encoding ABC transporters to be up-regulated by the *UPC2* gain-of-function mutation. In the resistant isolate S2, the ABC transporter gene *CDR12* is up-regulated 1.5- to 3.0-fold, and in SC5314 transformants expressing the *UPC2*^{S2-1} allele the ABC transporter gene *CDR11* is up-regulated 1.5- to 3.5-fold. According to the CGD, *CDR11* and *CDR12* are two different alleles of the gene *CDR11*. Because of the homology of *CDR11* to *ScaUS1*, Cdr11p is a strong candidate for the

transporter that mediates the uptake of exogenous sterols in *C. albicans*. This hypothesis is currently under investigation.

CHAPTER 5: DISCUSSION

With advancements such as highly active antiretroviral therapy (HAART), the morbidity and mortality of *Candida* infections have decreased. Incidences of OPC have decreased especially in developed countries where newer drugs are available (187, 188). However, in developing nations, the cost of the drugs and the long term course of treatment become prohibitive and impractical (7, 8). Without effective HAART, HIV patients develop OPC at pre-HAART rates (189). In these situations, OPC is still often treated with azole antifungals, and the development of resistance remains a problem.

Because azoles are fungistatic, the development of resistance can occur, especially when treatment spans a long period of time. In *C. albicans*, several common resistance mechanisms can develop through antifungal treatment. The azoles bind lanosterol demethylase, a cytochrome P-450 enzyme essential to the synthesis of ergosterol in the cell. One resistance mechanism occurs through mutations in *ERG11*, the gene that encodes lanosterol demethylase. Several nonsynonymous mutations have been identified in *ERG11* that affect drug resistance (44, 45, 46). Mutations in *ERG11* change the conformation or spatial structure of Erg11p, altering the enzyme-drug interaction without necessarily changing the enzyme function. Another mechanism of resistance is the overexpression of the azole target gene *ERG11* (27, 43, 168). By increasing the amount of drug target, fewer enzymes are bound by the drug. In order to achieve the same level of inhibition, the dosage of the drug would need to be increased. In *S. cerevisiae*, the regulation of ergosterol biosynthesis genes occurs through the zinc cluster transcription factors ScUpc2p and ScEcm22p (81). One homologous gene *UPC2* is present in *C. albicans*, and Upc2p regulates ergosterol genes in *C. albicans* (82, 90).

The major mechanism of azole resistance involves active efflux of the drug from the cell. The two major classes of efflux pumps in *C. albicans* are the ABC transporters and the MFS transporters. The ABC transporters responsible for azole drug resistance are Cdr1p and Cdr2p (32, 35, 37, 38, 51). The expression and regulation of *CDR1* and *CDR2* have been identified to be determined by the zinc cluster transcription factor Tac1p (88, 89). Gain-of-function mutations in *TAC1* result in azole resistance. One hyperactive allele of *TAC1* is insufficient to cause azole resistance. In high level azole resistance, two hyperactive *TAC1* alleles are present due to the loss of heterozygosity. *TAC1* is found on chromosome 5 near the mating type locus (*MTL*). Recent genetic studies have shown that the loss of heterozygosity of the *MTL* may be the mechanism by which *TAC1* alleles become homozygous, but the homozygosity of the *MTL* is not sufficient in itself to cause resistance (89). The overexpression of the MFS transporter Mdr1p also confers resistance (21, 35, 39, 43, 72, 168). *C. albicans MDR1* is not to be confused with the human *MDR1*, an ABC transporter. The substrate also differs between *C. albicans* Mdr1p and Cdr1p/Cdr2p. The *CDR* efflux pumps have the ability to transport all azole antifungals; Mdr1p is specific to fluconazole. The bZip transcription factor Cap1p was originally thought to be responsible for the expression of *MDR1* in resistance. However, deletion of *CAP1* in a resistance isolate overexpressing *MDR1* does

not abrogate *MDR1* gene expression. The deletion of *CAP1* also does not prevent the induction of *MDR1* in response to benomyl exposure (83, 84).

In *S. cerevisiae*, transcriptional regulators of drug resistance have been shown to be co-regulated with their target genes as well as autoregulated (75, 76, 78). As is the case with *S. cerevisiae*, we hypothesized that the azole resistance mechanisms developed in clinical isolates of *C. albicans* is due to gain-of-function mutations in a transcriptional regulator. Using custom Affymetrix microarrays specific to *C. albicans*, we were able to examine the genome-wide expression profile of clinical matched susceptible and resistant isolates. By studying matched isolates, we were able to identify specific changes that occurred between susceptible and resistant isolates. Because of the precedence of transcription factors being differentially expressed in *S. cerevisiae*, we looked for putative transcriptional regulators in our co-regulated genes.

Our first objective was to better characterize the genes co-regulated with *CDR1* and *CDR2* using our new microarrays designed from the newly completed *C. albicans* genome sequencing project. Previous studies have shown several genes consistently co-regulated with the expression of *CDR1* and *CDR2*. With the new arrays, we confirmed the co-regulation of *CDR1/CDR2* with previously identified genes. In addition, we also discovered the co-regulation of the zinc cluster transcription factor Tac1p. Although Tac1p had been shown to be the transcriptional regulator of *CDR1* and *CDR2*, it had not been shown to be autoregulated. Using expression profiling of four matched isolates from four different patients in conjunction with a *tac1Δ* mutant in a resistant isolate, we were able to narrow down the list of genes considered to be Tac1p dependent. The development of a Tac1p ChIP-chip assay allowed us to examine the genes whose promoters were directly bound by Tac1p. Tac1p-HA₃ binding was found to occur in both inducing (mutant *TAC1* allele) and noninducing (wild-type *TAC1* allele) conditions. This binding occurred independent of the HA₃ tagging as no gain- or loss-of-function mutation was detected by the Raymond lab. These data suggest that Tac1p is constitutively bound to the promoter region of the target genes. The gain-of-function mutation does not increase affinity of binding or act as a nuclear localization signal. Instead, the mutation could prevent the binding of a repressor element or act as an activator of transcription. By utilizing both expression and location profiling, we could identify the co-regulated genes that were directly bound by Tac1p (Figure 5-1).

Interestingly, there were genes that were up-regulated in the presence of the gain-of-function allele whose expression was abolished in the absence of *TAC1* but whose promoters were not bound by Tac1p. These data suggest the possibility of another transcriptional factor involved in gene regulation. Because Tac1p is a zinc cluster transcription factor, it binds to the DRE motif as homo- or hetero-dimers (64). The genes that are dependent on the presence of *TAC1* for expression could require a Tac1p hetero-dimer to bind to the promoter motif. Conversely, there were genes whose promoters were bound by Tac1p but the genes were not differentially expressed in resistant isolates. This suggests that Tac1p also acts as a transcriptional regulator for other cellular functions.

Figure 5-1. Proposed model for Tac1p-mediated fluconazole resistance in *C. albicans*.

(A) Schematic of a fluconazole susceptible isolate expressing baseline levels of Cdr1p. Tac1p is constitutively bound to the DRE of target genes, including *CDR1*, *CDR2*, and *TAC1*. The lack of Tac1p-mediated transcription of *CDR1*, *CDR2*, *TAC1*, and other co-regulated genes could be due to the inefficient recruitment of an enhancer or the binding of a repressor. (B) Schematic of a fluconazole resistant cell expressing increased levels of *CDR1*, *CDR2*, *TAC1*, and co-regulated genes. The gain-of-function mutation (amino acid substitution) results in increased expression of Tac1p target genes and fluconazole resistance possibly through preventing the binding of a repressor or through the increased recruitment of enhancers or cofactors. Additional Tac1p target genes may also contribute to fluconazole resistance.

A.

B.

The gain-of-function mutation in *TAC1* in addition to the constitutive binding of Tac1p to the promoter region of its target genes warrants further study. The gain-of-function mutation does not increase binding of Tac1p to the promoter region of target genes. However, the gain-of-function mutation in *TAC1* clearly increases the expression of *CDR1* and *CDR2*. The more efficient recruitment of an enhancer or the incompatibility of a repressor due to the mutation could result in the constitutive activation of Tac1p. The identification of this cofactor could provide further insight on how *C. albicans* responds to drug stresses.

Because the identification of transcriptional regulators was possible by gene expression profiling, we next examined three clinical matched isolate pairs known to overexpress *MDR1*. Our results in Chapter 3 showed a smaller number of genes co-regulated with *MDR1*, with significant overlap between all three resistant isolates. Our experiments confirmed previously identified co-regulated genes. In addition, we found a zinc cluster transcription factor we named *MRR1*. A gain-of-function mutation was identified in each resistant allele. The deletion of *MRR1* abrogates the expression of *MDR1* in resistant isolates and in response to cerulenin, brefeldin A, hydrogen peroxide, diamide, and fluconazole. Reintegration of gain-of-function *MRR1* in wild-type SC5314 restored the overexpression of *MDR1* in response to cerulenin, brefeldin A, diamide, and fluconazole. A loss of heterozygosity of *MRR1* alleles also contributes to resistance. In the fluconazole resistant isolates, both alleles of *MRR1* carry the gain-of-function mutation. Similar to *TAC1*, both *MRR1* alleles containing the gain-of-function mutation confer higher drug resistance than heterozygous *MRR1* alleles.

A large number of genes regulated by *MRR1* are homologous to *S. cerevisiae* genes implicated in the oxidative stress response. Additionally, these *S. cerevisiae* genes have also been identified as being controlled by Yrr1p and Yrm1p, two zinc cluster transcription factors found in the PDR family of drug resistance. These genes have not been shown to be regulated by *YAP1*. The oxidative stress response in *C. albicans* has been shown to be mediated by *CAP1*. The resistance to azole antifungal agents depends partly on *CAP1*, but the disruption of *CAP1* does not abrogate the expression of *MDR1*. Our group, in collaboration with the Morschhäuser lab, has shown that *MRR1* mediates the hydrogen peroxide and benomyl response through the control of *MDR1* gene expression; the disruption of *MRR1* completely abolishes *MDR1* gene expression in the presence of hydrogen peroxide and benomyl. However, in the absence of *MRR1*, the strains retain the ability to respond to hydrogen peroxide stress (data not shown). The redundancy of these two transcriptional regulators could be a consequence of the mechanism of transcriptional activation. Cap1p binds promoter regions of genes only if the transcription factor is localized to the nucleus in response to oxidative stress. Mutations in the nuclear localization signal domain can also cause constitutive activation of the Cap1p. The mechanism of *MRR1* activation by azole antifungals is still unknown. However, a gain-of-function mutation in *MRR1* constitutively activates the transcription factor and up-regulates *MDR1*. The schematics in Figure 5-2 show the possible mechanisms by which Mrr1p transcriptional regulation occurs.

Figure 5-2. Proposed model for Mrr1p-mediated fluconazole resistance in *C. albicans*.

(A) Schematic of a fluconazole susceptible isolate with no baseline Mdr1p. The lack of Mrr1p-mediated transcription of *MDR1*, *MRR1*, and co-regulated genes could be due to the cytoplasmic localization of Mrr1p, the inactivation of Mrr1p that prevents binding to the promoter, inefficient recruitment of an enhancer, or the binding of a repressor. (B) Schematic of a fluconazole resistant isolate expressing increased levels of *MDR1*, and co-regulated genes. The gain-of-function mutation (amino acid substitution) results in increased expression of Mrr1p target genes and fluconazole resistance possibly through a) increased nuclear localization of Mrr1p, b) by preventing the binding of a repressor or c) through the increased recruitment of enhancers or cofactors. Additional Mrr1p target genes may also contribute to fluconazole resistance.

A.

B.

Further study is needed to determine the relationship between the gain-of-function mutation and the activation of *MRR1*. Mrr1p could behave similar to Tac1p in that it binds constitutively to the promoter of the target genes. However, the gain-of-function mutation could result in increased affinity of the protein to the binding site. Mrr1p location profiling would allow us to study the binding of Mrr1p to promoter regions of *MDR1* co-regulated genes. By finding a core set of genes that are bound directly by Mrr1p, a binding motif can also be defined. Another important study would be to examine the interaction between *MRR1* and *CAP1* regulon. As it stands, *MRR1* seems to be responsible for drug resistance while *CAP1* is responsible for the oxidative stress response. However, these two transcription factors have overlapping functions and how they interact requires further study.

In our collection of clinical matched isolate pairs, we had a susceptible and resistant isolate which did not express known resistance genes (*CDR1*, *CDR2*, *MDR1*). Using genome-wide expression profiling, we looked to characterize the resistance mechanisms. Because high level azole resistance is most likely due to gain-of-function mutations in a *trans*-regulatory element, we examined the gene expression profile for putative transcriptional regulators. The resistant isolate overexpressed a large number of ergosterol biosynthesis genes including *ERG11*. Additionally, we found *UPC2* up-regulated in conjunction with the *ERG* genes (Figure 5-3). Sequencing of the *UPC2* alleles identified a gain-of-function mutation identical to the mutation found in *ScUPC2* (*upc2-1*) and *ScECM22*. The gain-of-function mutation reintegrated in a susceptible isolate conferred fluconazole resistance and increased the expression of *ERG* genes. The *UPC2* disruption strains conferred hypersusceptibility to fluconazole.

Interestingly, fluconazole resistance in clinical isolates as a result of gain-of-function mutations in *UPC2* is unlike the gain-of-function mutations in *TAC1*. One mutant allele of *UPC2* is sufficient in conferring fluconazole resistance in this clinical isolate. Loss of heterozygosity in addition to the gain-of-function mutations in the *TAC1* alleles contributed to high level azole resistance in fluconazole resistant clinical isolates. One *MRR1* allele containing a gain-of-function mutation is also sufficient in conferring azole resistance (Morschhäuser lab, personal communication), although the resistant isolates used in our study demonstrated a loss of heterozygosity and higher levels of azole resistance. It is possible that two mutant *UPC2* alleles could confer higher levels of azole resistance; however, we did not examine this possibility at this time.

Another gene of interest identified in this study was the gene encoding the ABC transporter *CDR11*. In our microarray data, we found the resistant clinical isolate to overexpress the ABC transporter Cdr12p; in the reintegrated gain-of-function mutant, Cdr11p was up-regulated. According to the newly assembled *Candida* genome database (CGD), *CDR11* and *CDR12* are actually two alleles of the *CDR11* gene. This ABC transporter may be responsible for uptake of exogenous sterols. In *S. cerevisiae*, the ABC transporter *ScaUS1* has been implicated in the utilization of exogenous sterols under sterol starvation conditions. While sterol uptake in *C. albicans* has not been well characterized, previous studies have shown that sterol uptake is possible (90). The expression of different *CDR11* alleles in response to the same *UPC2* mutation could be

Figure 5-3. Proposed model for Upc2p-mediated fluconazole resistance in *C. albicans*.

(A) Schematic of a fluconazole susceptible isolate with baseline ergosterol biosynthesis gene expression. The lack of overexpression of *ERG11*, *UPC2*, and other ergosterol biosynthesis genes could be due to the localization of Upc2p to the membrane of the endoplasmic reticulum (ER) or the binding of a repressor. (B) Schematic of a fluconazole resistant cell expressing increased levels of *ERG11*, other ergosterol biosynthesis genes, and other Upc2p target genes. The gain-of-function mutation (amino acid substitution) results in increased expression of Upc2p target genes and fluconazole resistance possibly through a) increased nuclear localization of Upc2p, b) by preventing the binding of a repressor or c) through the increased recruitment of enhancers or cofactors. Additional Upc2p target genes may also contribute to fluconazole resistance.

due to the background strain. The resistant clinical isolate and the genome strain SC5314 are not isogenic, so different *CDR11* alleles may be present in different *C. albicans* strains.

Further study is necessary to determine if *CDR11* is in fact responsible for the uptake of exogenous sterols by *C. albicans*. Because the *UPC2* gain-of-function mutation in *C. albicans* is the same as the *UPC2* and *ECM22* gain-of-function mutations in *S. cerevisiae* and the gain-of-function mutations in *S. cerevisiae* confers sterol uptake under aerobic conditions, the same phenotype may occur in *C. albicans*. The loss of heterozygosity of *UPC2* alleles in the development of azole resistance also requires further study. Our resistant clinical isolate only contained one gain-of-function mutation allele. Currently, no isolates have been characterized to contain two homozygous gain-of-function mutations in *UPC2*.

By studying matched susceptible and resistant clinical isolates, we were able to identify genes specific to the development of resistance. We successfully identified several gain-of-function mutations in transcriptional regulators of clinical azole resistance, and characterized core genes affected by these specific mutations. We have also identified a previously uncharacterized transcriptional regulator of *MDR1*. This transcription factor, Mrr1p, was also shown to contain gain-of-function mutations that constitutively activate its target genes conferring high level azole resistance. We also identified gain-of-function mutations in a known transcriptional regulator in a clinical matched set with an undefined resistance mechanism. These results demonstrate the importance of using genome-wide analysis to identify gain-of-function mutations in zinc cluster transcription factors in the development of azole resistance in *C. albicans*.

The discovery and identification of gain-of-function mutations in the transcriptional regulators of fluconazole resistance, combined with known azole resistance mutations in the *ERG11* gene itself, may allow for the development of more efficient diagnostic tools to better predict clinical success of azole therapy against a specific isolate. Theoretically, if all (or most) relevant mutations that confer azole resistance were identified in *C. albicans*, single nucleotide polymorphism (SNP) detection assays could be developed to quickly identify the presence of these mutations in clinical isolates. If such an assay could be created, it is quite possible that such genetic information would better correlate with clinical outcomes as compared to in vitro susceptibility testing. Moreover, the speed and efficiency with which such assays could be performed would allow for quicker initiation of optimal antifungal therapy. Major challenges will be the ability to first identify the majority of mutations that contribute to azole resistance in the overall clinical isolate population and the current cost of routine use of such technology.

The discovery of the transcription factors that regulate the major resistance mechanisms in *C. albicans* also has significant therapeutic ramifications. Tac1p, Mrr1p, and Upc2p are all zinc cluster transcription factors which are a class of zinc finger proteins unique to fungi. If inhibitors of this fungal-specific class of proteins could be identified, such compounds could be used in combination with the azole antifungals to

inhibit expression of azole resistance genes, or to prevent the development of azole resistance from the time of initiation of therapy.

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APPENDIX A: *TAC1* SUPPLEMENTAL DATA

Table A-1. Genes up-regulated in at least one matched clinical isolate.

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.6229	<i>CAT1</i>	<i>CTA1</i>	Catalase activity	1.7	1.1	-1.0	-1.1	-1.1
orf19.3682	<i>CWH8</i>	<i>CWH8</i>	Pyrophosphatase activity	2.0	1.1	-1.0	1.1	-1.1
orf19.7056	--	<i>DIP53.exon2</i>	Amino acid permease activity	2.1	-1.0	1.2	2.6	1.1
orf19.781	--	<i>DUR31</i>	Urea transporter activity	2.0	-1.4	-1.0	1.4	1.6
orf19.5017	--	<i>DUR32</i>	Urea transporter activity	1.8	-1.4	-1.2	1.2	1.1
orf19.767	<i>ERG3</i>	<i>ERG3</i>	C-5 sterol desaturase activity	1.5	1.1	1.4	-1.2	1.0
orf19.1631	<i>ERG6</i>	<i>ERG6</i>	Sterol 24-C-methyltransferase activity	1.8	1.2	1.4	-1.7	-1.3
orf19.460	<i>CEK2</i>	<i>ERK2</i>	MAP kinase activity	1.8	1.2	1.8	1.7	2.8
orf19.4015	<i>CAG1</i>	<i>GBA1</i>	GTPase activity	30.8	1.1	-1.3	2.3	30.8
orf19.1392	--	<i>IPF11068</i>	Protein disulfide isomerase activity	1.6	1.1	-1.6	-1.1	1.0
orf19.1616	<i>FGR23</i>	<i>IPF12262</i>	--	2.3	1.3	1.1	8.4	19.6
orf19.3180	--	<i>IPF12790</i>	Inositol-polyphosphate 5-phosphatase activity	1.9	1.0	1.4	1.5	1.2
orf19.2646	<i>ZCF13</i>	<i>IPF13024</i>	Specific RNA polymerase II transcription factor activity	1.7	1.4	1.2	2.8	-1.3
orf19.209	<i>DFG10</i>	<i>IPF13438</i>	--	1.7	-1.0	1.0	1.2	-1.0
orf19.3380	<i>HWP2</i>	<i>IPF14331</i>	--	1.5	1.2	1.3	-2.1	2.1
orf19.3586	--	<i>IPF14773.exon2</i>	--	1.5	-1.2	1.0	1.1	-1.3
orf19.4134	--	<i>IPF14985</i>	--	1.5	1.2	1.2	1.4	-1.1
orf19.4795	--	<i>IPF16501</i>	--	1.8	1.0	-2.1	5.9	2.5
orf19.720	<i>GST3</i>	<i>IPF17507</i>	Transcription corepressor activity	1.6	-1.2	-1.2	2.2	1.0
orf19.6469	--	<i>IPF19295.3f</i>	--	1.8	-1.0	-1.1	-1.1	1.9
orf19.5194.1	--	<i>IPF20014</i>	--	1.5	-1.1	1.3	-1.0	1.2
orf19.3563	--	<i>IPF3189</i>	--	1.8	-1.3	-1.1	2.5	1.1
orf19.7522	--	<i>IPF364</i>	Arylformamidase activity	1.6	1.2	1.1	-1.1	1.1
orf19.296	--	<i>IPF3833</i>	--	1.6	-1.1	1.4	1.3	1.7
orf19.538	--	<i>IPF4293</i>	--	1.7	1.0	1.4	-1.2	-1.1
orf19.6534	--	<i>IPF5158</i>	--	1.7	1.0	1.0	5.3	1.1
orf19.5655	--	<i>IPF5268.exon2</i>	--	1.7	-1.2	1.1	15.0	1.7
orf19.7105	<i>FAR1</i>	<i>IPF568</i>	Cyclin-dependent protein kinase inhibitor activity	16.9	1.1	1.5	1.3	12.0
orf19.5952	--	<i>IPF65</i>	--	2.0	-1.5	-2.0	2.5	-1.5
orf19.6468	--	<i>IPF6617.repeat1</i>	--	2.0	-1.7	1.5	-1.1	1.1
orf19.1171	--	<i>IPF7635</i>	--	1.8	-2.0	1.2	2.8	1.3
orf19.5342	--	<i>IPF768</i>	--	2.3	1.0	1.4	1.4	1.5
orf19.31	--	<i>IPF8878</i>	Structural constituent of cell wall	2.0	-1.3	-1.3	1.8	2.1
orf19.2836	--	<i>IPF9098</i>	--	1.9	-1.6	3.7	2.5	1.9
orf19.3713	--	<i>IPF9211.5f</i>	--	1.7	-1.2	1.3	7.2	1.2
orf19.2494	<i>STE3</i>	<i>IPF9230</i>	Mating-type a-factor pheromone receptor activity	4.1	-1.4	1.8	11.4	5.4
--	--	<i>IPF9851</i>	--	7.1	1.9	2.9	18.5	12.0

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.5172	<i>LIP9</i>	<i>LIP9.exon2</i>	Lipase activity	2.1	1.1	1.8	4.5	1.1
orf19.7420	--	<i>MED6</i>	RNA polymerase II transcription mediator activity	1.7	-1.0	1.2	1.1	-1.1
orf19.3197	<i>PAP1</i>	<i>PAP12</i>	RNA binding	1.8	-1.0	1.2	1.1	2.1
orf19.4599	<i>PHO89</i>	<i>PHO89</i>	High affinity phosphate transporter activity	1.6	-1.6	-1.6	-2.3	-1.1
orf19.3199	<i>PIKA</i>	<i>PIK1</i>	1-phosphatidylinositol 4-kinase activity	2.0	1.1	-1.1	1.3	2.3
orf19.756	<i>SAP7</i>	<i>SAP7</i>	Aspartic-type endopeptidase activity	2.7	-1.1	1.1	-1.4	-1.1
orf19.3893	<i>SCW11</i>	<i>SCW11.3eoc</i>	Glucan 1,3-beta-glucosidase activity	1.6	1.2	-1.0	1.1	-1.1
orf19.2179	<i>SIT1</i>	<i>SIT1</i>	Ferrichrome transporter activity	1.6	-1.3	-1.7	1.1	1.2
orf19.5820	<i>UGA6</i>	<i>UGA6.3eoc</i>	L-gamma-aminobutyric acid transporter activity	3.8	2.0	2.0	2.6	1.5
orf19.1163	--	<i>IPF13079</i>	--	-1.2	1.5	1.0	-1.0	-1.1
orf19.7034	--	<i>IPF3069</i>	--	-1.1	1.6	1.2	1.1	1.7
orf19.4608	<i>PDC12</i>	<i>PDC12.exon2</i>	Pyruvate carboxylase activity	1.2	2.1	1.3	3.7	-1.1
orf19.3796	--	<i>RNT1</i>	Ribonuclease III activity	-1.2	1.7	-1.5	1.1	1.1
orf19.6053	<i>CIS2</i>	<i>CIS2</i>	Protein-glutamine gamma-glutamyltransferase activity	1.2	1.3	1.9	1.1	1.3
orf19.7652	<i>CKA1</i>	<i>CKA1</i>	Protein kinase CK2 activity	1.1	1.2	1.9	-1.1	-1.1
orf19.1448.1	--	<i>DYN2.3</i>	--	-1.0	1.1	1.7	1.3	-1.5
orf19.5535	--	<i>FEN2</i>	Pantothenate transporter activity	1.6	1.4	1.7	-1.0	1.1
orf19.4182	--	<i>IPF11898</i>	--	1.1	1.1	1.5	-1.2	-1.1
orf19.3466	--	<i>IPF13112</i>	--	-1.0	-1.2	1.6	2.4	-1.3
orf19.1804	--	<i>IPF14864</i>	Transporter activity	-1.3	1.0	1.5	1.2	-1.5
orf19.1177	--	<i>IPF15134</i>	--	1.1	1.2	1.5	-1.1	1.5
orf19.4884	<i>WOR1</i>	<i>IPF15255</i>	Transcription regulator activity	2.1	2.2	2.3	2.4	1.2
orf19.4159	--	<i>IPF15660</i>	Transporter activity	-1.0	1.2	1.6	-1.7	-1.5
orf19.7468	--	<i>IPF2511</i>	--	1.0	1.0	1.7	1.8	-1.2
orf19.7456	--	<i>IPF2903</i>	--	1.3	1.5	2.2	4.4	1.1
orf19.5523	--	<i>IPF4325</i>	--	-1.2	-1.0	1.7	1.2	-1.0
orf19.5276	--	<i>IPF4708</i>	--	-1.0	1.3	1.6	1.7	-1.3
orf19.3615	--	<i>IPF5192</i>	--	1.0	1.2	1.5	2.1	1.1
orf19.1109	--	<i>IPF6338</i>	--	-1.3	-1.1	1.6	-1.5	-1.6
orf19.480	--	<i>IPF7097</i>	--	1.0	1.0	1.5	-1.0	-1.4
orf19.2104	--	<i>JAC1</i>	Chaperone binding	-1.1	1.0	1.6	-1.1	-1.1
orf19.6044	--	<i>MOB2</i>	Protein kinase activator activity	1.0	1.1	1.5	-1.2	-1.0
orf19.6041	<i>RPO41</i>	<i>RPO41</i>	DNA-directed RNA polymerase activity	-1.4	1.1	1.5	-2.1	-1.2
orf19.514	<i>SNP3</i>	<i>SNP3</i>	RNA splicing factor activity, transesterification mechanism	-1.1	1.0	1.7	-1.3	-2.4
orf19.854	--	<i>UGA11.exon2</i>	4-aminobutyrate transaminase activity	1.1	1.1	1.8	-1.2	1.1
orf19.7436	<i>AAF1</i>	<i>AAF1</i>	--	1.0	1.2	1.2	1.5	1.5
orf19.3417	<i>ACF2</i>	<i>ACF2</i>	Glucan 1,3-beta-glucosidase activity	1.3	1.1	1.1	1.9	1.1
orf19.2608	<i>ADH5</i>	<i>ADH5</i>	Alcohol dehydrogenase activity	1.3	1.2	1.4	1.7	1.2
orf19.4679	<i>AGP2</i>	<i>AGP2</i>	Hydrogen:amino acid symporter activity	1.4	1.3	1.4	1.8	-1.1
orf19.5742	<i>ALS9</i>	<i>ALS11.3f</i>	--	1.5	1.3	1.1	2.7	2.9
orf19.7400	<i>ALS7</i>	<i>ALS7</i>	Carbohydrate binding	1.0	-1.0	1.0	1.5	1.5

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.4413	<i>CMD1</i>	<i>CMD1</i>	Calcium ion binding	1.0	1.1	1.2	1.7	-1.2
orf19.4555	<i>ALS4</i>	<i>CPS2.3f</i>	Gly-X carboxypeptidase activity	1.1	1.2	1.2	1.6	1.3
orf19.4610	--	<i>CPS2.5f</i>	Gly-X carboxypeptidase activity	1.2	-1.0	1.2	1.9	1.5
orf19.6755	--	<i>DLD2</i>	D-lactate dehydrogenase (cytochrome) activity	1.3	1.2	1.1	2.4	1.5
orf19.780	<i>DUR1,2</i>	<i>DUR1,2</i>	Allophanate hydrolase activity	-1.1	1.1	1.1	1.9	2.8
orf19.5999	<i>DYN1</i>	<i>DYN1</i>	Minus-end-directed microtubule motor activity	1.1	1.1	1.0	2.9	2.7
orf19.7495	--	<i>EBP6</i>	NADPH dehydrogenase activity	-1.1	-1.2	2.3	3.5	-1.0
orf19.5816	<i>EBP7</i>	<i>EBP7</i>	NADPH dehydrogenase activity	1.2	1.1	-1.1	2.4	1.4
orf19.7379	<i>FAA2</i>	<i>FAA22</i>	Long-chain-fatty-acid-CoA ligase activity	1.4	1.1	1.3	5.1	-1.3
orf19.3653	<i>FAT1</i>	<i>FAT1</i>	Long-chain-fatty-acid-CoA ligase activity	1.1	1.1	1.2	1.5	-1.1
orf19.6178	<i>FBP1</i>	<i>FBP1</i>	Fructose-bisphosphatase activity	1.2	1.1	1.3	1.8	1.2
orf19.1153	<i>GAD1</i>	<i>GAD1</i>	Glutamate decarboxylase activity	1.0	-1.1	-1.0	2.0	1.1
orf19.5880	--	<i>GEF1.3f</i>	Voltage-gated chloride channel activity	1.3	1.2	1.1	1.6	1.6
orf19.734	<i>GLK1</i>	<i>GLK1</i>	Glucokinase activity	1.2	1.2	1.1	1.5	1.2
orf19.4309	<i>GRP2</i>	<i>GRP2</i>	Oxidoreductase activity	-1.1	1.2	1.3	3.1	1.4
orf19.5059	<i>GCS1</i>	<i>GSH1.exon1</i>	Glutamate-cysteine ligase activity	1.0	1.0	1.3	2.7	1.4
orf19.7419	<i>HNT2</i>	<i>HNT2</i>	Hydrolase activity	1.4	1.2	1.3	1.9	1.2
orf19.6323	--	<i>HPA3</i>	Histone acetyltransferase activity	1.4	1.3	1.4	3.0	1.1
orf19.1191	--	<i>HRD3</i>	Ubiquitin-protein ligase activity	1.2	1.2	-1.1	1.6	1.7
orf19.931	--	<i>IFA13</i>	--	1.0	1.2	1.3	2.3	1.1
orf19.996	--	<i>IFA15</i>	Adenylate cyclase activity	-1.0	-1.1	1.4	5.2	2.3
orf19.3919	--	<i>IFA23</i>	Cysteine-type endopeptidase activity	1.0	-1.0	-1.0	1.6	1.5
orf19.570	<i>IFF8</i>	<i>IFF8</i>	--	1.4	-1.2	1.4	1.6	1.4
orf19.428	--	<i>IKS1</i>	Protein kinase activity	1.2	-1.1	-1.0	1.6	1.1
orf19.4590	--	<i>IPF1009</i>	--	-1.1	1.7	1.3	2.1	-1.5
orf19.4575	--	<i>IPF1031</i>	--	1.3	-1.0	1.0	2.0	-1.1
orf19.3364	--	<i>IPF10394</i>	--	-1.1	-1.3	1.1	2.6	-1.3
orf19.424	<i>TRP99</i>	<i>IPF10482.exon1</i>	Alkyl hydroperoxide reductase activity	1.3	1.1	-1.5	1.5	-1.1
orf19.1287	--	<i>IPF10595</i>	--	-1.7	1.1	1.1	12.0	-1.3
orf19.5413	--	<i>IPF1104</i>	--	1.1	1.1	1.2	1.6	-1.1
orf19.1421	--	<i>IPF11234</i>	Ureidoglycolate hydrolase activity	1.8	1.2	-2.2	2.9	1.0
orf19.3661	--	<i>IPF11332</i>	Ubiquitin-specific protease activity	1.1	1.1	1.3	2.0	1.1
orf19.5295	--	<i>IPF11876</i>	--	1.4	1.4	2.0	4.5	-1.3
orf19.4234	--	<i>IPF11954</i>	--	1.1	1.1	1.0	1.8	-1.0
orf19.5644	--	<i>IPF12127</i>	--	1.1	1.4	1.2	2.0	1.5
orf19.3872	--	<i>IPF12179</i>	--	1.2	1.1	1.1	1.7	-1.3
orf19.3508	--	<i>IPF12233</i>	--	1.1	1.1	1.2	2.3	1.1
orf19.5070	--	<i>IPF12312</i>	--	1.2	2.9	1.7	11.0	1.3
orf19.7344	--	<i>IPF12537</i>	--	2.0	-1.4	1.5	3.3	1.8
orf19.2515	--	<i>IPF12799</i>	--	1.3	-1.1	-1.2	1.9	1.6
orf19.1162	--	<i>IPF13081</i>	--	-1.1	1.2	-1.3	1.9	1.9

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.1181	--	<i>IPF13097</i>	Phosphatidate phosphatase activity	3.6	-1.4	-1.0	11.3	14.4
orf19.5729	<i>FGR17</i>	<i>IPF13158</i>	Transcription factor activity	1.1	1.1	1.1	2.1	-1.3
orf19.6420	<i>PGA13</i>	<i>IPF1341</i>	--	1.3	-1.1	-1.1	4.9	-1.7
orf19.1656	--	<i>IPF13675</i>	Phosphatidylinositol binding	-1.0	-1.2	1.5	2.0	-1.2
orf19.6443	--	<i>IPF1380</i>	Dodecenoyl-CoA delta-isomerase activity	-1.2	-1.1	1.1	2.1	2.2
orf19.2344	<i>ASR1</i>	<i>IPF13836</i>	--	2.8	1.8	1.4	18.3	1.7
orf19.1120	<i>FAV2</i>	<i>IPF13879</i>	--	1.4	1.1	1.4	2.3	-1.8
orf19.914	--	<i>IPF14094.repeat4</i>	--	1.1	-1.0	1.1	1.5	-1.3
orf19.3586	--	<i>IPF14773</i>	--	1.4	-1.1	-1.2	1.8	1.1
orf19.4376	--	<i>IPF15098</i>	--	1.2	1.1	1.6	3.4	1.2
orf19.2607	--	<i>IPF15672</i>	--	1.4	-1.1	-1.3	1.9	-1.2
orf19.972	--	<i>IPF1588</i>	--	1.2	1.3	1.1	1.9	-1.5
orf19.1946	--	<i>IPF16194</i>	--	1.3	1.2	-1.1	1.8	-1.0
orf19.3922	--	<i>IPF16228</i>	Nucleotidase activity	1.1	-1.1	1.0	2.0	1.1
orf19.3932	--	<i>IPF16253</i>	--	1.2	1.2	-1.0	6.4	-1.2
orf19.5877	--	<i>IPF1837</i>	Alcohol O-acetyltransferase activity	1.3	1.2	-1.1	2.8	1.2
orf19.5620	--	<i>IPF18418</i>	--	1.3	1.5	1.1	2.3	-1.2
orf19.5727	--	<i>IPF18553</i>	--	1.5	1.1	-1.0	2.1	-1.5
orf19.2737	--	<i>IPF18712</i>	--	1.4	1.4	1.4	2.8	1.0
orf19.711	--	<i>IPF18732</i>	--	1.4	1.4	1.2	3.0	1.3
orf19.7317	<i>UGA33</i>	<i>IPF1960.5f</i>	Transcription factor activity	1.3	-1.1	1.1	1.6	1.0
orf19.7322	--	<i>IPF1969</i>	--	-1.1	1.1	1.2	1.8	-1.2
orf19.5517	--	<i>IPF20104</i>	Alcohol dehydrogenase (NADP+) activity	1.2	1.1	1.2	1.7	-1.2
orf19.4077	<i>MIT1</i>	<i>IPF2067</i>	Mannosyltransferase activity	1.0	1.2	1.1	1.8	2.1
orf19.5980	--	<i>IPF21</i>	--	1.4	1.1	-1.1	2.0	1.2
orf19.6662	--	<i>IPF2287</i>	--	1.1	-1.1	1.0	3.2	-1.8
orf19.2158	<i>NAG3</i>	<i>IPF2710.repeat2</i>	Monosaccharide transporter activity	-2.4	-1.7	-1.5	2.4	6.1
orf19.7297	--	<i>IPF2837</i>	--	1.1	1.2	-1.1	2.1	1.1
orf19.3273	--	<i>IPF285.3f</i>	--	1.3	-1.1	1.0	2.0	1.2
orf19.7281	--	<i>IPF2861</i>	--	1.3	1.4	1.8	12.6	3.3
orf19.4305.1	--	<i>IPF2930</i>	--	1.2	-1.2	-1.1	2.1	-1.0
orf19.3282	--	<i>IPF300</i>	--	1.0	1.1	1.3	1.5	-1.2
orf19.4438	<i>RME1</i>	<i>IPF3121</i>	Transcription factor activity	1.2	1.1	-1.3	5.7	1.7
orf19.6758	--	<i>IPF3484</i>	Aldo-keto reductase activity	2.4	-1.1	-1.5	2.9	1.1
orf19.6757	--	<i>IPF3485</i>	Aldo-keto reductase activity	1.1	1.2	1.4	2.0	-1.1
orf19.675	--	<i>IPF3964</i>	--	1.2	1.1	1.2	4.2	2.4
orf19.535	<i>RBR1</i>	<i>IPF4299</i>	--	1.3	-1.1	1.3	1.7	1.3
orf19.2204	--	<i>IPF5496</i>	--	1.3	1.1	1.1	1.8	-1.3
orf19.6325.1	--	<i>IPF5730</i>	--	1.3	1.1	1.1	1.6	-1.8
orf19.5213	--	<i>IPF5866</i>	--	1.1	1.2	1.2	2.7	1.4
orf19.7631	--	<i>IPF610</i>	--	1.1	-1.1	1.0	1.7	-1.1

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.3292	--	<i>IPF6231</i>	Protein-methionine-R-oxide reductase activity	1.2	1.0	-1.3	2.0	1.1
orf19.1461	--	<i>IPF6257</i>	--	1.2	-1.1	1.0	2.5	1.1
orf19.6318	--	<i>IPF6280</i>	--	1.3	1.1	1.1	1.6	-1.6
orf19.2724	--	<i>IPF6339</i>	--	1.2	1.3	-1.3	4.3	-1.6
orf19.384	--	<i>IPF6367</i>	--	1.2	1.2	1.3	1.7	1.6
orf19.1573	--	<i>IPF6387.3</i>	--	1.2	1.1	-2.3	1.6	-1.1
orf19.6489	<i>FMP45</i>	<i>IPF6624</i>	--	1.3	-1.4	1.2	4.8	2.2
orf19.2762	<i>AHP1</i>	<i>IPF6629</i>	Alkyl hydroperoxide reductase activity	1.2	1.2	-1.2	2.5	-1.3
orf19.7596	--	<i>IPF673</i>	--	1.7	1.0	-2.0	15.2	1.1
orf19.320	--	<i>IPF6755</i>	--	1.4	-1.1	1.3	4.6	1.0
orf19.3813	--	<i>IPF6945.3f</i>	--	1.3	-1.0	1.3	2.4	1.2
orf19.5770	<i>OPT8</i>	<i>IPF6990</i>	Oligopeptide transporter activity	-1.0	-1.1	1.1	2.0	-1.0
orf19.1562	--	<i>IPF7141</i>	--	1.2	1.4	1.4	2.3	1.2
orf19.1564	--	<i>IPF7145</i>	--	1.2	1.3	2.4	1.8	-1.2
orf19.860	--	<i>IPF7647</i>	--	1.2	1.3	1.4	2.4	1.6
orf19.2693	--	<i>IPF7968</i>	Transcription corepressor activity	-1.1	1.1	1.3	5.0	1.6
orf19.1719	<i>SGA1</i>	<i>IPF8402</i>	Glucan 1,4-alpha-glucosidase activity	1.1	1.0	1.6	2.4	-1.4
orf19.6952	--	<i>IPF8666</i>	--	1.1	1.3	1.2	1.9	1.2
orf19.4903	--	<i>IPF8860</i>	N-acetylglucosaminylphosphatidylinositol deacetylase activity	-1.1	1.1	-1.1	1.7	-1.3
orf19.5943	--	<i>IPF91</i>	--	1.2	1.1	-1.0	1.8	1.2
orf19.4370	--	<i>IPF9450</i>	--	1.1	1.1	-1.1	2.4	1.0
orf19.4911	--	<i>IPF9618</i>	--	1.2	1.1	1.0	2.3	-1.3
orf19.4593.1	--	<i>IPF995</i>	--	-1.1	-1.0	1.4	1.6	-1.5
orf19.184	--	<i>MDM10</i>	--	1.0	1.4	1.3	2.0	1.2
orf19.478	--	<i>MON1.3</i>	--	1.3	-1.1	-1.0	1.9	-2.4
orf19.4044	<i>MUM2</i>	<i>MUM2</i>	--	1.1	1.3	3.8	2.4	1.5
orf19.5005	<i>OSM2</i>	<i>OSM2</i>	Fumarate reductase (NADH) activity	1.3	-1.0	-1.3	2.0	1.2
orf19.2769	--	<i>PBI2</i>	Endopeptidase inhibitor activity	1.3	1.2	1.1	3.3	-1.2
orf19.4186	<i>PCT1</i>	<i>PCT1</i>	Choline-phosphate cytidyltransferase activity	-1.2	1.3	-1.2	1.9	1.2
orf19.2094	<i>PDR6</i>	<i>PDR6</i>	Protein carrier activity	-1.1	-1.0	-1.0	1.7	-1.1
orf19.1089	--	<i>PEX11</i>	--	-1.0	1.3	1.0	1.8	-2.4
orf19.5285	<i>PST3</i>	<i>PST2</i>	--	1.1	-1.1	1.2	2.3	1.0
orf19.6341	--	<i>RIB7</i>	--	1.1	1.0	-1.1	2.2	1.2
orf19.2087	--	<i>SAS2</i>	Acetyltransferase activity	-1.2	1.3	1.4	1.9	-1.3
orf19.2770.1	<i>SOD1</i>	<i>SOD1.3</i>	Copper, zinc superoxide dismutase activity	-1.0	1.1	-1.1	2.2	-1.1
orf19.2299	--	<i>URM1</i>	--	-1.1	1.0	1.0	1.7	1.6
orf19.5634	<i>FRP1</i>	<i>FRE5</i>	Ferric-chelate reductase activity	2.6	2.6	1.6	1.7	-1.2
orf19.2176	<i>IFM3</i>	<i>IFM3</i>	--	1.7	1.5	1.1	1.2	1.0
orf19.6219	--	<i>IPF7432</i>	--	1.8	1.5	1.1	-1.1	1.1
orf19.1878	--	<i>IPF9160</i>	RNA polymerase II transcription mediator activity	1.5	2.4	-1.0	1.0	-1.5

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.5169	--	<i>AMD21</i>	Amidase activity	1.8	-1.5	1.5	3.5	1.3
orf19.393	--	<i>APS3</i>	--	1.7	1.2	1.5	1.5	1.0
orf19.7053	<i>GAC1</i>	<i>GAC1</i>	Protein phosphatase type 1 activity	2.0	-1.2	1.7	1.0	1.5
orf19.5431	--	<i>IPF1129</i>	--	1.7	1.1	2.2	1.2	1.1
orf19.7316	--	<i>IPF5971</i>	--	1.5	1.3	1.5	1.0	-1.1
orf19.5362	<i>PSO2</i>	<i>IPF730</i>	--	6.9	-1.1	1.5	1.7	10.0
orf19.1725	--	<i>IPF9336.3eoc</i>	--	1.8	1.1	1.5	1.1	1.5
orf19.3112	<i>ZRT1</i>	<i>ZRT1</i>	Low-affinity zinc ion transporter activity	1.7	-1.6	1.5	3.9	1.2
orf19.4774	<i>AOX1</i>	<i>AOX1</i>	Alternative oxidase activity	2.2	-1.1	1.3	3.2	-1.1
orf19.4788	<i>ARG5,6</i>	<i>ARG5,6</i>	N-acetyl-gamma-glutamyl-phosphate reductase activity	1.6	1.1	2.3	2.3	-1.9
orf19.6214	<i>ATC1</i>	<i>ATH1</i>	Alpha, alpha-trehalase activity	1.8	1.3	1.1	5.1	2.6
orf19.2401	--	<i>AUT2</i>	Microtubule binding	1.5	-1.2	1.1	1.7	1.1
orf19.5604	<i>MDR1</i>	<i>BMR1</i>	Multidrug transporter activity	1.8	-1.1	1.1	1.8	1.3
orf19.113	<i>CIP1</i>	<i>CIP1</i>	--	1.5	1.2	1.4	2.2	1.7
orf19.6956	<i>DAL9</i>	<i>DAL51</i>	Allantoate transporter activity	1.9	1.1	1.2	2.9	1.6
orf19.3234	--	<i>EBP5</i>	NADPH dehydrogenase activity	1.8	1.0	1.2	2.5	-1.1
orf19.6958	<i>ECM18</i>	<i>ECM18</i>	--	2.7	1.1	-1.0	3.4	1.2
orf19.4589	--	<i>FMS1</i>	--	1.6	1.1	-1.1	1.8	-1.2
orf19.6995	--	<i>FRP7</i>	--	1.7	1.3	1.7	2.2	1.5
orf19.313	<i>DAL4</i>	<i>FUR4</i>	Allantoin permease activity	2.4	-1.2	1.3	4.5	2.1
orf19.7021	<i>GPH1</i>	<i>GPH1</i>	Glycogen phosphorylase activity	1.8	1.2	1.2	1.6	1.8
orf19.1582	--	<i>HOL5.5f</i>	Transporter activity	1.5	-1.3	1.3	5.3	6.2
orf19.1048	--	<i>IFD1</i>	Aryl-alcohol dehydrogenase activity	2.1	1.3	-1.1	3.5	1.2
orf19.3311	<i>IFD3</i>	<i>IFD3</i>	Aryl-alcohol dehydrogenase activity	1.7	-1.1	1.3	2.1	1.2
orf19.1048	<i>IFD6</i>	<i>IFD5</i>	Aryl-alcohol dehydrogenase activity	1.6	1.2	1.5	3.7	1.5
orf19.7472	<i>IFF4</i>	<i>IFF4</i>	--	2.6	1.3	1.2	3.0	-1.1
orf19.5840	--	<i>IPF11222</i>	--	1.8	1.2	1.4	2.4	-1.3
orf19.5210	--	<i>IPF11259</i>	Transcription factor activity	2.3	1.7	1.2	1.8	2.1
orf19.1405	<i>PHO13</i>	<i>IPF11589</i>	4-nitrophenylphosphatase activity	1.7	1.1	1.2	1.6	-1.8
orf19.1152	--	<i>IPF11713</i>	--	1.6	1.2	-1.3	1.7	-1.1
orf19.2076	--	<i>IPF1197</i>	--	1.7	1.1	1.1	2.3	1.6
orf19.1950	--	<i>IPF12138</i>	--	1.6	-1.0	-1.0	3.4	1.6
orf19.2064	--	<i>IPF1216</i>	Transcription factor activity	1.5	-1.1	2.7	1.7	1.5
orf19.2131	--	<i>IPF12244</i>	--	1.6	1.1	1.4	1.7	1.2
orf19.4356	<i>HGT3</i>	<i>IPF12946</i>	Glucose transporter activity	1.5	1.2	-1.0	1.8	1.3
orf19.6366	--	<i>IPF13450</i>	--	1.6	2.7	2.0	10.6	1.6
orf19.5925	--	<i>IPF138</i>	--	2.4	1.1	-1.0	3.0	1.7
orf19.5204	--	<i>IPF13971</i>	--	1.7	1.2	1.5	2.5	1.8
orf19.4688	<i>DAG7</i>	<i>IPF14119</i>	--	2.4	1.1	1.1	1.5	1.2
orf19.4132	--	<i>IPF14871</i>	--	1.6	1.3	-1.1	2.1	-1.3

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.348	--	IPF1505	--	2.6	1.2	1.2	3.7	1.8
orf19.3053	--	IPF15297	--	1.5	1.2	-1.1	1.8	1.1
orf19.3910	--	IPF15494	--	1.6	-1.3	1.1	3.0	2.2
orf19.6586	--	IPF1617	--	1.5	1.1	1.2	9.9	-1.0
orf19.69	--	IPF17131	--	1.8	1.1	1.3	5.5	1.4
orf19.5879	--	IPF1839	Acylglycerone-phosphate reductase activity	1.6	1.0	1.4	6.0	-1.0
orf19.4334	PGA58	IPF19706	--	2.3	1.3	-1.3	1.6	1.1
orf19.7288	--	IPF2852	--	1.9	-1.2	1.0	2.8	2.6
orf19.4439	--	IPF3105	--	1.8	1.2	1.0	2.5	-1.2
orf19.2870	--	IPF3250	--	1.5	-1.4	-1.2	3.3	-1.0
orf19.3839	SAP10	IPF4089	Aspartic-type signal peptidase activity	1.6	1.0	-1.3	2.5	1.6
orf19.5449	--	IPF4176	--	1.5	1.3	1.2	3.3	1.1
orf19.1632	--	IPF4256	--	2.3	1.5	1.4	2.7	-1.0
orf19.6329	--	IPF5723.exon1	--	1.6	1.0	1.3	1.6	-1.5
orf19.1307	--	IPF6678	--	6.9	-1.4	-2.3	3.5	1.2
orf19.4246	--	IPF6881	--	1.7	1.3	1.3	2.2	-1.1
orf19.2966	--	IPF7778	Carboxymethylenebutenolidase activity	1.6	1.2	1.3	2.0	1.2
orf19.6025	--	IPF7900	--	1.6	1.2	1.0	2.4	-1.3
orf19.6869	--	IPF8041	Peptidase activity	1.6	-1.1	1.6	1.6	1.9
orf19.4543	UGA2	IPF8048	Succinate-semialdehyde dehydrogenase [NAD(P)+] activity	1.8	1.4	1.1	2.7	1.1
orf19.7235	--	IPF846	--	1.6	1.4	1.2	2.0	1.2
orf19.2302	--	IPF8814	--	1.5	1.1	-1.3	2.0	1.1
orf19.7214	--	IPF885	--	2.4	1.4	1.3	4.6	1.5
orf19.4905	--	IPF8862	--	1.5	1.1	-1.4	1.7	1.2
orf19.1360	--	IPF8914	--	1.8	1.4	-1.7	8.2	4.1
orf19.7210	--	IPF893	--	1.8	-1.3	1.4	2.6	1.3
orf19.2737	--	IPF9167	--	1.6	1.3	1.4	2.3	1.2
orf19.5942	--	IPF96	--	1.6	1.4	1.1	1.7	1.5
orf19.4910	FGR41	IPF9616	--	1.5	1.2	1.3	1.6	1.1
orf19.3499	--	IPF9955	--	2.1	1.3	-1.1	4.8	-1.0
orf19.7447	JEN1	JEN1	Lactate transporter activity	1.7	-1.1	1.1	2.0	1.2
orf19.7498	LEU1	LEU1	3-isopropylmalate dehydratase activity	1.6	-1.0	1.3	1.6	1.9
orf19.4584	PHO114	PHO12	Acid phosphatase activity	1.5	-1.2	1.1	1.9	-1.1
orf19.1586	FGR22	PLC3	Phosphoinositide phospholipase C activity	1.6	1.2	-1.0	1.5	-2.4
orf19.2969	RAD16	RAD16	DNA-dependent ATPase activity	1.6	1.2	1.0	1.9	1.6
orf19.5636	RBT5	RBT5	Heme binding	2.4	1.2	1.3	3.3	1.7
orf19.5714	SAP1	SAP1	Aspartic-type endopeptidase activity	2.0	1.3	1.1	2.2	1.5
orf19.5542	SAP6	SAP6	Aspartic-type endopeptidase activity	2.1	1.1	1.1	3.6	1.2
orf19.6928	SAP9	SAP9	Aspartic-type endopeptidase activity	1.5	1.2	1.0	1.6	1.3
orf19.2812	--	SNG2	--	2.7	-1.3	1.3	3.6	1.2

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.2897	<i>SOU2</i>	<i>SOU2</i>	2,4-dienoyl-CoA reductase (NADPH) activity	1.7	1.1	1.1	1.6	1.2
orf19.4980	<i>HSP70</i>	<i>SSA4</i>	Unfolded protein binding	2.1	1.1	1.5	3.1	3.2
orf19.7637	<i>YHB4</i>	<i>YHB2</i>	Nitric oxide dioxygenase activity	1.5	-1.1	1.1	1.9	1.6
orf19.4476	--	<i>IFD6</i>	Aryl-alcohol dehydrogenase activity	1.6	4.8	2.3	2.2	1.2
orf19.1865	--	<i>IPF4059</i>	--	1.2	1.8	1.6	-1.4	-1.1
orf19.6996	--	<i>IPF4514</i>	Alpha-1,3-mannosyltransferase activity	1.1	1.6	2.1	1.1	1.0
orf19.3737	--	<i>IPF4721</i>	--	1.3	1.5	1.9	1.1	-1.2
orf19.2652	<i>TEF4</i>	<i>TEF41</i>	Translation elongation factor activity	1.3	3.7	2.9	-1.1	-2.4
orf19.3195	<i>HIP1</i>	<i>GAP7.3eoc</i>	Amino acid permease activity	1.2	1.5	1.1	1.6	1.5
orf19.3133	<i>GUT2</i>	<i>GUT2</i>	Glycerol-3-phosphate dehydrogenase activity	1.0	1.5	1.3	1.8	1.2
orf19.4763	--	<i>IPF10916</i>	--	-1.1	1.5	1.0	3.7	-1.3
orf19.1395	--	<i>IPF11059</i>	Inorganic phosphate transporter activity	1.3	1.5	-1.1	9.7	-1.4
orf19.1698	--	<i>IPF13217</i>	--	1.1	1.5	1.0	1.5	1.3
orf19.956	--	<i>IPF1566</i>	--	-1.2	1.5	1.4	1.7	1.3
orf19.5145	<i>SSP96</i>	<i>IPF18316.3f</i>	--	-1.0	1.6	-1.4	3.4	1.5
orf19.909	<i>STP4</i>	<i>IPF4553</i>	Transcription factor activity	-1.0	1.9	1.0	1.9	-1.5
orf19.2184	--	<i>IPF5534</i>	--	-1.0	1.6	1.5	1.9	1.8
orf19.3338	--	<i>IPF7374</i>	--	-1.1	2.1	2.3	5.8	1.5
orf19.4670	<i>CASS</i>	<i>IPF7414</i>	Transcription factor activity	1.3	1.6	1.5	4.2	1.9
orf19.1424	--	<i>IPF9370</i>	--	1.3	1.8	1.9	2.8	1.6
orf19.4478	--	<i>MSD1</i>	Aspartate-tRNA ligase activity	1.1	1.7	1.1	1.6	1.2
orf19.5614	--	<i>RNH1</i>	Ribonuclease H activity	-1.0	1.5	1.1	1.5	-1.0
orf19.6951	--	<i>DPL1</i>	--	1.2	1.1	1.7	2.0	1.0
orf19.932	--	<i>DRS24</i>	Phospholipid-translocating ATPase activity	1.2	1.3	1.6	1.6	1.1
orf19.629	<i>IFD7</i>	<i>IFD7</i>	Aryl-alcohol dehydrogenase activity	5.2	1.7	2.0	1.8	-1.1
orf19.2574	--	<i>IFU4</i>	--	1.2	1.0	1.5	2.2	1.8
orf19.1676	--	<i>IPF12501</i>	--	1.1	1.2	1.8	1.7	-1.3
orf19.2910	<i>PGA43</i>	<i>IPF13139</i>	--	1.3	1.1	2.2	2.2	-1.2
orf19.4347	--	<i>IPF14273</i>	Receptor signaling protein serine/threonine kinase activity	-1.8	-1.1	1.5	2.1	1.2
orf19.3461	--	<i>IPF14968</i>	--	1.1	1.5	2.4	2.6	1.1
orf19.5612	--	<i>IPF15015</i>	--	1.5	1.4	4.8	3.3	1.3
orf19.6754	--	<i>IPF3490</i>	--	-1.1	1.3	1.6	1.7	-1.1
orf19.3743	--	<i>IPF4729</i>	--	1.4	1.2	1.6	2.5	-1.0
orf19.3826	--	<i>IPF5830</i>	--	1.1	1.3	1.9	1.6	-1.2
orf19.3859	--	<i>IPF6600</i>	--	1.1	1.3	1.7	2.0	1.2
orf19.5587	--	<i>IPF6812</i>	--	1.4	1.0	1.5	3.7	-1.3
orf19.4539	--	<i>IPF8055</i>	Rho GDP-dissociation inhibitor activity	-1.1	1.1	2.3	2.1	1.5
orf19.6220.3	--	<i>MMD1.3</i>	--	1.0	1.2	1.5	1.6	1.1
orf19.5731	--	<i>PAD1</i>	Carboxy-lyase activity	1.4	1.2	2.5	3.0	-2.4
orf19.7389	<i>REV3</i>	<i>REV3.3f</i>	Zeta DNA polymerase activity	1.0	1.1	1.7	1.7	-1.0

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.732	--	<i>SOU3</i>	2,4-dienoyl-CoA reductase (NADPH) activity	1.3	1.2	1.7	2.3	-1.1
orf19.6026	<i>ERG2</i>	<i>ERG2</i>	C-8 sterol isomerase activity	1.8	3.0	2.5	1.6	-1.4
orf19.1598	<i>ERG24</i>	<i>ERG24</i>	Delta 14-sterol reductase activity	1.6	1.8	2.1	-1.2	-1.0
orf19.3421.1	--	<i>IPF17743</i>	RNA polymerase II transcription mediator activity	1.6	1.6	1.9	1.2	-1.5
orf19.2849	<i>AQY1</i>	<i>AQY1</i>	Water channel activity	3.6	2.4	1.7	8.4	2.4
orf19.3442	--	<i>EBP2</i>	NADPH dehydrogenase activity	1.7	1.5	2.1	2.4	1.4
orf19.4255	<i>ECM331</i>	<i>ECM331</i>	--	2.7	1.6	1.3	4.2	-1.4
orf19.2613	<i>ECM4</i>	<i>ECM41.3</i>	--	1.6	2.0	2.0	4.6	-1.0
orf19.2990	<i>XOG1</i>	<i>EXG1</i>	Glucan 1,3-beta-glucosidase activity	2.9	2.2	1.2	1.8	1.0
orf19.2952	<i>EXG2</i>	<i>EXG2</i>	Glucan 1,3-beta-glucosidase activity	1.5	1.5	-1.2	2.4	1.0
orf19.6659	<i>GAP6</i>	<i>GAP6</i>	Amino acid permease activity	1.6	1.5	1.2	4.0	1.1
orf19.4477	<i>CSH1</i>	<i>IFD4</i>	Aryl-alcohol dehydrogenase activity	2.9	2.3	-1.0	4.6	1.6
orf19.6678	--	<i>IFJ5</i>	--	2.1	1.5	1.2	12.3	1.6
orf19.2280	<i>ZCF10</i>	<i>IPF10079</i>	Transcription factor activity	1.6	1.8	1.5	6.1	2.1
orf19.5635	<i>PGA7</i>	<i>IPF12101</i>	--	3.2	1.7	1.5	2.8	1.0
orf19.251	--	<i>IPF17186</i>	--	2.0	1.8	1.2	4.2	1.1
orf19.2175	--	<i>IPF19998</i>	Oxidoreductase activity	2.4	1.6	1.3	3.3	1.3
orf19.7284	<i>ASR2</i>	<i>IPF2857</i>	--	5.2	2.3	10.5	17.6	1.5
orf19.7042	--	<i>IPF3080</i>	--	2.1	1.5	1.2	5.5	1.3
orf19.1862	--	<i>IPF4065</i>	--	3.5	1.7	1.1	9.0	-1.1
orf19.5525	--	<i>IPF4328</i>	--	1.5	1.6	1.2	2.3	1.1
orf19.2296	--	<i>IPF7715</i>	--	2.3	1.5	1.2	4.1	1.7
orf19.3131	--	<i>IPF7817</i>	NADPH dehydrogenase activity	1.7	1.8	1.4	5.1	1.4
orf19.2691	--	<i>IPF7970</i>	--	1.6	1.6	1.2	4.9	3.0
orf19.744	<i>GDB1</i>	<i>IPF9740</i>	4-alpha-glucanotransferase activity	1.7	1.5	1.1	3.1	1.7
orf19.1240	--	<i>IPF9887.3eoc</i>	--	1.5	1.6	1.2	2.6	1.6
orf19.2285	--	<i>IPF9996</i>	--	4.6	2.2	1.6	8.1	1.2
orf19.1149	<i>MRF1</i>	<i>MRF1</i>	DNA binding	1.8	1.6	-1.1	8.0	1.5
orf19.1655	<i>PXP2</i>	<i>PXP2</i>	Acyl-CoA oxidase activity	2.1	2.1	1.1	3.0	1.2
orf19.6202	<i>RBT4</i>	<i>RBT4</i>	--	1.7	1.7	-1.1	2.6	-1.1
orf19.5585	<i>SAP5</i>	<i>SAP5</i>	Aspartic-type endopeptidase activity	1.8	1.5	-1.1	5.4	-1.0
orf19.2991	<i>HOL1</i>	<i>HOL1</i>	Transporter activity	2.3	1.3	1.8	2.5	1.1
orf19.7585	<i>INO1</i>	<i>INO1</i>	Inositol-3-phosphate synthase activity	3.1	1.3	1.8	6.1	1.3
orf19.1266	--	<i>IPF10280</i>	--	1.6	1.4	2.0	2.2	1.1
orf19.1180	--	<i>IPF13098</i>	--	1.7	1.2	1.5	1.8	2.2
orf19.2296	--	<i>IPF14282</i>	--	1.8	1.4	2.3	6.1	2.4
orf19.3929	--	<i>IPF14603</i>	--	1.9	1.0	1.9	1.9	-1.1
orf19.347	<i>RSN1</i>	<i>IPF1506</i>	--	2.0	1.1	1.7	2.0	1.8
orf19.3190	<i>HAL9</i>	<i>IPF16067</i>	Specific RNA polymerase II transcription factor activity	1.9	1.2	1.6	1.8	1.6
orf19.94	--	<i>IPF19749</i>	--	2.0	1.1	1.5	2.8	-1.0

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.3846	--	IPF20118	--	1.6	-1.1	3.8	2.6	-1.5
orf19.6627	--	IPF2557	--	1.7	1.2	2.2	2.6	-1.0
orf19.3043	--	IPF3594	Triacylglycerol lipase activity	1.5	-1.1	1.8	1.6	1.1
orf19.3544	--	IPF6438	--	1.6	1.3	2.8	2.7	1.6
orf19.4533	--	IPF7533	--	1.5	1.3	1.6	1.6	1.1
orf19.1395	--	IPF7686	Inorganic phosphate transporter activity	1.5	1.4	2.1	6.3	-1.5
orf19.3188	TAC1	IPF9191.5f	--	2.5	1.5	2.4	45.4	-11.7
orf19.3071	--	IPF9605	Protein tyrosine phosphatase activity	1.5	1.0	1.5	1.7	1.5
orf19.5102	PLB5	PLB5	Lysophospholipase activity	1.5	1.3	1.6	3.6	1.1
orf19.2046	POT1-2	POT13	Acetyl-CoA C-acyltransferase activity	1.5	1.3	1.6	1.7	1.2
orf19.7319	SUC1	SUC1	Transcription factor activity	2.0	1.2	1.8	5.1	1.2
orf19.1974	TFS1	TFS1	Lipid binding	1.5	1.4	1.7	2.4	-1.0
orf19.345	--	UGA2	Succinate-semialdehyde dehydrogenase activity	1.8	1.3	1.6	4.0	3.0
orf19.4082	DDR48	DDR48	--	1.2	2.5	2.6	2.5	-1.6
orf19.6445	EC11	IPF1384	Dodecenoyl-CoA delta-isomerase activity	1.3	1.8	1.6	4.4	1.2
orf19.354	--	IPF1493	--	-1.0	1.5	1.7	1.8	1.2
orf19.1365	--	IPF16016	--	1.2	1.6	1.6	2.1	-1.2
orf19.5799	--	IPF20010	--	1.4	1.8	1.6	1.7	1.7
orf19.6770	--	IPF2804	Cytoskeletal adaptor activity	1.3	1.6	1.5	2.7	-1.0
orf19.3045	--	IPF3597	Aldehyde dehydrogenase (NAD) activity	1.4	1.6	1.6	1.6	1.2
orf19.6501	--	IPF3931	--	2.0	3.1	7.4	4.6	1.1
orf19.6734	TCC1	IPF6067	General transcriptional repressor activity	-1.1	1.8	1.6	1.7	-1.0
orf19.4056	GAT2	IPF7666	Transcription factor activity	1.2	2.1	2.3	5.4	-1.7
orf19.2248	ARE2	ARE2	Sterol O-acyltransferase activity	2.1	1.7	1.5	3.0	1.7
orf19.6000	CDR1	CDR1	Multidrug transporter activity	1.7	3.0	2.4	4.4	-1.0
orf19.5958	CDR2	CDR2	Transporter activity	50.6	14.1	17.1	50.5	-1.3
orf19.86	--	GPX1	Glutathione peroxidase activity	2.3	3.0	2.3	4.0	-1.2
orf19.896	CHK1	HK1	Protein histidine kinase activity	2.5	1.9	1.8	4.5	1.3
orf19.3160	HSP12	HSP12	--	5.3	2.9	3.1	16.7	-1.7
orf19.157	--	IFA24.3	--	1.5	1.7	1.6	2.1	1.1
orf19.2568	IFU5	IFU5	--	3.3	3.0	3.7	5.4	1.5
orf19.2726	--	IPF10262	--	2.5	2.8	1.9	6.2	-2.9
orf19.1267	--	IPF10278	Chaperone regulator activity	1.8	1.7	1.8	2.5	-1.2
orf19.4459	--	IPF11849	--	2.0	4.1	4.3	5.0	-1.5
orf19.2244	--	IPF12897	--	1.8	1.7	1.5	5.4	-1.7
orf19.851	--	IPF14030	--	1.7	1.9	1.9	4.0	-1.3
orf19.344	--	IPF1514	--	15.5	4.7	4.0	28.9	1.9
orf19.4907	--	IPF17283	--	3.2	1.6	2.3	2.0	1.1
orf19.5313	--	IPF19810	--	36.0	29.4	18.1	143.1	1.7
orf19.5777	--	IPF19961	--	2.0	1.7	2.2	4.2	-1.3

Table A-1 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.7166	--	<i>IPF2186</i>	--	4.2	2.2	1.5	5.0	1.2
orf19.6840	--	<i>IPF3535</i>	--	1.8	2.3	1.9	2.3	-1.4
orf19.3047	--	<i>IPF3598</i>	Transcription cofactor activity	1.5	1.5	1.7	3.0	1.3
orf19.2531	<i>CSP37</i>	<i>IPF4991</i>	ER to Golgi vesicle-mediated transport	2.5	1.8	5.5	6.8	-1.3
orf19.3007.2	--	<i>IPF5360.3</i>	--	2.0	1.6	1.9	2.8	1.1
orf19.7310	--	<i>IPF5981</i>	--	4.9	2.7	2.0	16.8	1.2
orf19.1887	--	<i>IPF6464</i>	--	2.3	1.8	1.7	4.5	1.2
orf19.2790	--	<i>IPF7260</i>	Histone-lysine N-methyltransferase activity	1.8	1.6	1.6	2.8	-1.0
orf19.4531	--	<i>IPF7530</i>	ATPase activity	2.7	3.8	3.5	4.0	-1.6
orf19.4898	--	<i>IPF8950</i>	--	2.4	1.6	1.8	3.4	1.1
orf19.3188	<i>TAC1</i>	<i>IPF9191.3f</i>	--	2.7	2.8	2.8	4.2	-11.0
orf19.3644	--	<i>IPF9683</i>	--	2.5	1.5	2.1	3.4	1.1
orf19.5257	--	<i>LCB4</i>	D-erythro-sphingosine kinase activity	2.2	1.7	2.2	4.0	1.1
orf19.278	--	<i>MTR</i>	--	3.1	1.8	1.9	5.2	-1.1
orf19.5713	<i>YMX6</i>	<i>NDH2</i>	NADH dehydrogenase activity	5.3	3.1	2.9	15.3	1.3
orf19.7218	<i>RBE1</i>	<i>PRY2</i>	--	2.1	1.8	1.7	1.7	1.2
orf19.23	<i>RTA3</i>	<i>RTA3</i>	Phospholipid-translocating ATPase activity	40.4	15.9	22.0	41.0	1.0
orf19.2896	<i>SOU1</i>	<i>SOU1</i>	Sorbose dehydrogenase activity	2.0	1.5	1.5	7.0	-2.0
orf19.1290	--	<i>XKS1</i>	Xylulokinase activity	1.6	1.7	1.6	2.7	1.6
orf19.3104	--	<i>YDC1</i>	--	3.3	1.6	2.1	5.3	1.4

^a orf19 nomenclature according to the Assembly 19 version.

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Gene expression values in bold designate statistical significance (p-value < 0.05) in all three experiments.

Table A-2. Genes down-regulated in at least one matched clinical isolate.

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.7043	--	ACB1.exon2	Acyl-CoA binding	-2.0	1.6	1.9	1.4	1.1
orf19.798	--	ANC1	General RNA polymerase II transcription factor activity	-1.8	-1.0	1.1	-1.1	-2.5
orf19.5345	--	DSK2	Protein binding	-1.5	-1.0	1.2	-1.1	1.6
orf19.4295	--	HIR2	Transcription corepressor activity	-2.3	1.1	1.7	1.0	1.2
orf19.4051	<i>HTS1</i>	HTS1	Histidine-tRNA ligase activity	-1.5	1.1	1.2	-1.5	1.2
orf19.3691	--	IPF10716	--	-1.5	-1.0	1.3	1.1	1.1
orf19.2356	<i>CRZ2</i>	IPF10795	Transcription factor activity	-2.2	2.1	-1.4	1.4	1.3
orf19.2827	--	IPF10963	Structural constituent of cytoskeleton	-2.2	1.5	-1.0	5.3	3.9
orf19.4658	--	IPF11006	--	-1.5	1.0	2.5	2.6	1.4
orf19.3198	<i>OBPA</i>	IPF11167	Oxysterol binding	-85.5	1.1	1.3	2.1	-1.8
orf19.5241	<i>SNT1</i>	IPF12584	NAD-dependent histone deacetylase activity	-1.5	1.1	1.3	-1.2	1.2
orf19.7371	<i>ZCF35</i>	IPF1264	Transcription factor activity	-1.7	1.8	1.3	1.1	-1.6
orf19.2363	--	IPF12845	--	-1.5	1.2	1.2	-1.4	1.1
orf19.3551	--	IPF13370	Structural constituent of cytoskeleton	-1.8	-1.0	1.4	1.6	-1.7
orf19.4474	--	IPF14379	--	-1.8	-1.7	4.2	-1.1	1.1
orf19.4342	--	IPF14623	Specific RNA polymerase II transcription factor activity	-3.2	1.3	1.1	-1.0	-1.7
orf19.954	--	IPF1557	--	-1.5	1.1	-1.2	-1.3	-1.2
orf19.4698	--	IPF15977	--	-1.8	-1.0	1.2	-1.9	-1.2
orf19.5297	--	IPF19753	General RNA polymerase II transcription factor activity	-1.8	1.3	1.6	-1.1	-1.0
orf19.6920	--	IPF2195	--	-2.2	-1.2	-1.3	1.1	1.3
orf19.3260	--	IPF256	--	-1.7	2.0	1.1	1.9	1.7
orf19.871	--	IPF3950	--	-2.1	-1.0	1.6	1.1	1.0
orf19.7506	--	IPF404.5f	Protein binding	-1.7	1.2	1.1	-1.2	-1.0
orf19.6934	--	IPF4206	--	-1.5	1.1	2.4	-1.0	-1.6
orf19.5908	<i>TEC1</i>	IPF4351	Specific RNA polymerase II transcription factor activity	-1.6	1.0	-1.1	1.1	-1.6
orf19.5910	--	IPF4356	--	-1.6	-1.3	1.4	1.5	-1.2
orf19.7675	--	IPF4955	Structural constituent of ribosome	-1.6	1.2	1.2	-1.5	1.0
orf19.2529	--	IPF4988	--	-1.6	-1.4	1.2	1.1	1.0
orf19.7256	--	IPF5243	--	-1.6	1.0	1.5	1.2	-1.5
orf19.7085	--	IPF525	--	-1.5	1.0	-1.0	1.2	1.8
orf19.7314	<i>CDG1</i>	IPF5972	--	-1.9	1.5	-1.1	-1.2	-1.5
orf19.1542	<i>HEX3</i>	IPF6497	--	-1.7	-1.0	-1.3	-1.1	-1.1
orf19.7545	--	IPF7010.3	--	-1.7	1.1	1.2	-1.0	-1.6
orf19.5368	--	IPF714	--	-1.9	1.2	4.6	1.1	1.4
orf19.1449	--	IPF7863	--	-1.7	-1.2	1.1	2.7	1.4
orf19.2939	--	IPF8114	--	-1.8	-1.3	-1.4	1.2	-2.8
orf19.3714	--	IPF8295	--	-1.5	1.0	-1.8	-1.4	-1.2
orf19.4066	--	IPF9466	--	-2.1	-1.3	-1.1	1.1	1.0
orf19.4144	--	IPF9825	--	-2.1	1.1	1.3	1.5	1.0
orf19.585	--	MRPL17	Structural constituent of ribosome	-1.8	1.6	1.2	1.0	-1.7
orf19.3321	--	MRS7	--	-1.8	1.3	1.7	1.4	-1.1

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.6294	--	MYO1	Microfilament motor activity	-1.6	1.0	1.7	1.1	-1.2
orf19.3181.1	--	NCE11	--	-2.7	-1.1	1.3	-1.0	-1.5
orf19.7279	--	NIT2	--	-1.6	-1.1	-1.1	-1.3	1.1
orf19.3197	PAP1	PAP11	RNA binding	-108.1	1.2	-1.1	-1.2	-50.0
orf19.3974	PUT2	PUT2	1-Pyrroline-5-carboxylate dehydrogenase activity	-1.6	-1.0	1.1	-1.3	-1.1
orf19.607	--	RAD26	DNA-dependent ATPase activity	-1.7	-1.0	1.8	1.6	1.1
orf19.5968	RDI1	RDI1	Rho GDP-dissociation inhibitor activity	-1.6	1.0	-1.0	1.0	-1.4
orf19.2823	RFG1	ROX1	DNA bending activity	-1.5	1.2	1.0	-1.0	-1.0
orf19.6933	--	RRD2	Protein phosphatase type 2A regulator activity	-1.5	-1.2	1.0	-1.1	-1.1
orf19.2555	URA5	URA5	Orotate phosphoribosyltransferase activity	-1.5	1.1	1.1	-1.3	-2.0
orf19.1495	--	UTR4	--	-2.0	1.4	-1.2	1.8	-2.2
orf19.6169	--	FRP2	--	1.2	-1.6	1.5	2.2	-1.1
orf19.4570	--	IPF1038	--	1.1	-2.0	6.7	1.6	-1.6
orf19.6308	--	IPF12227.3f	--	1.2	-1.5	-1.0	1.1	1.1
orf19.3905	--	IPF17642	--	-1.0	-3.9	1.7	1.6	1.6
orf19.2336	--	IPF8682	--	1.7	-1.7	1.4	2.8	-1.2
orf19.2028	MXR1	MXR1	Protein-methionine-S-oxide reductase activity	-1.2	-1.6	-1.3	-1.5	-1.2
orf19.6031	--	VPS27	--	-1.2	-1.5	-1.1	1.3	1.2
orf19.1816	ALS3	ALS3.3eoc	Cell adhesion molecule binding	1.0	-1.4	-2.4	1.0	-1.5
orf19.2419	--	DOM34	--	1.1	1.1	-1.7	-1.1	-1.1
orf19.3433	OYE23	EBP4	NADPH dehydrogenase activity	1.0	1.0	-2.0	-1.7	2.0
orf19.3374	ECE1	ECE1	--	1.9	1.5	-3.8	5.6	1.5
orf19.1774	--	FDH13.5f	Formate dehydrogenase activity	-1.1	-1.7	-1.5	-1.1	-1.8
orf19.6947	--	GTT1.3	Glutathione transferase activity	-1.4	-1.2	-1.5	1.3	-1.0
orf19.6706	GYP7	GYP7	Rab GTPase activator activity	-1.4	-1.1	-1.9	1.2	1.6
orf19.779	--	HAT1	H3/H4 histone acetyltransferase activity	-1.3	1.1	-1.6	1.0	-1.0
orf19.1979	GIT1	IFN1	Phospholipid transporter activity	-1.3	-1.1	-1.5	-1.0	1.2
orf19.2283	--	IPF10071	--	1.1	-1.1	-1.5	1.1	-1.2
orf19.1392	--	IPF11068	Protein disulfide isomerase activity	1.6	1.1	-1.6	-1.1	1.0
orf19.4771	--	IPF11667	--	-1.4	-1.4	-1.7	-1.4	1.2
orf19.4457	BNI4	IPF11847	--	-1.0	1.2	-1.5	-1.1	1.6
orf19.3222	--	IPF162	--	-1.2	-1.1	-1.6	-1.0	1.9
orf19.2989	--	IPF16795	--	-1.0	1.0	-1.9	1.5	2.0
orf19.4997	KIS2	IPF1792	AMP-activated protein kinase activity	-1.0	-1.0	-1.6	-1.1	1.5
orf19.730	--	IPF20142	--	-1.1	-1.0	-1.5	-1.3	1.7
orf19.588	--	IPF6380	--	1.2	1.2	-1.8	1.3	-1.6
orf19.1573	--	IPF6387.3	--	1.2	1.1	-2.3	1.6	-1.1
orf19.6077	--	IPF8369	--	1.2	-1.2	-1.7	-1.1	1.5
orf19.1187	CPH2	IPF9062	Double-stranded DNA binding	-1.1	-1.0	-1.6	-1.5	-1.1
orf19.4374	--	IPF9438	--	-1.0	-1.0	-1.5	-1.3	-1.7
orf19.6671	LAP4	LAP42	Aminopeptidase I activity	1.0	-1.2	-1.7	-1.1	1.5

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.3796	--	RNT1	Ribonuclease III activity	-1.2	1.7	-1.5	1.1	1.1
orf19.5750	<i>SHM2</i>	SHM2	Glycine hydroxymethyltransferase activity	-1.2	-1.1	-1.6	-1.5	1.2
orf19.2179	<i>SIT1</i>	SIT1	Ferrichrome transporter activity	1.6	-1.3	-1.7	1.1	1.2
orf19.6260	--	UBP12	Ubiquitin-specific protease activity	-1.1	1.1	-1.6	1.9	1.2
orf19.559	<i>FGR14</i>	Zorro1a	--	1.1	1.1	-2.9	-1.1	-1.1
orf19.3331	<i>ABC1</i>	ABC1	--	-1.1	1.2	1.7	-2.1	-1.2
orf19.6632	<i>ACO2</i>	ACO2	Aconitate hydratase activity	-1.2	-1.0	1.1	-1.9	1.1
orf19.3468	<i>ALG11</i>	ALG11	Alpha-1,2-mannosyltransferase activity	1.3	-1.0	-1.3	-1.9	1.0
orf19.7551	<i>ALO1</i>	ALO1	D-arabinono-1,4-lactone oxidase activity	-1.2	-1.0	1.1	-2.0	1.1
orf19.2848	--	APG13	Protein binding	-1.0	-1.1	-1.1	-1.5	1.3
orf19.5487	<i>CDC46</i>	CDC46	ATP-dependent DNA helicase activity	1.1	-1.2	1.1	-1.7	1.1
orf19.5870	<i>CTP1</i>	CTP1	Tricarboxylate carrier activity	-1.2	-1.0	1.0	-1.7	-1.3
orf19.4870	--	DBP3	ATP-dependent RNA helicase activity	-1.2	1.1	-1.0	-1.9	-1.0
orf19.3393	--	DBP9	ATP-dependent RNA helicase activity	-1.3	1.1	1.1	-2.2	-1.6
orf19.6702	<i>DED81</i>	DED81	ATP binding	-1.1	-1.0	-1.1	-1.9	1.2
orf19.5164	<i>ECM39</i>	ECM39	Alpha-1,6-mannosyltransferase activity	1.0	1.0	-1.2	-2.3	1.7
orf19.7332	<i>ELF1</i>	ELF1	ATP binding	-1.3	1.5	1.1	-2.2	-1.6
orf19.333	<i>FCY2</i>	FCY22	Cytosine-purine permease activity	-1.3	1.1	-1.3	-2.0	1.3
orf19.7231	<i>FTR2</i>	FTR2	Iron ion binding	-1.2	1.1	-1.1	-2.2	1.0
orf19.481	<i>GCD1</i>	GCD1	Translation initiation factor activity	-1.3	1.1	1.1	-1.9	-1.0
orf19.500	--	GCD10	tRNA methyltransferase activity	-1.2	1.2	-1.2	-2.2	-1.3
orf19.2003	<i>HNM1</i>	HNM1	Choline transporter activity	1.0	-1.4	-1.0	-2.3	-1.7
orf19.4889	--	HOL2	Transporter activity	-1.3	-1.1	-1.2	-2.6	-1.2
orf19.3476	--	HRR25	Casein kinase activity	-1.2	1.3	1.1	-1.5	-1.0
orf19.6515	<i>HSP90</i>	HSP90	ATPase activity, coupled	-1.1	-1.0	1.1	-1.7	1.3
orf19.7488	--	IMP3	snoRNA binding	-1.1	1.1	-1.1	-1.7	-2.6
orf19.5071	<i>NRP1</i>	IPF10092	--	-1.1	1.1	-1.0	-1.8	-1.2
orf19.5034	--	IPF10424	--	-1.2	-1.1	-1.2	-1.6	-1.0
orf19.5987	--	IPF11	--	-1.1	1.1	-1.1	-1.6	-1.1
orf19.4623	--	IPF11090.exon2	--	-1.3	1.1	-1.1	-1.6	1.2
orf19.51	--	IPF11177	Translation initiation factor activity	-1.4	1.1	1.2	-1.9	-1.5
orf19.1418	<i>SEC15</i>	IPF11236.3f	--	-1.1	1.1	-1.1	-1.5	-1.1
orf19.4518	--	IPF11424	--	1.0	1.1	-1.1	-1.6	-1.0
orf19.6463	--	IPF11432	--	-1.3	1.1	-1.1	-1.5	1.0
orf19.1646	--	IPF11615	rRNA primary transcript binding	-1.2	-1.1	1.1	-2.3	-1.5
orf19.3412	<i>ATG15</i>	IPF12033	Lipase activity	1.0	-1.0	-1.2	-1.7	1.2
orf19.4787	--	IPF12091	ATPase activator activity	-1.1	1.3	1.1	-2.0	-1.2
orf19.2667	<i>RPF1</i>	IPF12428	--	-1.3	1.1	-1.1	-1.9	-1.7
orf19.5238	--	IPF12577	--	-1.2	-1.0	-1.0	-1.9	1.2
orf19.2898	--	IPF13465	--	1.1	1.0	-1.2	-2.3	1.5
orf19.2451	<i>PGA45</i>	IPF14109	--	1.3	1.2	1.1	-2.2	1.1

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.2452	--	IPF14850	--	-1.1	-1.1	-1.4	-3.8	-1.1
orf19.2108	<i>SOD6</i>	IPF15423	Copper, zinc superoxide dismutase activity	1.5	-1.0	1.1	-1.7	1.5
orf19.2256	--	IPF15468	--	-1.2	-1.0	-1.2	-1.8	-1.3
orf19.6539	--	IPF15737	ATPase activity	-1.2	1.0	-1.2	-1.7	1.4
orf19.6581	--	IPF1629	--	1.1	1.1	1.2	-1.6	1.0
orf19.6566	--	IPF1660	--	1.0	-1.1	1.2	-1.8	1.2
orf19.2917	--	IPF16748	--	-1.2	1.9	-1.0	-1.8	-1.4
orf19.2664	--	IPF16761	--	-1.0	1.1	1.5	-1.8	1.2
orf19.2143	--	IPF17094	--	-1.2	1.1	1.2	-2.0	-1.2
orf19.3553	--	IPF17139	--	-1.3	1.2	-1.4	-1.8	-1.5
orf19.6707	--	IPF17904.3	--	-1.3	1.1	-1.1	-4.8	1.2
orf19.7028	--	IPF18125	--	1.2	-1.0	-1.1	-1.7	1.9
orf19.5884	--	IPF1846	--	-1.1	1.3	-1.2	-1.8	-1.1
orf19.3159	<i>UTP20</i>	IPF18641.exon2	--	1.0	1.1	1.0	-2.0	-1.4
orf19.7154	--	IPF1948	--	-1.1	-1.0	1.1	-2.1	-1.1
orf19.5559	<i>STE23</i>	IPF19660	--	1.1	-1.2	-1.4	-1.7	-1.3
orf19.6789	--	IPF19818	Structural constituent of cytoskeleton	-1.1	1.1	2.0	-2.0	-1.1
orf19.7182	--	IPF2166	--	-1.2	1.1	-1.1	-2.1	-1.0
orf19.6902	--	IPF2228	ATP-dependent RNA helicase activity	-1.3	-1.1	-1.1	-2.8	1.2
orf19.7022	--	IPF2359	--	-1.0	-1.1	-1.2	-1.6	1.2
orf19.6818	--	IPF2409	Helicase activity	-1.1	1.1	1.3	-2.1	-1.4
orf19.7424	--	IPF2441	--	-1.3	1.3	-1.1	-2.3	-1.5
orf19.6625	--	IPF2560	--	-1.1	-1.2	-1.1	-1.6	-1.3
orf19.3559	--	IPF3184.exon1	--	-1.3	-1.0	-1.1	-1.7	-1.4
orf19.3559	--	IPF3184.exon2	--	-1.0	1.0	1.0	-1.8	-1.6
orf19.4384	<i>HXT5</i>	IPF3282	Fructose transporter activity	1.2	1.0	-1.1	-1.6	-1.3
orf19.7398	--	IPF3309.3eoc	--	-1.2	1.2	-1.4	-2.0	-1.4
orf19.7388	<i>PBS2</i>	IPF3329	MAP kinase kinase activity	-1.1	1.1	1.2	-2.0	1.1
orf19.6752	--	IPF3492	Structural constituent of ribosome	-1.1	-1.0	1.0	-1.5	-1.5
orf19.28	--	IPF3853	Transporter activity	-1.2	-1.2	-1.2	-1.9	-1.3
orf19.768	<i>SYG1</i>	IPF3887	--	1.1	-1.1	-1.2	-2.5	1.7
orf19.6503	--	IPF3928	--	-1.3	1.5	1.0	-1.8	1.2
orf19.671	<i>PSP1</i>	IPF3970	--	-1.3	-1.1	-1.2	-1.6	1.2
orf19.1630	--	IPF4253	--	-1.2	1.1	-1.0	-1.6	2.2
orf19.1633	--	IPF4257	snoRNA binding	-1.3	1.1	-1.0	-1.7	-1.3
orf19.549	--	IPF4276	Structural constituent of ribosome	-1.2	1.1	1.1	-1.8	-1.2
orf19.541	--	IPF4290	--	-1.2	-1.4	-1.3	-2.1	1.0
orf19.5279	--	IPF4703	--	-1.3	1.0	1.1	-1.7	-1.9
orf19.6276	--	IPF4859	--	1.1	1.2	-1.4	-1.6	1.7
orf19.6392	--	IPF5129	--	1.0	1.3	1.2	-1.7	-1.2
orf19.5681	--	IPF5469	--	1.2	-1.1	1.2	-1.8	-1.3

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.7098	--	IPF556	--	-1.1	-1.2	-1.2	-1.8	1.2
orf19.7107	--	IPF5615	--	-1.1	1.7	-1.1	-1.9	-1.0
orf19.4176	--	IPF6175	Structural constituent of ribosome	-1.3	1.0	-1.1	-2.2	-1.5
orf19.1971	--	IPF6286	DNA binding	-1.1	1.0	1.5	-2.3	-1.5
orf19.1964	--	IPF6298	--	-1.1	1.3	1.1	-2.3	-1.3
orf19.7618	--	IPF630	--	-1.2	1.3	-1.0	-2.3	-1.4
orf19.1693	<i>CAS4</i>	IPF6521.3eoc	Transcription factor activity	-1.2	1.2	1.1	-1.6	1.1
orf19.7599	--	IPF668	snoRNA binding	-1.3	1.2	-1.0	-1.9	-1.1
orf19.1565	--	IPF7147	tRNA dihydrouridine synthase activity	-1.2	-1.2	-1.0	-1.9	1.2
orf19.386	<i>SAM4</i>	IPF7279	Homocysteine S-methyltransferase activity	-1.2	-1.0	-1.0	-2.2	1.0
orf19.2447	--	IPF7472	--	-1.3	-1.2	-1.3	-1.5	-1.4
orf19.2936	--	IPF8110	--	-1.0	-1.3	-1.1	-2.3	-1.7
orf19.3327	--	IPF8326	tRNA methyltransferase activity	-1.2	1.1	1.0	-1.8	-1.7
orf19.3977	--	IPF8652	--	-1.4	-1.1	-1.2	-1.6	-1.4
orf19.2487	--	IPF9225	--	-1.1	1.0	1.3	-1.6	-1.0
orf19.4375	--	IPF9435	--	-1.2	1.1	-1.1	-1.8	-1.2
orf19.6092	<i>KEL1</i>	KEL1	--	-1.4	-1.0	-1.1	-2.1	1.8
orf19.6086	<i>LEU4</i>	LEU41	2-isopropylmalate synthase activity	1.2	1.2	-1.1	-2.0	1.2
orf19.3540	<i>MAK5</i>	MAK5	ATP-dependent RNA helicase activity	-1.2	-1.2	-1.1	-1.8	-1.5
orf19.2551	<i>MET6</i>	MET6	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity	1.0	-1.1	-1.1	-1.9	-1.0
orf19.7383	<i>MNN9</i>	MNN9	Mannosyltransferase activity	-1.0	1.2	1.2	-1.7	-1.1
orf19.1662	--	MRP1.3f	Structural constituent of ribosome	-1.3	1.2	1.1	-1.5	-1.4
orf19.185	--	MRP51	Structural constituent of ribosome	-1.2	1.1	-1.1	-1.8	-2.7
orf19.3532	<i>MRPL10</i>	MRPL10.3	Structural constituent of ribosome	-1.5	1.7	1.2	-2.1	-1.5
orf19.2019	--	MRPL16	Peptidyltransferase activity	-1.1	1.1	-1.1	-1.7	-1.3
orf19.6129	<i>MRPL8</i>	MRPL8	Structural constituent of ribosome	-1.1	1.1	-1.2	-1.6	-1.0
orf19.706	<i>NMD3</i>	NMD3	RNA binding	-1.2	1.2	1.0	-2.5	-1.0
orf19.1199	<i>NOP5</i>	NOP58	snoRNA binding	-1.1	1.2	1.2	-1.6	1.0
orf19.3000	--	ORC1	ATPase activity	-1.0	3.1	1.1	-3.2	-1.1
orf19.2199	<i>PHO86</i>	PHO86	--	-1.1	-1.3	-1.2	-1.8	1.1
orf19.5847	--	RET1	DNA-directed RNA polymerase activity	-1.2	1.2	1.0	-2.0	1.0
orf19.2320	--	RIO1	Amino acid kinase activity	-1.4	1.3	-1.1	-2.1	-1.6
orf19.5420	--	RML2	Structural constituent of ribosome	-1.2	1.2	1.2	-1.8	-1.0
orf19.2594	--	RPA43	DNA-directed RNA polymerase activity	-1.3	1.1	1.0	-2.2	-1.8
orf19.443	--	RPC25	DNA-directed RNA polymerase activity	-1.3	1.2	1.3	-1.8	-1.5
orf19.2830	--	RRP9	snoRNA binding	-1.3	-1.0	1.5	-1.7	-1.7
orf19.6355	--	RSA2	--	-1.3	1.2	-1.1	-1.9	-1.2
orf19.24	<i>RTA2</i>	RTA2	Phospholipid-translocating ATPase activity	1.5	-1.2	-1.1	-2.1	1.1
orf19.642	--	SAP155	Protein serine/threonine kinase activity	-1.0	-1.2	1.2	-2.1	-1.1
orf19.6558	--	SEC231	GTPase activator activity	-1.2	1.4	1.1	-1.7	1.4
orf19.5263	<i>SER33</i>	SER33	Phosphoglycerate dehydrogenase activity	-1.0	-1.2	-1.1	-1.7	1.2

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.5953	--	SFP1	Transcription factor activity	1.0	1.2	1.2	-1.6	1.3
orf19.7569	--	SIK1	snoRNA binding	-1.2	1.2	-1.0	-1.5	-1.0
orf19.4367	<i>SMC1</i>	SMC1	ATP DNA binding	1.2	-1.1	1.1	-1.6	-1.1
orf19.5407	--	SOF1	snoRNA binding	-1.2	1.1	-1.0	-1.6	-1.6
orf19.4960	--	SPE4	--	-1.2	1.0	1.0	-2.5	-1.2
orf19.1139	--	SVL3	--	-1.2	1.1	1.2	-1.6	-1.1
orf19.1352	--	TIM22	Protein transporter activity	-1.2	1.0	-1.0	-1.9	-1.7
orf19.5143	--	TIM54	Protein transporter activity	-1.3	1.2	1.5	-2.0	1.2
orf19.1532	--	TOM37	--	1.2	1.4	1.5	-2.0	-1.5
orf19.429	--	TRF4	DNA-directed DNA polymerase activity	-1.4	1.2	1.4	-2.1	-1.5
orf19.802	--	UGA12.5f	4-aminobutyrate transaminase activity	-1.3	-1.1	1.1	-2.3	1.8
orf19.3363	<i>VTC4</i>	VTC4	--	-1.1	-1.0	-1.0	-1.6	1.0
orf19.7036	--	WHI2	--	-1.1	-1.1	1.3	-1.5	1.1
orf19.3300	<i>ZPR1</i>	ZPR1	Transcription factor activity	-1.3	1.0	-1.1	-1.8	-1.1
orf19.4773	<i>AOX2</i>	AOX2	Alternative oxidase activity	-2.6	-2.1	-1.1	1.1	-2.7
orf19.1078	<i>HBR2</i>	IPF14203.5f	--	-1.6	-1.5	-1.1	-1.1	-1.2
orf19.2158	<i>NAG3</i>	IPF2710.repeat2	Monosaccharide transporter activity	-2.4	-1.7	-1.5	2.4	6.1
orf19.2948	<i>SNO1</i>	SNO1	Imidazoleglycerol-phosphate synthase activity	-2.6	-1.6	-1.4	-1.2	1.3
orf19.7111	--	SOD21.5f	Manganese superoxide dismutase activity	-1.6	-1.8	-1.1	2.3	2.0
orf19.7111	--	SOD22.3f	Manganese superoxide dismutase activity	-1.6	-2.4	1.3	1.0	1.9
orf19.5531	<i>CDC37</i>	CDC37	Unfolded protein binding	-1.7	-1.0	-2.4	-1.0	-1.2
orf19.3915	--	IPF10029.5eoc	--	-1.8	-1.3	-1.5	-1.4	1.5
orf19.2246	--	IPF12900	--	-1.6	-1.2	-1.7	1.0	-1.5
orf19.3050	<i>AGE1</i>	IPF3610	ARF GTPase activator activity	-1.5	-1.3	-1.5	-1.3	1.2
orf19.4900	--	IPF8854	Alpha-1,3-mannosyltransferase activity	-1.9	1.1	-1.6	-1.3	-1.3
orf19.1769	--	PLP2	--	-1.9	-1.0	-2.0	-1.4	-1.0
orf19.5906	<i>ADE2</i>	ADE2	Phosphoribosylaminoimidazole carboxylase activity	-1.5	-1.3	-1.3	-1.8	1.6
orf19.5806	<i>ALD5</i>	ALD5	3-chloroallyl aldehyde dehydrogenase activity	-1.6	-1.3	1.2	-4.1	-1.0
orf19.1623	<i>CAP1</i>	CAP1	Transcription factor activity	-1.7	1.1	-1.0	-1.7	-1.1
orf19.2876	<i>CBF1</i>	CBF1	Centromeric DNA binding	-1.8	-1.1	-1.2	-1.7	1.1
orf19.6948	<i>CCC1</i>	CCC1	--	-1.8	-1.4	-1.0	-2.3	-1.2
orf19.1055	<i>CDC3</i>	CDC3	Nucleotide binding	-1.6	1.2	-1.1	-1.6	1.2
orf19.1996	<i>CHA1</i>	CHA12	L-serine ammonia-lyase activity	-5.3	-1.1	-1.2	-2.6	-1.9
orf19.7478	--	COQ1	Trans-hexaprenyltransferase activity	-1.7	1.1	1.0	-1.6	-1.6
orf19.6652	<i>DBP8</i>	DBP8	ATP-dependent RNA helicase activity	-1.5	1.2	-1.1	-2.1	-1.1
orf19.7635	<i>DRS1</i>	DRS1	ATP-dependent RNA helicase activity	-1.7	1.0	-1.1	-2.1	1.2
orf19.3670	<i>GAL1</i>	GAL1	Galactokinase activity	-1.6	-1.1	-1.3	-1.8	1.3
orf19.385	<i>GCV2</i>	GCV2	Glycine dehydrogenase (decarboxylating) activity	-1.7	-1.1	-1.1	-1.9	1.1
orf19.2396	<i>IFR2</i>	IFR2	--	-1.7	-1.3	-1.2	-1.7	1.3
orf19.473	<i>TPO4</i>	IPF11142	--	-1.5	-1.4	-1.1	-1.7	1.6
orf19.512	--	IPF11315	--	-1.6	1.2	1.0	-2.0	1.1

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.1715	<i>IRO1</i>	IPF11363	--	-1.7	-1.0	-1.2	-1.7	1.3
orf19.4337	--	IPF11698	Monocarboxylic acid transporter activity	-1.6	-1.3	-1.3	-1.6	1.8
orf19.2067	--	IPF1210	--	-1.5	1.1	1.3	-1.6	-1.2
orf19.2185	--	IPF12457	--	-1.7	1.5	1.1	-2.1	-1.4
orf19.2330	--	IPF13361	snoRNA binding	-1.6	1.1	-1.1	-1.8	1.0
orf19.4746	<i>JIP5</i>	IPF14019	--	-1.7	1.1	1.1	-2.2	-1.8
orf19.4931	--	IPF15618	Cysteine-tRNA ligase activity	-1.5	1.0	-1.1	-1.7	1.1
orf19.4751	--	IPF16405	Structural constituent of ribosome	-1.6	1.2	-1.3	-2.4	-1.6
orf19.3547	--	IPF16996	--	-1.6	1.2	-1.1	-1.8	-2.1
orf19.2778	--	IPF18002	--	-1.7	1.2	-1.2	-1.8	-1.4
orf19.4929	--	IPF19785	Translation regulator activity	-1.6	1.3	1.9	-1.6	1.0
orf19.4093	--	IPF2093	--	-1.5	1.1	-1.1	-2.5	1.0
orf19.4105	--	IPF2109	--	-2.1	-1.2	-1.3	-1.9	-1.3
orf19.4835	--	IPF4485	snoRNA binding	-1.8	1.1	1.1	-1.9	-1.1
orf19.6648	--	IPF5009	--	-1.8	-1.2	1.1	-1.9	-1.2
orf19.3448	--	IPF5330	--	-10.5	1.3	1.2	-3.0	-1.6
orf19.7091	--	IPF538	--	-1.6	1.1	1.0	-1.8	-1.1
orf19.5680	--	IPF5471	--	-1.6	-1.2	-1.3	-1.7	1.3
orf19.4805	--	IPF6117	--	-1.5	1.1	-1.1	-2.5	1.1
orf19.7621	--	IPF625	--	-1.7	1.5	2.0	-1.8	-1.3
orf19.4191	--	IPF7998	--	-1.6	1.1	-1.1	-2.3	-1.6
orf19.4268	--	IPF9525	snoRNA binding	-1.5	1.2	-1.3	-1.6	-1.2
orf19.3406	--	IPF9898	--	-1.9	-1.2	-1.5	-2.7	-1.5
orf19.1244	<i>GYP2</i>	MDR1	Rab GTPase activator activity	-1.6	-1.1	1.0	-1.7	1.2
orf19.7384	--	NOG1	GTPase activity	-1.5	1.3	1.0	-2.8	-1.5
orf19.1030	--	NPI46	Peptidyl-prolyl cis-trans isomerase activity	-2.2	1.0	1.2	-1.6	-1.0
orf19.1687	--	PRP43	ATP-dependent RNA helicase activity	-1.5	1.3	-1.0	-1.6	-1.1
orf19.4274	<i>PUT1</i>	PUT1	Proline dehydrogenase activity	-2.1	1.0	1.9	-1.5	1.2
orf19.4593	<i>RGA2</i>	RGA2	Rho GTPase activator activity	-1.7	1.1	1.1	-2.0	-1.3
orf19.3867	<i>RPL7</i>	RPL7	rRNA binding	-1.8	1.0	-1.2	-1.9	-1.7
orf19.6041	<i>RPO41</i>	RPO41	DNA-directed RNA polymerase activity	-1.5	1.1	1.5	-2.1	-1.2
orf19.1923	<i>RRN3</i>	RRN3	RNA polymerase I transcription factor activity	-1.7	1.6	1.6	-2.2	-1.0
orf19.1161	<i>PLD1</i>	SPO14.5eoc	Phospholipase D activity	-1.6	1.3	1.0	-2.4	1.6
orf19.6109	<i>TUP1</i>	TUP1	General transcriptional repressor activity	-1.8	1.1	1.1	-1.7	1.5
orf19.1536	--	ZRC1	Di-, tri-valent inorganic cation transporter activity	-1.6	1.0	-1.0	-1.7	-1.1
orf19.1816	<i>ALS3</i>	ALS10	Cell adhesion molecule binding	-1.1	-1.5	-3.2	1.2	-1.5
orf19.1816	<i>ALS3</i>	ALS3.5eoc	Cell adhesion molecule binding	1.1	-1.6	-2.3	1.0	1.0
orf19.1264	<i>CFL2</i>	CFL2	Ferric-chelate reductase activity	1.6	-2.0	-1.6	-1.3	-1.6
orf19.1587	<i>HGT20</i>	IPF13131.3	Glucose transporter activity	1.1	-1.6	-1.9	1.2	2.9
orf19.1121	--	IPF2329	--	-1.4	-1.7	-1.5	-1.1	-1.3
orf19.6636	--	IPF2524	--	-1.5	-1.6	-1.7	-1.4	1.0

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.6249	<i>HAK1</i>	IPF9136.5eoc	Potassium ion transporter activity	1.1	-3.6	-3.1	-1.3	-1.5
orf19.5591	<i>ADO1</i>	ADO1	Adenosine kinase activity	1.0	-1.5	-1.1	-2.1	-1.2
orf19.4099	<i>ECM17</i>	ECM17	Sulfite reductase (NADPH) activity	-1.2	-1.7	-1.4	-2.2	1.2
orf19.6570	<i>NUP</i>	IPF1651	Nucleoside permease activity	-1.1	-1.7	-1.1	-1.8	2.1
orf19.6957.3	--	IPF19538	--	-1.3	-1.6	-1.3	-1.5	1.1
orf19.7046	<i>MET28</i>	IPF3087	Specific RNA polymerase II transcription factor activity	-1.1	-1.9	-1.1	-1.8	1.3
orf19.1789.1	<i>LYS1</i>	LYS1.5eoc	Saccharopine dehydrogenase (NAD ⁺ , L-lysine-forming) activity	-1.2	-1.5	-1.4	-2.4	1.0
orf19.3870	<i>ADE13</i>	ADE13	Adenylosuccinate lyase activity	-1.2	-1.2	-1.5	-1.9	1.4
orf19.5789	<i>ADE8</i>	ADE8	Phosphoribosylglycinamide formyltransferase activity	-1.3	-1.4	-1.5	-2.0	-1.2
orf19.2337	<i>ALP1</i>	ALP1	Basic amino acid transporter activity	-1.1	-1.5	-1.8	-2.2	-1.0
orf19.6459	--	DPP3	Phosphatidase phosphatase activity	-1.2	-1.2	-1.8	-2.2	1.4
orf19.4215	<i>FET34</i>	FET5	Ferroxidase activity	1.1	-1.4	-2.7	-1.8	1.0
orf19.4456	<i>GAP4</i>	GAP5	Amino acid permease activity	1.0	-1.1	-1.5	-2.9	-1.0
orf19.7566	--	GNP1	Amino acid permease activity	-1.0	-1.5	-1.6	-2.8	1.2
orf19.2020	<i>HGT6</i>	HXT61	Fructose transporter activity	1.1	-1.0	-1.7	-4.3	1.1
orf19.2923	--	IPF11448	--	-1.1	-1.2	-1.6	-2.5	1.3
orf19.1961	--	IPF14506	--	1.2	1.1	-3.0	-1.5	-2.8
orf19.3581	--	IPF3227	--	1.0	1.0	-1.9	-1.7	-1.7
orf19.6936	<i>RAD53</i>	RAD53	Protein serine/threonine kinase activity	-1.1	1.1	-1.5	-1.6	-1.0
orf19.5838	--	SER2	Phosphoserine phosphatase activity	-1.3	-1.3	-1.6	-2.1	1.1
orf19.844	<i>STE11</i>	STE11	MAP kinase kinase kinase activity	-1.1	1.2	-1.5	-1.5	1.1
orf19.34	--	GIT1	Phospholipid transporter activity	-2.0	-2.8	-2.5	-1.4	-1.0
orf19.7370	--	IPF1261	--	-1.8	-1.5	-1.5	-1.0	-1.3
orf19.2738	<i>SUL2</i>	SUL1	Sulfate transporter activity	-2.0	-4.3	-2.1	-1.0	1.5
orf19.4669	<i>AAT22</i>	AAT22	Aspartate transaminase activity	-1.8	-1.5	-1.3	-2.8	-1.6
orf19.993	--	IPF20135	--	-1.5	-1.5	-1.3	-1.7	1.1
orf19.847	--	IPF3415	Mitochondrial inner membrane peptidase activity	-1.8	-1.7	-1.0	-1.5	1.7
orf19.3040	--	IPF3589	--	-1.8	-1.5	-1.0	-1.5	1.2
orf19.3529	<i>ABP2</i>	IPF4463	--	-2.8	-1.5	1.0	-2.0	-1.2
orf19.1847	<i>ARO10</i>	IPF4667	Carboxy-lyase activity	-3.4	-1.7	-1.0	-2.6	1.5
orf19.7422	--	LCP5	--	-1.5	-1.9	-1.1	-2.0	1.0
orf19.5519	<i>GCV1</i>	GCV1	Glycine dehydrogenase (decarboxylating) activity	-1.8	-1.1	-1.8	-1.7	1.7
orf19.5392	<i>NGT1</i>	IPF1065	N-acetylglucosamine transporter activity	-1.5	-1.4	-1.8	-2.7	-1.2
orf19.4779	--	IPF12884	Multidrug transporter activity	-2.2	-1.1	-1.7	-2.2	1.3
orf19.4450.1	--	IPF6551	--	-1.7	-1.3	-1.5	-1.9	-1.0
orf19.1510	--	IPF9282	--	-1.6	-1.4	-1.9	-3.4	1.7
orf19.3810	--	MTD1	Methylenetetrahydrofolate dehydrogenase (NAD ⁺) activity	-1.5	-1.4	-1.9	-1.9	1.6
orf19.3599	<i>TIF4631</i>	TIF4631	Translation initiation factor activity	-1.6	1.0	-1.5	-1.5	-1.1
orf19.3122	<i>ARR3</i>	ARR3	Arsenite transporter activity	-1.2	-1.6	-1.6	-1.8	-1.1
orf19.111	<i>CAN2</i>	CAN2	Basic amino acid transporter activity	-1.0	-2.1	-2.5	-2.2	1.1
orf19.4215	<i>FET34</i>	FET34.3eoc	Ferroxidase activity	1.1	-1.5	-2.2	-3.2	-1.1

Table A-2 (continued).

Systematic Name ^a	CGD Name ^b	CandidaDB Name ^c	GO Annotation (Molecular Function) ^d	C56 vs. C43	Gu5 vs. Gu2	17 vs. 3	5674 vs. 5457	SZY31 vs. 5457
orf19.1978	<i>GIT2</i>	IFN3	Phospholipid transporter activity	1.4	-1.6	-2.6	-2.3	1.1
orf19.3902	--	IPF11503	--	1.2	-2.6	-2.3	-2.2	1.6
orf19.2475	<i>PGA26</i>	IPF7204	--	-1.2	-2.0	-1.9	-4.0	1.3
orf19.288	--	MET13	Structural constituent of ribosome	-1.2	-2.4	-1.8	-2.6	-1.1
orf19.946	<i>MET14</i>	MET14	Adenylylsulfate kinase activity	-1.2	-2.7	-2.0	-2.1	-2.0
orf19.638	<i>FDH1</i>	FDH12	Formate dehydrogenase activity	-1.6	-3.8	-1.8	-2.4	-1.5
orf19.7219	<i>FTR1</i>	FTR1	Ferrous iron transporter activity	-2.7	-3.0	-2.1	-4.0	-1.6
orf19.4527	<i>HGT1</i>	HGT11	Glucose transporter activity	-1.7	-1.5	-1.6	-2.5	-1.1
orf19.2461	<i>PRN4</i>	IFS1	--	-1.7	-1.7	-1.6	-1.8	-1.2
orf19.5760	<i>IHD1</i>	IPF10662	--	-1.9	-2.2	-3.1	-9.0	1.7
orf19.3475	--	IPF11725	--	-3.9	-2.1	-2.7	-5.8	-1.2
orf19.4655	<i>OPT6</i>	IPF12193	Oligopeptide transporter activity	-1.8	-2.0	-2.4	-3.1	-1.4
orf19.2060	<i>SOD5</i>	IPF1222	Copper, zinc superoxide dismutase activity	-2.3	-8.6	-17.8	-26.3	-1.4
orf19.2059	--	IPF1228	--	-2.0	-1.7	-1.7	-2.9	1.2
orf19.5673	<i>OPT7</i>	IPF12736	Oligopeptide transporter activity	-1.6	-1.7	-1.5	-1.6	1.8
orf19.4749	--	IPF13921	--	-2.3	-1.7	-2.4	-3.0	-1.4
orf19.670.2	--	IPF20159	--	-2.3	-2.0	-1.7	-4.9	-1.9
orf19.6679	--	IPF2314	--	-1.5	-2.1	-1.8	-2.2	1.1
orf19.7296	--	IPF2839	--	-1.6	-3.4	-2.5	-5.4	1.2
orf19.7561	<i>DEF1</i>	IPF946	--	-3.4	-1.5	-1.6	-2.8	-1.4
orf19.2618	<i>MET2</i>	MET2	Homoserine O-acetyltransferase activity	-1.5	-1.5	-1.6	-1.6	1.2
orf19.5025	<i>MET3</i>	MET3	Sulfate adenylyltransferase (ATP) activity	-2.0	-7.9	-1.8	-2.5	-1.2
orf19.700	<i>SEO1</i>	SEO2	Transporter activity	-2.3	-2.5	-3.0	-2.1	-1.2
orf19.2947	<i>SNZ1</i>	SNZ1	--	-3.7	-1.8	-1.7	-2.2	1.3

^a orf19 nomenclature according to the Assembly 19 version.

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Gene expression values in bold designate statistical significance (p-value < 0.05) in all three experiments.

APPENDIX B: *MRR1* SUPPLEMENTAL DATA

Table B-1. Genes up-regulated in at least one clinical matched isolate overexpressing *MDR1*.

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>AAF1</i>	orf19.7436	--	1.4	1.3	1.0	1.9	2.1	2.2	1.2	1.8	1.2
<i>AAH1</i>	orf19.2251	Adenine deaminase activity	-2.0	-1.6	1.6	1.5	1.7	2.6	1.5	-1.2	3.0
<i>ACB1.3f</i>	orf19.7043	Acyl-CoA binding	-1.0	-2.3	4.9	1.6	2.3	10.6	1.7	-1.1	2.0
<i>ADH5</i>	orf19.2608	Alcohol dehydrogenase activity	1.3	2.0	1.0	-1.0	1.3	-1.1	1.6	1.7	2.9
<i>ALD4</i>	orf19.6306	Aldehyde dehydrogenase (NAD) activity	-1.4	-1.2	1.2	1.5	1.9	1.9	1.2	1.6	-1.1
<i>ALS10</i>	orf19.2355	Cell adhesion molecule binding	1.2	1.7	1.1	-1.6	-2.0	-2.0	5.9	5.3	8.7
<i>ALS10</i>	orf19.2355	Cell adhesion molecule binding	1.3	1.8	1.2	-1.4	-1.5	-1.6	4.9	4.8	18.0
<i>ALS3.5eoc</i>	orf19.1816	Cell adhesion molecule binding	1.4	1.8	1.2	-1.1	-1.3	-4.9	4.1	4.3	7.9
<i>ALS6</i>	orf19.7414	Cell adhesion molecule binding	1.8	2.7	3.0	-8.1	-10.4	-8.1	1.2	1.1	2.3
<i>AMD21</i>	orf19.5169	Amidase activity	1.0	1.5	-1.2	1.8	2.4	2.1	1.1	-1.0	1.2
<i>ARE2</i>	orf19.2248	Sterol O-acyltransferase activity	-1.3	2.0	1.6	1.6	2.1	1.2	1.8	1.6	3.0
<i>ARO9</i>	orf19.1237	Aromatic-amino-acid transaminase activity	2.2	2.3	20.0	-1.1	1.0	1.2	-1.0	1.0	-2.5
<i>AUT1</i>	orf19.6020	--	-1.5	-1.1	-1.3	1.6	1.5	1.8	-1.2	-1.5	1.3
<i>BMR1</i>	orf19.5604	Multidrug transporter activity	47.8	152.1	112.2	116.2	181.2	210.3	97.1	169.5	132.3
<i>CBP1</i>	orf19.7323	Steroid binding	1.6	2.1	1.4	1.6	2.0	1.8	1.5	1.5	1.8
<i>CDR11.5f</i>	orf19.918	ATP binding	2.1	2.3	1.4	1.3	1.9	-1.3	1.7	2.3	1.7
<i>CDR12</i>	orf19.2802	ATPase activity	1.7	2.3	2.0	2.4	2.6	2.7	2.1	1.6	-1.3
<i>CFL11</i>	orf19.701	Ferric-chelate reductase activity	1.6	1.7	1.9	-1.7	-1.7	1.3	-4.8	1.7	1.1
<i>CHA12.3f</i>	orf19.1996	L-serine ammonia-lyase activity	-1.3	-1.5	-1.6	-1.1	1.2	1.4	1.7	1.8	2.7
<i>Cirt2</i>	orf19.3820	--	1.1	1.4	-1.1	1.8	1.6	1.8	1.7	1.0	-1.3
<i>CLN2</i>	orf19.1960	Cyclin-dependent protein kinase regulator activity	-1.0	1.4	1.4	-1.6	-1.2	-3.5	1.5	2.0	2.1
<i>CLN21</i>	orf19.6028	Cyclin-dependent protein kinase regulator activity	7.2	2.6	1.9	-3.2	1.1	1.1	1.4	4.7	2.2
<i>CRD1</i>	orf19.4784	Copper ion binding	1.2	1.7	-1.1	-1.7	1.5	1.5	1.5	1.6	1.5
<i>CRN1.3f</i>	orf19.6534.2	Actin filament binding	-1.3	1.1	1.1	1.7	1.8	2.7	1.4	1.4	1.4
<i>CTR9</i>	orf19.7067	RNA polymerase II transcription factor activity	-1.4	-2.0	1.5	1.6	1.6	1.9	1.1	1.1	2.1
<i>DAL1</i>	orf19.5454	Allantoinase activity	1.6	1.2	2.8	2.7	2.4	2.5	2.0	1.5	4.9
<i>DDR48</i>	orf19.4082	--	1.9	4.3	2.3	-1.4	-1.1	1.0	-2.2	-1.2	-1.9
<i>DFR1</i>	orf19.5142	Dihydrofolate reductase activity	1.3	1.1	-1.3	1.9	1.7	2.1	1.5	-1.1	-1.7
<i>DIP51.5f</i>	orf19.2942	Amino acid permease activity	1.7	2.5	1.6	3.0	-1.0	-6.3	-2.0	-1.3	1.0
<i>DLD2</i>	orf19.6755	D-lactate dehydrogenase (cytochrome) activity	1.3	1.2	-1.1	2.9	2.2	1.9	1.2	2.1	-1.2
<i>DPB11</i>	orf19.1434	Epsilon DNA polymerase activity	1.3	1.7	1.6	1.9	1.5	2.0	1.4	1.1	-3.3
<i>DSK2</i>	orf19.5345	Protein binding	1.3	-1.2	-1.3	1.5	1.8	1.7	1.3	1.1	1.0
<i>DURI.2</i>	orf19.780	Allophanate hydrolase activity	1.7	1.9	2.2	2.1	4.0	2.6	1.7	2.0	1.5
<i>EBP4</i>	orf19.3433	NADPH dehydrogenase activity	1.1	2.4	2.8	1.8	2.5	2.0	7.9	8.0	11.4
<i>ECE1</i>	orf19.3374	--	25.6	16.4	38.9	1.4	1.1	2.4	2.7	-1.3	2.1
<i>ECM29.5f</i>	orf19.6773	--	2.6	1.6	1.5	-1.0	-1.0	-22.7	-1.1	1.0	2.7
<i>ERG24</i>	orf19.1598	Delta 14-sterol reductase activity	1.5	1.6	1.6	1.4	1.6	1.4	1.0	-1.2	1.5

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>ERG26</i>	orf19.2909	C-3 sterol dehydrogenase (C-4 sterol decarboxylase) activity	1.6	1.5	2.1	-1.4	1.4	1.8	-1.3	-1.2	-1.3
<i>ERG27</i>	orf19.3240	3-keto sterol reductase activity	1.5	1.6	1.9	1.1	1.2	1.1	1.0	-1.1	-1.4
<i>EXG2</i>	orf19.2952	Glucan 1,3-beta-glucosidase activity	2.2	2.4	1.8	1.9	2.0	1.6	1.3	1.5	-1.0
<i>FAA24</i>	orf19.7156	Long-chain-fatty-acid-CoA ligase activity	1.4	2.7	1.4	2.3	1.9	3.0	-1.3	-1.1	2.5
<i>FEN2</i>	orf19.5535	Pantothenate transporter activity	1.5	2.1	1.5	1.7	1.4	1.1	-1.1	-1.0	-1.4
<i>FET34.3eoc</i>	orf19.4215	Ferroxidase activity	-2.2	-1.4	-1.0	1.6	1.6	4.0	-1.9	-2.5	-1.7
<i>FMS1</i>	orf19.4589	--	1.7	1.6	-1.0	2.6	2.4	2.3	1.0	1.2	-1.3
<i>FRE30.3f</i>	orf19.6139	Ferric-chelate reductase activity	-1.8	1.3	-2.5	1.5	1.5	1.5	1.6	1.5	1.9
<i>FRE30.53f</i>	orf19.6140	Ferric-chelate reductase activity	-1.2	1.2	-1.8	2.7	1.6	-5.2	1.5	2.0	1.7
<i>FRE7</i>	orf19.7077	Ferric-chelate reductase activity	-1.5	1.2	-2.1	1.7	1.7	2.8	-1.0	1.3	2.4
<i>FRP2</i>	orf19.6169	--	1.7	7.4	2.5	1.1	-1.5	-1.5	-1.8	1.3	-1.4
<i>FTH2</i>	orf19.3227	Iron ion transporter activity	1.5	1.9	2.3	-1.2	1.2	2.1	1.2	1.3	1.4
<i>GAL1</i>	orf19.3670	Galactokinase activity	1.5	2.4	2.4	-1.5	-1.4	-1.4	-1.5	-1.2	-1.2
<i>GBA1</i>	orf19.4015	GTPase activity	-1.6	-1.2	1.3	41.8	21.8	20.5	-1.2	-1.2	1.3
<i>GDI1</i>	orf19.7261	Rab GDP-dissociation inhibitor activity	1.4	1.6	-1.1	1.7	1.6	2.0	1.2	1.1	-1.1
<i>GPH1</i>	orf19.7021	Glycogen phosphorylase activity	-1.2	1.5	-1.7	-1.5	1.3	1.5	1.6	1.6	3.8
<i>GPR1</i>	orf19.1944	G-protein coupled receptor activity	2.5	1.6	1.8	1.5	-1.7	-1.7	-1.6	-1.2	-1.0
<i>GPX1</i>	orf19.86	Glutathione peroxidase activity	2.1	3.5	5.2	3.1	3.5	4.9	2.4	3.2	8.8
<i>GRP2</i>	orf19.4309	Oxidoreductase activity	3.0	5.3	3.4	4.9	6.1	5.5	7.6	8.6	18.6
<i>HBS1</i>	orf19.7144	--	-1.1	-1.0	-1.4	1.7	1.6	1.7	-1.2	1.5	1.4
<i>HEM1</i>	orf19.2601	5-aminolevulinate synthase activity	1.7	2.2	1.8	-1.4	1.1	-1.7	-1.2	1.1	1.1
<i>HGT11</i>	orf19.4527	Glucose transporter activity	3.0	4.4	1.7	1.7	-1.3	-1.6	-2.1	-1.5	-2.5
<i>HGT12</i>	orf19.3668	Glucose transporter activity	2.4	14.0	4.4	6.8	2.6	1.8	-3.1	-3.1	-3.0
<i>HIK1</i>	orf19.5181	Osmosensor activity	1.6	1.8	1.6	3.8	1.5	-1.6	1.2	1.3	2.0
<i>HIK1</i>	orf19.5181	Osmosensor activity	2.5	1.8	1.8	2.5	1.4	-7.0	-1.2	1.5	1.7
<i>HWP1</i>	orf19.1321	--	1.5	3.0	1.5	1.0	-1.1	-1.1	3.2	1.4	-1.6
<i>IFA18.53f</i>	orf19.4508	--	2.0	1.8	1.6	-1.2	1.3	-1.2	1.6	-1.5	-2.0
<i>IFA24.3eoc</i>	orf19.156	--	1.1	1.4	2.2	1.7	2.1	2.8	-1.1	-1.4	2.3
<i>IFD2</i>	orf19.771	Aryl-alcohol dehydrogenase activity	13.8	14.7	23.7	5.4	8.2	10.9	3.5	3.8	5.1
<i>IFD4</i>	orf19.4477	Aryl-alcohol dehydrogenase activity	21.3	37.9	75.5	81.0	115.9	149.7	129.0	97.7	212.5
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	76.2	200.7	141.5	151.6	195.9	299.8	263.9	333.2	247.6
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	140.8	361.9	144.3	137.7	155.8	201.1	307.3	274.7	964.2
<i>IFD6</i>	orf19.4476	Aryl-alcohol dehydrogenase activity	965.7	1290.0	3064.8	565.4	818.4	1916.6	1220.6	2580.4	2890.9
<i>IFD7</i>	orf19.629	Aryl-alcohol dehydrogenase activity	113.1	338.2	332.4	55.2	66.8	139.0	575.5	311.7	656.4
<i>IFK2</i>	orf19.856	--	2.0	1.4	1.2	1.5	1.7	2.2	1.8	-1.2	1.2
<i>IFK2</i>	orf19.856	--	2.6	2.5	4.3	1.8	-1.0	-1.3	1.3	-1.3	5.2
<i>IFK3.3f</i>	orf19.857	--	2.0	2.2	2.2	1.1	1.3	1.8	-1.4	-1.4	1.5
<i>IFM1</i>	orf19.5167	Translation initiation factor activity	1.8	-1.3	-1.0	2.6	1.7	2.7	1.2	-1.1	1.5
<i>IFU6.5f</i>	orf19.5167	--	2.2	2.8	1.8	1.5	2.0	1.6	-1.3	1.3	-1.4
<i>IFV9.5f</i>	orf19.3144	--	1.7	1.7	1.6	2.6	-1.1	-8.1	-1.0	1.1	1.9
<i>INO1</i>	orf19.7585	Inositol-3-phosphate synthase activity	1.1	1.8	1.0	1.9	2.3	1.6	-1.2	1.2	1.2

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF1009</i>	orf19.4590	--	1.2	1.5	1.9	-1.4	-1.3	-1.2	2.1	2.0	1.7
<i>IPF10124</i>	orf19.276	--	1.7	1.8	1.5	1.1	1.0	-1.2	1.3	1.8	4.6
<i>IPF10168</i>	orf19.643	--	1.9	1.5	2.0	2.0	1.5	1.5	-1.3	-1.1	2.5
<i>IPF10262</i>	orf19.2726	--	1.2	1.9	8.3	1.7	2.3	1.8	-1.0	1.7	2.2
<i>IPF10300</i>	orf19.2274	--	2.1	1.7	1.9	2.0	-1.5	-2.1	1.0	1.3	2.0
<i>IPF10333</i>	orf19.1835	Cytoskeletal regulatory protein binding	1.6	1.5	1.7	1.2	-1.7	-1.3	1.1	2.3	-1.9
<i>IPF10394</i>	orf19.3364	--	1.0	-1.1	1.9	1.5	1.7	2.9	1.5	1.5	2.1
<i>IPF10595</i>	orf19.1287	--	-1.7	-1.4	-1.1	-1.3	1.0	1.6	2.1	1.7	1.7
<i>IPF10735</i>	orf19.6790	RNA binding	1.9	2.9	5.0	-1.0	1.2	-1.0	-1.1	-1.1	-1.2
<i>IPF10795</i>	orf19.2356	Transcription factor activity	18.2	-1.3	1.4	2.1	1.6	1.9	1.8	2.0	-1.9
<i>IPF11006</i>	orf19.4658	--	1.6	1.1	1.5	3.4	2.0	4.9	-1.0	1.1	3.2
<i>IPF11101</i>	orf19.4615	--	1.2	1.6	1.6	1.9	1.6	1.7	-1.1	1.3	-1.1
<i>IPF11161.3</i>	orf19.3201	Transcription factor activity	1.9	-1.5	-2.6	4.4	2.0	2.6	-1.2	-1.1	1.5
<i>IPF11167</i>	orf19.3198	Oxysterol binding	2.2	3.2	-1.7	1.8	1.8	2.6	1.1	1.0	1.1
<i>IPF11118</i>	orf19.5422	--	-1.5	1.0	-1.2	1.0	1.2	-1.8	3.4	2.4	7.3
<i>IPF11222</i>	orf19.5840	--	-1.1	1.0	1.3	-1.1	-1.0	-1.2	2.3	2.7	4.6
<i>IPF11432</i>	orf19.6463	--	-1.3	-1.8	-1.1	1.8	1.7	1.9	-1.2	-1.1	1.3
<i>IPF11435</i>	orf19.6461	--	-1.1	1.4	1.5	2.6	1.6	1.5	1.5	-1.1	-1.1
<i>IPF11694</i>	orf19.4355	Nicotinamide mononucleotide permase activity	1.8	9.9	8.4	-1.4	-1.1	-1.2	-2.1	-2.0	1.4
<i>IPF11821</i>	orf19.6552	Thiol oxidase activity	-2.0	-1.4	1.3	1.9	2.0	3.2	1.4	-1.1	2.3
<i>IPF1264</i>	orf19.7371	Transcription factor activity	3.8	3.2	2.8	2.0	1.7	1.2	1.5	2.2	2.4
<i>IPF1272</i>	orf19.7376	--	-1.5	-3.7	2.4	2.3	1.5	2.0	1.2	1.3	1.5
<i>IPF12963</i>	orf19.5094	--	2.6	3.3	2.8	-1.3	1.4	-1.4	1.7	2.1	21.4
<i>IPF1321</i>	orf19.6413	--	1.7	-1.1	-1.2	4.8	2.8	1.9	1.0	1.0	1.9
<i>IPF13319</i>	orf19.740	Transcriptional activator activity	2.5	1.9	2.1	1.6	-1.8	-1.4	-1.7	-1.3	-1.4
<i>IPF13423</i>	orf19.4128	--	2.1	1.4	1.2	1.7	2.2	3.9	1.9	-1.9	-5.0
<i>IPF13609</i>	orf19.6526	--	-1.8	-1.3	1.1	1.8	1.6	1.8	1.3	1.3	1.8
<i>IPF13723</i>	orf19.260	--	1.5	1.5	1.6	-1.5	2.5	1.9	1.5	-1.2	1.7
<i>IPF13883</i>	orf19.5502	--	-1.3	-1.4	-1.6	2.3	1.9	1.7	1.4	1.4	-1.9
<i>IPF14030</i>	orf19.851	--	-1.5	-1.0	-1.9	5.7	2.4	1.6	-1.3	-1.2	-4.4
<i>IPF14119</i>	orf19.4688	--	-1.6	-1.2	1.4	1.2	1.2	-1.2	1.8	2.1	2.6
<i>IPF14379</i>	orf19.4474	--	-1.1	-4.2	3.0	1.6	1.5	2.9	1.3	-1.2	1.2
<i>IPF14448</i>	orf19.5601	--	2.1	1.3	1.8	1.6	1.7	3.3	1.2	-1.1	-1.5
<i>IPF14968</i>	orf19.3461	--	1.7	1.5	2.0	1.6	1.1	1.4	-1.1	1.3	-1.3
<i>IPF14981</i>	orf19.3483	--	5.1	1.8	2.0	3.3	1.8	3.0	1.8	1.1	1.7
<i>IPF15255</i>	orf19.4884	Transcription regulator activity	2.1	3.3	1.6	-1.7	-3.8	-1.7	1.2	-2.1	1.2
<i>IPF15273</i>	orf19.1822	Transcription factor activity	2.5	2.1	2.1	-1.4	-1.8	-1.3	1.4	4.8	-2.1
<i>IPF15890</i>	orf19.6349	Cytoskeletal protein binding	1.9	1.5	1.6	1.1	3.0	2.9	1.5	-1.4	1.3
<i>IPF1621</i>	orf19.6585	--	-1.9	1.1	-1.1	2.8	2.4	2.8	-1.2	1.4	5.8
<i>IPF1632</i>	orf19.6579	--	1.2	-1.3	1.0	1.5	1.6	1.6	1.3	-1.1	-1.5
<i>IPF16320</i>	orf19.5131	--	-1.1	1.1	1.0	1.6	2.6	2.2	1.5	1.3	-1.1

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF16323</i>	orf19.6460	ATPase activity	1.7	-1.8	2.3	3.1	1.6	1.6	1.7	-1.8	-1.2
<i>IPF1651</i>	orf19.6570	Nucleoside permease activity	-1.4	1.2	-1.4	3.8	2.7	2.5	-1.3	1.2	1.6
<i>IPF1674</i>	orf19.6559	--	-1.0	-1.1	-1.8	2.1	3.2	2.7	1.5	-1.0	-1.3
<i>IPF1674</i>	orf19.6559	--	1.0	1.4	-1.5	1.9	2.5	3.2	1.6	-1.3	-1.0
<i>IPF17186</i>	orf19.251	--	20.7	38.9	39.7	9.2	14.8	14.6	20.8	31.4	21.0
<i>IPF17255</i>	orf19.696	Mating-type alpha-factor pheromone receptor activity	-1.5	5.6	-3.4	8.6	41.6	17.3	-3.9	-1.9	-4.3
<i>IPF18161</i>	orf19.6587	--	-2.1	-2.4	1.1	2.6	1.6	2.0	1.2	1.1	-2.0
<i>IPF18316</i>	orf19.5145	--	1.3	1.4	1.5	4.0	2.2	1.5	-1.6	-1.1	-1.6
<i>IPF1837</i>	orf19.5877	Alcohol O-acetyltransferase activity	2.1	1.9	1.9	1.7	1.6	2.1	3.8	4.3	6.0
<i>IPF18385</i>	orf19.4143	--	4.4	3.4	1.4	1.0	2.4	1.0	1.5	1.5	3.0
<i>IPF18418</i>	orf19.5620	--	1.3	2.4	2.4	2.0	2.8	1.7	2.4	2.7	3.6
<i>IPF1862.5f</i>	orf19.5894	--	1.7	1.3	-1.6	2.4	1.7	2.1	-7.7	1.9	1.4
<i>IPF18641.5f</i>	orf19.3159	--	1.9	1.9	14.6	-1.5	-2.2	-9.5	-1.1	-1.2	2.0
<i>IPF1899</i>	orf19.7131	--	-1.1	1.5	-1.5	2.0	1.8	2.1	1.0	1.1	2.1
<i>IPF1912</i>	orf19.7140	--	-1.6	-1.3	-1.6	1.8	1.7	1.9	-1.5	1.2	1.6
<i>IPF19295.5f</i>	orf19.4649	--	-1.3	-1.5	-2.7	1.9	3.0	2.2	1.4	1.0	-1.8
<i>IPF19513</i>	orf19.6476	--	-1.0	-1.4	-1.2	1.5	1.6	1.7	-1.5	-1.0	-1.3
<i>IPF19538.3f</i>	orf19.6957.3	--	2.2	1.7	6.0	8.5	4.4	2.3	3.1	2.2	2.7
<i>IPF19908</i>	orf19.1344	--	1.2	2.7	1.8	3.2	2.2	4.2	-1.6	-1.5	-1.0
<i>IPF19997</i>	orf19.6458.1	--	-1.6	-1.3	1.5	1.9	1.8	2.4	1.5	1.5	1.9
<i>IPF19998</i>	orf19.2175	Oxidoreductase activity	1.0	1.1	-1.0	1.6	1.9	1.8	1.3	1.2	1.8
<i>IPF20010</i>	orf19.5799	--	1.9	1.9	1.6	1.6	1.5	1.4	1.0	1.2	-1.4
<i>IPF20015</i>	orf19.499	--	1.5	1.6	1.5	1.1	-1.1	-1.1	-1.2	-1.3	-1.3
<i>IPF20157</i>	orf19.6696	--	1.9	3.8	2.0	1.2	2.4	10.5	1.8	1.6	-10.0
<i>IPF2021</i>	orf19.5245	--	1.6	2.2	1.6	-1.3	-1.2	-1.4	-1.3	1.4	2.2
<i>IPF2050</i>	orf19.5265	Structural constituent of cytoskeleton	1.7	1.7	2.3	1.2	-1.0	-1.3	1.4	1.5	-1.9
<i>IPF2130</i>	orf19.7204	--	1.8	1.8	-1.4	17.2	37.0	15.2	5.0	7.4	3.0
<i>IPF2150</i>	orf19.7193	--	-1.1	1.2	-1.1	1.7	1.8	2.8	-1.3	1.1	-1.3
<i>IPF2170</i>	orf19.7179	--	1.1	-1.2	-1.1	3.0	1.5	2.5	2.5	-1.7	-1.0
<i>IPF2175</i>	orf19.7175	--	1.1	-1.1	-1.2	1.5	1.6	1.6	1.1	-1.1	-1.1
<i>IPF2186</i>	orf19.7166	--	3.0	6.0	7.3	14.0	15.8	11.7	11.8	11.1	22.5
<i>IPF2189</i>	orf19.7165	--	1.1	-1.5	-1.1	3.1	2.5	1.9	1.2	1.3	-1.0
<i>IPF2190</i>	orf19.7164	--	-1.5	1.4	-1.5	1.5	1.7	2.0	-1.2	-1.4	-1.0
<i>IPF2228</i>	orf19.6902	ATP-dependent RNA helicase activity	-1.2	-1.8	-1.0	2.1	1.5	1.6	-1.0	1.0	1.7
<i>IPF2280</i>	orf19.6658	--	1.1	2.6	-1.0	1.9	2.0	1.5	-1.2	1.1	-1.3
<i>IPF2361</i>	orf19.7023	--	1.2	-1.5	1.1	1.6	2.5	1.7	1.2	1.5	2.2
<i>IPF2557</i>	orf19.6627	--	-1.1	1.3	1.1	1.5	1.7	1.7	1.4	2.0	2.2
<i>IPF2582</i>	orf19.5504	--	-1.3	-1.7	-1.1	1.8	1.6	2.8	1.2	1.4	1.8
<i>IPF2905</i>	orf19.7457	--	1.8	-1.4	1.4	1.7	2.5	2.0	-1.4	-1.2	1.1
<i>IPF300</i>	orf19.3282	--	1.0	-1.3	1.2	1.5	2.2	1.7	-1.1	1.4	3.6
<i>IPF3014</i>	orf19.5030	--	2.2	1.6	2.3	1.4	1.4	-1.5	1.2	-1.4	3.1

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF3069</i>	orf19.7034	--	1.0	1.3	-1.2	1.6	1.5	1.5	-1.1	1.4	1.5
<i>IPF3080</i>	orf19.7042	--	2.3	1.0	2.7	6.2	4.7	5.5	10.7	11.3	12.2
<i>IPF3081</i>	orf19.7043	--	-1.2	-1.1	1.4	1.5	2.3	2.9	1.1	-1.6	1.6
<i>IPF3121</i>	orf19.4438	Transcription factor activity	-1.1	1.9	-1.1	3.0	8.0	3.9	1.1	1.1	-7.2
<i>IPF3277</i>	orf19.4384	Fructose transporter activity	2.5	5.3	2.3	-1.1	1.2	-2.0	-1.5	-1.4	-1.5
<i>IPF3277</i>	orf19.4384	Fructose transporter activity	1.7	4.0	1.7	-1.0	-1.8	-3.3	-1.4	-1.2	-1.7
<i>IPF3282.3eoc</i>	orf19.4384	Fructose transporter activity	1.7	4.5	1.6	-1.3	-1.7	-2.5	-1.6	-1.3	-1.6
<i>IPF3352</i>	orf19.4013	--	1.1	1.3	1.3	1.8	2.2	2.8	-1.0	-1.1	-1.0
<i>IPF3491</i>	orf19.6753	--	1.0	1.3	1.3	1.8	1.7	1.5	-1.1	1.1	-1.1
<i>IPF3535</i>	orf19.6840	--	1.8	2.2	4.2	2.4	1.7	1.8	-1.6	-1.5	1.7
<i>IPF3679</i>	orf19.889	Transcription factor activity	2.3	1.5	1.8	1.0	1.0	-1.0	-1.6	-1.6	-1.1
<i>IPF3836</i>	orf19.279	Hydrolase activity, acting on ester bonds	-1.2	1.2	-1.4	-1.1	-1.1	1.5	1.5	1.5	1.7
<i>IPF3866</i>	orf19.21	--	-1.4	1.0	-1.0	2.0	1.6	1.6	-1.0	1.3	1.5
<i>IPF3876</i>	orf19.775	--	1.9	1.8	1.1	1.5	1.9	2.9	1.6	1.0	1.3
<i>IPF3927</i>	orf19.6506	--	1.3	-1.6	-1.4	1.6	1.5	3.1	1.0	1.1	-1.5
<i>IPF4059</i>	orf19.1865	--	1.5	1.6	1.5	1.3	1.3	1.3	-1.1	1.0	1.1
<i>IPF4325</i>	orf19.5523	--	1.5	1.6	1.9	1.5	2.3	2.9	1.1	-1.4	-1.8
<i>IPF448</i>	orf19.7051	--	-1.8	-1.7	1.6	2.6	2.2	1.9	-1.6	-1.2	6.7
<i>IPF4567.3f</i>	orf19.6525	--	-1.1	-1.1	-1.2	1.9	1.5	1.9	-1.3	-1.1	-1.1
<i>IPF474</i>	orf19.7060	--	1.4	1.6	-2.0	1.5	2.0	2.0	-1.3	-1.3	-1.2
<i>IPF480</i>	orf19.7063	--	-1.4	-1.0	-1.2	2.0	1.8	1.8	-1.1	-1.0	1.2
<i>IPF4835</i>	orf19.5992	Transcription factor activity	1.3	1.3	1.1	2.8	2.0	3.9	2.3	1.7	1.7
<i>IPF4890</i>	orf19.6263	Monocarboxylic acid transporter activity	-1.1	1.1	-1.0	-1.8	-1.0	1.0	1.5	1.7	3.4
<i>IPF511</i>	orf19.7078	--	-1.6	1.2	1.2	1.9	1.7	1.8	1.0	1.2	1.8
<i>IPF525</i>	orf19.7085	--	1.3	1.4	1.0	2.5	1.7	2.3	1.5	1.4	-1.6
<i>IPF538</i>	orf19.7091	--	-1.1	-1.4	-1.2	1.5	1.7	2.3	1.0	-1.0	1.2
<i>IPF539</i>	orf19.7092	--	1.3	1.4	-1.1	1.5	1.7	2.0	1.2	1.2	1.2
<i>IPF5426</i>	orf19.6378	--	-1.1	-1.3	-1.2	1.7	1.5	2.3	1.5	-1.0	-1.6
<i>IPF553</i>	orf19.7096	--	-1.1	1.4	-1.3	1.5	1.9	1.9	-1.2	-1.1	1.1
<i>IPF5567</i>	orf19.5160	Protein phosphatase regulator activity	1.0	-1.4	1.1	2.0	1.7	2.4	1.3	1.0	1.4
<i>IPF5625</i>	orf19.7112	Ferric-chelate reductase activity	1.4	1.3	1.8	2.0	2.2	2.8	-1.3	-1.2	1.3
<i>IPF568</i>	orf19.7105	Cyclin-dependent protein kinase inhibitor activity	1.3	1.1	-2.0	30.5	21.8	21.0	-1.1	1.1	-1.5
<i>IPF5723</i>	orf19.6329	--	-1.4	1.2	-1.3	-2.4	-1.3	-1.3	1.6	1.5	3.7
<i>IPF5987</i>	orf19.7306	--	48.6	49.5	63.3	41.5	50.1	81.4	45.5	38.2	35.7
<i>IPF6032</i>	orf19.4607	--	1.6	2.0	3.3	1.7	1.6	1.4	1.5	2.0	1.6
<i>IPF6041</i>	orf19.4612	--	-1.3	1.6	1.2	1.7	2.3	2.2	2.2	-1.2	3.1
<i>IPF6050</i>	orf19.6737	--	1.5	1.5	2.0	-1.1	-1.4	-1.8	1.0	1.0	1.3
<i>IPF610</i>	orf19.7631	--	1.5	1.8	1.6	-1.0	-1.2	-1.6	-1.2	1.5	-1.1
<i>IPF6203</i>	orf19.4166	--	1.6	2.1	1.5	1.1	-1.1	1.2	-1.0	1.2	-1.4
<i>IPF6235</i>	orf19.5372	--	1.0	1.2	-1.1	1.9	3.4	3.4	-1.0	1.1	4.5
<i>IPF6238</i>	orf19.2374	--	1.2	1.0	1.3	2.2	3.4	2.8	1.3	1.0	3.9

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF6318</i>	orf19.1664	--	1.0	1.4	-1.2	1.5	1.9	3.5	1.5	-1.1	1.3
<i>IPF643</i>	orf19.7610	Protein tyrosine phosphatase activity	1.3	-1.1	1.2	-1.3	1.1	1.8	1.9	1.7	3.0
<i>IPF6444</i>	orf19.1900	--	1.2	-1.3	1.4	2.3	3.1	1.6	1.3	1.1	-1.5
<i>IPF6518</i>	orf19.1691	--	1.5	3.5	3.5	2.0	3.2	4.4	1.2	-1.2	2.6
<i>IPF7171.3f</i>	orf19.5372	--	1.9	2.2	1.8	2.6	-1.2	-1.0	-1.1	-1.2	-2.0
<i>IPF730</i>	orf19.5362	--	-1.1	-1.1	-1.9	13.3	3.7	3.9	1.1	-1.1	-1.5
<i>IPF7397</i>	orf19.1800	--	2.2	1.9	2.4	1.6	1.5	1.3	-1.2	-1.6	-2.5
<i>IPF7456</i>	orf19.2047	--	-1.2	1.7	-1.1	1.6	2.1	1.7	1.3	1.6	1.4
<i>IPF7493</i>	orf19.4682	Glucose transporter activity	1.5	11.3	9.5	5.8	2.1	3.2	-2.8	-2.0	-2.7
<i>IPF7602</i>	orf19.6837	--	-1.1	1.1	-1.3	-1.8	-1.0	1.1	1.6	1.8	1.9
<i>IPF7666</i>	orf19.4056	Transcription factor activity	2.5	3.9	3.3	2.3	1.0	1.3	3.2	2.3	3.5
<i>IPF7686</i>	orf19.1395	Inorganic phosphate transporter activity	1.9	3.3	2.3	1.4	1.3	-1.0	2.6	2.0	5.0
<i>IPF7686</i>	orf19.1395	Inorganic phosphate transporter activity	1.7	2.3	1.9	-1.2	1.2	1.4	-1.1	1.1	-1.0
<i>IPF7770</i>	orf19.3881	--	1.5	1.6	-1.1	1.8	1.6	2.1	1.0	1.0	-1.7
<i>IPF7817</i>	orf19.3131	NADPH dehydrogenase activity	9.4	13.7	13.4	7.7	11.0	8.2	11.8	13.3	84.2
<i>IPF7863</i>	orf19.1449	--	6.1	9.4	9.1	7.2	7.2	5.7	6.5	9.5	5.7
<i>IPF7940</i>	orf19.6608	--	5.4	4.3	10.3	4.3	5.2	20.2	6.4	2.6	-1.0
<i>IPF7947</i>	orf19.6602	--	-1.5	-1.2	2.1	1.6	1.5	1.9	1.3	-1.1	1.6
<i>IPF7968</i>	orf19.2693	Transcription corepressor activity	-1.1	1.4	1.3	1.5	1.7	1.8	1.4	1.5	-1.1
<i>IPF8016</i>	orf19.1336.2	--	-1.0	-1.9	1.8	1.7	1.7	4.3	2.2	-1.2	13.7
<i>IPF8024</i>	orf19.1334	--	-1.5	-1.4	-1.3	1.6	1.8	2.3	-1.2	-1.0	1.0
<i>IPF8067</i>	orf19.3697	--	1.6	1.4	2.6	3.0	2.4	1.7	3.5	1.5	1.2
<i>IPF8147</i>	orf19.6200	--	2.4	8.5	1.6	-2.6	-1.9	3.6	2.6	1.2	-1.7
<i>IPF8378</i>	orf19.6072	--	1.9	-1.4	1.3	1.9	1.5	1.6	1.2	1.1	1.1
<i>IPF8439</i>	orf19.6498	--	1.3	-1.0	-1.5	1.7	1.8	2.7	-1.5	-1.1	1.2
<i>IPF8591</i>	orf19.5862	Arginase activity	2.5	3.1	7.0	1.0	2.0	1.9	1.8	-1.3	-1.2
<i>IPF8627</i>	orf19.3969	Specific transcriptional repressor activity	2.8	2.7	1.8	1.2	-1.1	1.3	1.3	1.3	2.3
<i>IPF867</i>	orf19.7221	NAD-dependent histone deacetylase activity	2.4	1.6	1.5	2.1	1.5	1.6	-1.1	1.0	-2.0
<i>IPF8951</i>	orf19.4897	--	1.7	1.3	2.1	2.1	2.1	2.3	2.0	1.6	-1.1
<i>IPF8970</i>	orf19.496	--	2.8	2.1	1.4	3.6	2.5	2.9	1.0	-1.1	-1.6
<i>IPF8976</i>	orf19.1369	--	5.2	5.7	14.9	6.2	7.3	4.0	1.2	2.3	-1.2
<i>IPF9370</i>	orf19.1424	--	1.6	2.0	2.1	1.1	1.2	1.3	-1.3	1.2	-4.2
<i>IPF9410</i>	orf19.6148	Structural constituent of cytoskeleton	2.5	1.6	1.7	1.4	1.4	1.1	-1.8	-1.1	-2.1
<i>IPF9450</i>	orf19.4370	--	-1.1	1.1	-1.0	2.3	1.6	1.9	1.4	1.9	1.3
<i>IPF9496</i>	orf19.2809	Carnitine O-acetyltransferase activity	2.3	5.8	2.5	2.2	1.5	2.9	-2.2	-2.2	-1.2
<i>IPF9538</i>	orf19.271	2,4-dienoyl-CoA reductase (NADPH) activity	108.9	279.5	131.7	7.8	24.5	18.1	29.2	29.3	27.6
<i>IPF9704</i>	orf19.4882	General RNA polymerase II transcription factor activity	1.5	-1.3	1.2	1.5	1.8	2.1	1.2	-1.0	1.5
<i>IPF9748</i>	orf19.746	--	2.8	-1.2	1.5	1.5	1.8	3.8	1.5	-1.5	-2.1
<i>IPF9828</i>	orf19.6861	Protein binding	1.1	-2.5	-1.1	1.8	1.9	3.5	1.1	-1.7	-2.4
<i>IPF9887</i>	orf19.1240	--	2.8	3.1	3.8	2.9	4.2	5.8	4.1	4.2	5.5
<i>IQG1</i>	orf19.6536	Cytoskeletal protein binding	-1.0	-1.0	1.2	1.7	1.8	2.8	1.1	1.2	2.7

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>KAP114</i>	orf19.7086	Protein carrier activity	-1.1	-1.2	-1.1	1.5	1.9	2.5	1.0	-1.1	1.9
<i>KEX1</i>	orf19.7020	Carboxypeptidase D activity	1.0	1.0	-1.0	1.5	1.8	2.2	1.3	1.4	3.1
<i>KIN3</i>	orf19.5325	Protein kinase activity	-1.3	-1.2	-1.1	2.0	1.8	3.6	1.4	1.4	-1.1
<i>LPI9</i>	orf19.6544	--	1.1	1.0	-1.3	2.2	1.8	1.8	-1.1	1.2	-4.2
<i>LYS21</i>	orf19.4506	Homocitrate synthase activity	-2.3	-2.5	-1.2	-1.2	-1.2	-1.2	1.5	2.8	3.4
<i>MDM12</i>	orf19.6900	--	1.4	-1.2	-1.1	2.0	1.7	2.3	1.3	-1.3	1.2
<i>MDN1</i>	orf19.4697	ATPase activity	2.5	1.6	3.7	2.1	-1.2	-1.7	1.1	-1.3	-1.0
<i>MET13</i>	orf19.288	Structural constituent of ribosome	-1.0	-1.2	1.4	-4.1	-1.4	-1.3	1.8	1.7	2.8
<i>MGM1</i>	orf19.2690	GTPase activity	1.1	-1.1	1.4	1.7	1.5	2.2	1.4	1.1	1.5
<i>MRE11</i>	orf19.6915	3'-5' exonuclease activity	1.2	-1.3	-1.2	2.0	1.9	1.7	-1.0	1.0	2.1
<i>MRF1</i>	orf19.1149	DNA binding	1.6	1.7	2.7	-1.7	-1.1	1.1	-1.0	-1.0	1.2
<i>MRR1</i>	orf19.7372	RNA polymerase II transcription factor activity	2.8	1.8	5.7	2.9	1.8	1.9	2.3	3.4	4.7
<i>MSD1</i>	orf19.4478	Aspartate-tRNA ligase activity	1.1	1.0	-1.1	1.5	1.5	1.8	-1.0	-1.1	-1.2
<i>MSK1</i>	orf19.6533	Lysine-tRNA ligase activity	-1.1	-1.0	-1.2	1.5	1.6	2.4	1.0	1.0	-1.3
<i>NDH2</i>	orf19.5713	NADH dehydrogenase activity	2.0	2.6	1.6	2.9	4.0	4.7	2.1	2.5	10.9
<i>OPT1</i>	orf19.2602	Oligopeptide transporter activity	-1.6	1.1	-2.2	-4.5	-2.4	-4.4	1.8	2.4	3.0
<i>PAP11</i>	orf19.3197	RNA binding	4.7	-3.8	-1.5	2.2	2.3	2.5	-1.3	1.5	2.2
<i>PET112</i>	orf19.2494	--	1.2	1.4	-1.4	2.4	1.5	1.7	1.4	1.1	1.3
<i>PET8</i>	orf19.7082	Transporter activity	-1.0	1.1	-1.2	1.5	1.7	2.3	1.1	1.1	-2.5
<i>PEX17</i>	orf19.3605	--	2.5	1.6	1.5	1.7	1.2	1.6	-1.5	-1.4	1.1
<i>PEX4</i>	orf19.4041	Ubiquitin conjugating enzyme activity	-1.0	1.8	-1.5	1.5	1.5	1.8	-1.6	-1.1	-3.6
<i>PGA10</i>	orf19.5674	Heme binding	1.8	4.3	1.2	2.0	3.9	3.2	1.2	-1.1	-1.1
<i>PGA26</i>	orf19.2475	--	-3.2	-1.4	-1.6	2.8	6.0	2.9	1.5	1.1	1.4
<i>PHR1</i>	orf19.3829	1,3-beta-glucanosyltransferase activity	1.9	2.4	1.5	-1.1	-1.4	-2.1	-1.0	1.4	1.2
<i>PIB1</i>	orf19.7547	Ubiquitin-protein ligase activity	1.5	1.5	-1.4	1.7	2.0	2.9	-1.0	-1.3	-1.5
<i>PLB4.3f</i>	orf19.1442	Lysophospholipase activity	-1.8	1.1	1.0	1.9	2.6	4.3	-1.2	-1.3	-1.2
<i>POL0</i>	orf19.5373	--	1.4	1.3	1.4	3.0	4.2	3.9	-1.0	-1.0	1.7
<i>POL3</i>	orf19.5182	Delta DNA polymerase activity	1.8	1.9	1.6	2.9	1.7	-4.3	1.5	-1.2	-1.2
<i>POT13</i>	orf19.2046	Acetyl-CoA C-acyltransferase activity	1.1	1.8	1.4	1.5	1.5	2.2	-1.1	-1.0	-1.1
<i>PRE10</i>	orf19.6582	Endopeptidase activity	-1.2	-1.3	1.7	1.9	1.9	2.4	1.3	-1.1	-1.2
<i>PRE5</i>	orf19.7178	Endopeptidase activity	-1.2	1.1	-1.0	1.5	1.5	1.6	-1.3	-1.1	-2.2
<i>PRY2</i>	orf19.7218	--	1.1	1.1	-1.1	3.4	2.7	2.6	-1.3	1.1	-1.3
<i>PYC2.3f</i>	orf19.789	Pyruvate carboxylase activity	1.3	-1.3	1.1	-1.4	1.2	1.7	1.6	1.8	1.5
<i>PYC2.5f</i>	orf19.789	Pyruvate carboxylase activity	2.1	1.5	4.8	1.4	1.5	-5.8	1.6	1.7	2.1
<i>RBT1</i>	orf19.1327	--	-1.2	1.2	-1.2	-1.4	-1.3	-2.3	1.8	1.5	2.1
<i>RBT4</i>	orf19.6202	--	3.6	3.3	3.2	1.2	-1.5	-2.4	-1.1	1.4	1.4
<i>RPN4</i>	orf19.1069	Transcription factor activity	2.4	2.6	2.2	2.8	2.8	2.0	1.5	1.4	1.1
<i>SLA2</i>	orf19.7201	Structural constituent of cytoskeleton	-1.6	-1.5	-1.2	2.0	2.2	4.1	1.4	1.1	1.2
<i>SNF31</i>	orf19.7094	Glucose transporter activity	-1.0	14.4	3.2	1.9	1.7	3.4	-3.0	-1.1	-1.5
<i>SNZ1</i>	orf19.2947	--	1.5	2.1	1.9	2.3	-1.2	-1.3	-1.5	1.3	1.0
<i>SOD21</i>	orf19.7111	Manganese superoxide dismutase activity	-1.1	2.7	-2.0	2.0	2.3	1.6	3.2	2.2	2.6

Table B-1 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>SOD21</i>	orf19.7111	Manganese superoxide dismutase activity	-1.0	4.8	-5.0	5.9	2.6	3.1	2.9	2.3	1.7
<i>SOU3</i>	orf19.732	2,4-dienoyl-CoA reductase (NADPH) activity	1.9	2.5	1.5	2.2	2.2	2.0	-1.5	-1.1	-1.4
<i>STL2</i>	orf19.7093	Glucose transporter activity	1.4	1.9	-1.1	1.5	2.0	2.2	-1.3	-1.1	-2.4
<i>TAF19</i>	orf19.5174	General RNA polymerase II transcription factor activity	1.0	1.1	1.2	2.0	1.8	2.1	-1.2	-1.0	-1.5
<i>TEF41</i>	orf19.2652	Translation elongation factor activity	3.4	3.9	13.6	5.0	5.2	7.0	-1.1	-1.5	-1.7
<i>TERT2</i>	orf19.5089	Telomeric template RNA reverse transcriptase activity	2.0	2.5	1.4	8.1	2.7	1.7	-1.6	-2.1	-3.2
<i>TES12</i>	orf19.4122	--	2.4	1.5	1.7	-2.8	-2.1	-2.0	1.2	-1.1	-1.4
<i>TIM9</i>	orf19.6696	Protein transporter activity	-1.3	1.3	1.6	3.0	1.8	3.1	1.2	-1.4	2.1
<i>TOM72</i>	orf19.3700	Protein transporter activity	1.0	-1.5	1.0	1.9	1.7	1.8	1.3	-1.2	3.1
<i>TRP4</i>	orf19.3099	Anthranilate phosphoribosyl transferase activity	1.5	1.2	1.2	1.2	1.2	2.2	1.5	1.7	1.9
<i>UFD1</i>	orf19.5833	--	-1.5	1.0	-1.1	1.5	1.5	2.0	-1.1	1.0	-2.1
<i>UGA2</i>	orf19.345	Succinate-semialdehyde dehydrogenase activity	1.8	2.9	16.0	1.2	1.0	-1.1	2.4	2.5	3.4
<i>UGT51</i>	orf19.2616	Sterol 3-beta-glucosyltransferase activity	1.1	1.8	-4.4	3.3	1.8	4.7	1.2	1.1	5.7
<i>YAR1</i>	orf19.7160	--	-1.6	-1.9	2.2	1.6	1.7	2.5	1.6	-1.0	1.1
<i>YOX1</i>	orf19.7017	--	1.5	1.5	1.7	1.3	1.2	-1.1	1.3	1.9	1.9

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b orf19 nomenclature according to the Assembly 19 version.

^c GO annotation found at CGD (<http://www.candidagenome.org/>).

Table B-2. Genes down-regulated in at least one clinical matched isolate overexpressing *MDR1*.

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>AAT1</i>	orf19.3554	Aspartate transaminase activity	-1.3	1.1	-1.0	-1.5	-1.2	-1.1	-2.1	-1.6	-1.8
<i>AAT21</i>	orf19.6287	Aspartate transaminase activity	-1.4	1.1	-1.2	-1.9	-1.6	-2.0	-1.4	1.0	1.1
<i>ADE1</i>	orf19.7484	Phosphoribosylaminoimidazolesuccino carboxamide synthase activity	-1.7	-1.8	-1.5	-1.3	-1.2	-1.1	-2.1	-1.9	-4.8
<i>ADE13</i>	orf19.3870	Adenylosuccinate lyase activity	-1.7	-1.7	-1.8	-1.4	-1.2	-1.1	-2.2	-1.9	-1.8
<i>ADE2</i>	orf19.5906	Phosphoribosylaminoimidazole carboxylase activity	-1.3	-1.3	-2.2	-1.1	-1.3	-1.4	-2.1	-1.7	-2.3
<i>ADE6</i>	orf19.6317	Phosphoribosylformylglycinamide synthase activity	-1.5	-1.6	-1.5	-1.8	-1.2	1.1	-1.6	-1.7	-1.4
<i>ADE8</i>	orf19.5789	Phosphoribosylglycinamide formyltransferase activity	-2.2	-1.8	-1.5	-1.1	-1.6	-1.7	-2.2	-1.8	-3.1
<i>AGP2</i>	orf19.4679	Hydrogen:amino acid symporter activity	-2.5	-2.0	-2.2	-6.9	-6.0	-7.6	-1.1	1.6	4.6
<i>AKR1</i>	orf19.4950	--	-1.2	-1.2	-2.4	-1.8	1.0	1.5	-1.5	-1.7	-1.6
<i>ALP1</i>	orf19.2337	Basic amino acid transporter activity	-2.8	-2.3	-1.9	-3.3	-1.6	-1.6	1.0	-1.1	-1.0
<i>ALS1</i>	orf19.5741	Cell adhesion molecule binding	-1.9	-1.0	-2.4	-2.3	-2.1	-3.1	1.2	1.6	1.0
<i>ALS6</i>	orf19.7414	Cell adhesion molecule binding	1.0	2.2	1.3	-6.3	-7.2	-1.9	-1.1	-1.2	1.2
<i>AOX1</i>	orf19.4774	Alternative oxidase activity	1.1	-1.9	-2.4	-1.6	-1.3	1.2	-1.6	-2.2	-2.2
<i>AOX2.3f</i>	orf19.4773	Alternative oxidase activity	1.4	1.6	1.7	-3.2	-6.3	-4.9	-2.0	-1.6	1.3
<i>AQY1</i>	orf19.2849	Water channel activity	-3.2	1.1	-2.2	-6.6	-3.3	-2.9	-3.2	-2.3	-2.0
<i>ARO3</i>	orf19.1517	3-deoxy-7-phosphoheptulonate synthase activity	-1.4	1.2	-1.4	-1.5	-1.5	-1.7	-1.0	1.9	1.8
<i>ARR3</i>	orf19.3122	Arsenite transporter activity	-2.1	-1.5	-1.5	-4.5	-2.0	-2.1	-1.5	-1.4	-1.9
<i>AUR1</i>	orf19.1945	Inositol phosphoceramide synthase activity	-1.4	-1.1	-1.0	-3.0	-1.5	-2.3	-1.3	-1.0	1.5
<i>AXL2</i>	orf19.5292	--	1.2	-1.6	3.4	-2.2	-1.7	-1.6	1.6	1.2	-2.7
<i>AYR2</i>	orf19.5615	Acylglycerone-phosphate reductase activity	-1.8	-1.1	-1.7	-3.1	-1.5	-2.1	-1.3	-1.2	-1.5
<i>CAN1</i>	orf19.97	L-arginine transporter activity	-1.7	-1.1	-1.5	-2.8	-1.9	-2.7	-1.8	-2.1	1.1
<i>CAN2</i>	orf19.111	Basic amino acid transporter activity	-1.6	1.0	-4.0	-4.9	-2.8	-4.9	-3.7	-3.0	-6.3
<i>CAP1</i>	orf19.1623	Transcription factor activity	1.2	1.1	-1.0	-1.9	-1.6	-1.8	-1.2	1.2	-1.1
<i>CAT2</i>	orf19.4591	Carnitine O-acetyltransferase activity	-1.0	1.0	-1.3	1.1	-1.4	-1.6	-1.7	-1.9	-2.5
<i>CFL1</i>	orf19.1263	Ferric-chelate reductase activity	-1.5	-1.5	-2.0	-1.4	1.1	-1.5	-1.7	-2.4	-2.4
<i>CPA1</i>	orf19.4630	Carbamoyl-phosphate synthase activity	-1.8	-2.0	-1.9	-2.4	-1.3	-1.7	-1.4	-1.1	-1.3
<i>CPA2</i>	orf19.3221	Carbamoyl-phosphate synthase activity	-1.4	-1.7	1.4	-2.2	-2.4	-1.5	1.1	-1.0	-1.2
<i>CTA1</i>	orf19.6229	Catalase activity	-1.9	1.6	-1.8	-1.6	-2.0	-2.5	-2.3	-1.3	-1.2
<i>CTA211.5f</i>	orf19.362	--	-1.8	-1.7	-1.8	-2.2	-1.3	1.8	-1.2	1.1	17.7
<i>CYB3</i>	orf19.5000	L-lactate dehydrogenase activity	1.4	2.9	2.5	1.2	1.0	-1.1	-2.4	-1.9	-1.7
<i>CYT2</i>	orf19.4578	Holocytochrome-c synthase activity	1.0	1.3	-1.3	-2.4	-2.1	-2.4	-1.0	1.0	-1.2
<i>DAK2</i>	orf19.4777	Glycerone kinase activity	-1.5	-1.6	-2.7	-1.9	1.1	1.7	2.4	1.1	-1.7
<i>DBP2</i>	orf19.171	RNA helicase activity	1.0	-1.4	1.1	-1.5	-1.8	-3.3	-1.0	1.3	-1.1
<i>DOG2</i>	orf19.3392	2-deoxyglucose-6-phosphatase activity	-1.1	-1.0	1.0	-1.9	-1.5	-2.1	1.4	1.4	1.6
<i>DPP1</i>	orf19.656	Phosphatidase phosphatase activity	-1.4	1.3	1.7	-2.3	-1.6	-1.8	-1.2	1.1	1.0
<i>DRS21.5eoc</i>	orf19.783	Calcium-transporting ATPase activity	-1.1	-1.2	-1.3	-1.6	-1.2	-1.1	-1.8	-1.5	-1.6
<i>DUR32</i>	orf19.5017	Urea transporter activity	2.0	1.3	1.1	-4.2	-1.6	-2.1	-1.4	-1.8	-1.9
<i>DUR33.5eoc</i>	orf19.7205	Urea transporter activity	-1.6	1.1	1.0	-2.3	-1.8	-1.9	1.3	-1.1	-2.2
<i>EBP1</i>	orf19.125	NADPH dehydrogenase activity	-5.5	-4.2	-2.9	-4.5	-2.8	-2.7	-2.9	-2.8	-3.9
<i>ECM17</i>	orf19.4099	Sulfite reductase (NADPH) activity	1.1	-1.1	-1.3	-1.6	-1.7	-2.1	-1.2	-1.1	1.4

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>ECM22</i>	orf19.2623	Transcription factor activity	-2.1	-1.3	-2.2	-2.8	-2.3	-2.5	-1.8	-1.5	-1.3
<i>ECM42</i>	orf19.6500	Amino-acid N-acetyltransferase activity	-2.2	-1.8	-1.8	1.2	1.4	1.7	1.1	1.1	-1.0
<i>ENA22</i>	orf19.6070	ATPase activity	-1.7	-1.4	-2.1	-2.9	-1.5	-1.7	-1.5	-1.4	-1.0
<i>FAA21</i>	orf19.272	Long-chain-fatty-acid-CoA ligase activity	1.6	1.1	1.5	-3.0	-2.4	-1.8	-1.4	-1.1	1.6
<i>FCR1</i>	orf19.6817	Transcription factor activity	-2.0	-1.5	-2.0	-2.1	-1.6	-2.3	1.0	1.1	1.1
<i>FDH12</i>	orf19.638	Formate dehydrogenase activity	-1.7	1.8	-2.2	-2.4	-3.0	-2.4	-2.4	-2.4	-1.8
<i>FDH2</i>	orf19.1117	Formate dehydrogenase activity	1.2	-4.1	-12.0	-2.3	-7.5	-4.6	-2.5	-1.5	-2.0
<i>FDH3.3f</i>	orf19.1774	Formate dehydrogenase activity	-1.9	-1.1	-2.2	-2.5	-4.7	-4.7	-3.1	-3.6	-4.3
<i>FDH3.5f</i>	orf19.1774	Formate dehydrogenase activity	1.4	-12.4	-5.1	-2.6	-4.6	-2.1	-2.3	-4.4	-2.8
<i>FET33</i>	orf19.4211	Ferroxidase activity	1.5	1.4	1.1	-1.8	-1.7	-2.8	-1.7	-1.5	-2.3
<i>FOX2</i>	orf19.1288	3-hydroxyacyl-CoA dehydrogenase activity	-1.2	3.8	-1.5	-2.0	-1.9	-1.8	-1.9	-1.4	-1.4
<i>FTR1</i>	orf19.7219	Ferrous iron transporter activity	-4.3	-3.0	-2.8	-3.8	-3.2	-5.2	-5.6	-3.9	-5.4
<i>GAD1</i>	orf19.1153	Glutamate decarboxylase activity	-1.4	-1.3	-1.7	-2.3	-1.2	-1.0	-1.6	-1.5	-2.6
<i>GAL10</i>	orf19.1153	UDP-glucose 4-epimerase activity	1.2	2.1	1.6	-2.4	-1.8	-1.7	-1.7	-1.2	-2.0
<i>GAL7</i>	orf19.3675	UTP:galactose-1-phosphate uridylyltransferase activity	-1.0	1.8	2.3	-2.2	-2.4	-2.3	-1.7	-1.3	-1.7
<i>GAP4</i>	orf19.1799	Amino acid permease activity	-1.6	-1.6	-1.6	-1.5	-1.1	-1.3	-1.2	-1.2	5.0
<i>GCV1</i>	orf19.5519	Glycine dehydrogenase (decarboxylating) activity	-1.9	-2.1	-2.2	-1.6	-1.2	1.2	-2.3	-3.1	-2.5
<i>GCV2</i>	orf19.385	Glycine dehydrogenase (decarboxylating) activity	-1.4	-1.5	-2.4	1.3	-1.1	-1.1	-2.8	-1.8	-2.5
<i>GNP1</i>	orf19.7566	Amino acid permease activity	1.0	2.4	-1.7	-1.5	-1.4	-1.7	-2.2	-1.7	-1.8
<i>GPT1</i>	orf19.4063	L-gamma-aminobutyric acid transporter activity	1.9	-1.5	-1.6	-2.1	-1.6	-1.7	1.1	-1.1	-1.8
<i>GRP4</i>	orf19.3150	Oxidoreductase activity	-1.4	1.3	1.7	-1.9	-1.5	-2.3	-1.9	-1.7	-1.1
<i>GSL22</i>	orf19.2495	1,3-beta-glucan synthase activity	-1.4	1.1	1.0	-2.0	-1.5	-2.4	-1.1	1.2	1.9
<i>GSL23.3f</i>	orf19.3269	1,3-beta-glucan synthase activity	-1.0	-1.1	-1.2	-1.9	-1.6	-2.2	-1.1	-1.1	-1.3
<i>GTT1</i>	orf19.6947	Glutathione transferase activity	-1.5	1.2	1.0	-1.8	-1.5	-1.7	-1.1	1.1	-1.3
<i>GYP1</i>	orf19.3811	Rab GTPase activator activity	1.1	1.4	-1.3	-1.1	-1.1	1.2	-1.6	-1.5	-1.9
<i>HGT11</i>	orf19.4527	Glucose transporter activity	3.1	4.4	1.7	1.8	-1.2	-1.5	-2.3	-1.5	-3.0
<i>HGT12</i>	orf19.3668	Glucose transporter activity	2.4	14.0	4.4	6.8	2.6	1.8	-3.1	-3.1	-3.0
<i>HIP1</i>	orf19.4940	L-histidine transporter activity	-1.6	-1.6	1.1	-2.2	-1.7	-1.7	-1.0	1.0	-1.6
<i>HIS3</i>	orf19.183	Imidazoleglycerol-phosphate dehydratase activity	1.3	-1.2	1.7	-2.0	-3.0	-2.9	-1.1	-1.1	1.7
<i>HNMI</i>	orf19.2003	Choline transporter activity	-1.4	-1.1	-1.1	-1.7	-1.6	-2.1	-1.4	-1.1	-1.2
<i>HOL1</i>	orf19.2991	Transporter activity	-1.2	-1.1	-1.6	-2.2	-1.7	-2.2	1.3	1.5	2.1
<i>HSP12</i>	orf19.3160	--	-2.2	1.1	-3.0	-12.3	-4.4	-3.5	-7.0	-1.7	-1.6
<i>HSP31</i>	orf19.3664	--	-2.8	-2.3	-2.5	-1.5	-1.3	-1.6	1.4	-1.2	-3.1
<i>HXT5.3f</i>	orf19.2021	Fructose transporter activity	-1.6	-1.2	-1.6	-3.8	-2.5	-3.1	-1.6	-1.1	-1.3
<i>HXT5.5f</i>	orf19.2021	Fructose transporter activity	-1.6	-1.2	-2.0	-3.7	-3.0	-6.2	-1.4	-1.3	-1.5
<i>IFC3</i>	orf19.3749	Oligopeptide transporter activity	-2.1	-1.6	-3.0	-6.7	-1.4	1.0	-1.2	-2.1	-1.7
<i>IFF4</i>	orf19.7472	--	1.2	1.4	-1.5	-1.6	-1.1	1.5	-2.0	-1.8	-12.4
<i>IFN1</i>	orf19.1979	Phospholipid transporter activity	-3.1	-1.9	-1.8	-2.8	-1.5	-1.6	-1.0	-1.2	-1.9
<i>IFN3</i>	orf19.1978	Phospholipid transporter activity	-1.5	-1.4	-1.5	-3.9	-2.0	-2.0	-1.1	1.1	-1.1
<i>IFR2</i>	orf19.2396	--	-1.8	-1.6	-1.8	-2.0	-1.5	-2.4	-1.1	-1.1	1.1
<i>IFY2.5f</i>	orf19.2158	Monosaccharide transporter activity	-2.0	-1.9	-2.1	-3.1	-1.5	-1.6	-1.6	-1.7	-1.5

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF10029.5eoc</i>	orf19.3915	--	-1.4	-1.7	-1.1	-1.5	-1.5	-5.3	1.1	-1.0	-1.0
<i>IPF10153</i>	orf19.2170	--	-1.4	-1.0	-1.2	-2.3	-1.7	-2.2	-1.2	-1.1	-1.6
<i>IPF10355</i>	orf19.3809	Transcription factor activity	1.5	1.1	-1.3	1.5	1.2	-1.6	-2.0	-1.6	-1.8
<i>IPF11105</i>	orf19.2262	--	-2.4	1.6	1.1	-1.9	-1.6	-1.9	-2.5	-1.9	-2.4
<i>IPF11142</i>	orf19.473	--	-1.5	-1.9	-2.4	-1.8	-1.2	-1.5	-1.1	1.1	-1.1
<i>IPF11448</i>	orf19.2923	--	-1.5	-1.7	-1.8	-2.1	-1.3	-1.3	1.0	1.0	1.3
<i>IPF11503</i>	orf19.3902	--	-2.1	-1.1	-1.9	-4.1	-1.7	-2.6	-4.8	-3.2	-3.6
<i>IPF11548</i>	orf19.3854	Protein kinase activity	1.0	-2.4	1.0	-1.6	-1.9	-1.6	-1.1	-1.3	-1.4
<i>IPF11713.3f</i>	orf19.1152	--	-2.1	-1.2	-2.3	-5.1	-2.2	-3.5	-1.2	-1.0	1.5
<i>IPF11725</i>	orf19.3475	--	-1.3	1.0	-1.1	-1.9	-1.6	-1.8	-1.1	-1.2	1.1
<i>IPF11759.3f</i>	orf19.4737	--	-1.8	-1.9	-4.3	-2.7	-1.0	-1.7	-1.1	-1.2	-2.2
<i>IPF11849</i>	orf19.4559	--	1.1	1.4	1.4	-1.2	-5.5	-2.1	-1.5	-1.5	-2.8
<i>IPF11858</i>	orf19.1277	--	-1.0	1.1	-1.2	-2.0	-1.8	-2.4	-1.4	-1.6	1.1
<i>IPF1193</i>	orf19.2079	--	-1.4	-1.1	1.1	-2.4	-1.8	-2.0	-1.0	-1.1	1.1
<i>IPF12082.3f</i>	orf19.6832	--	-1.7	-2.1	-2.1	1.2	1.7	-1.0	1.3	-1.0	1.4
<i>IPF12568</i>	orf19.3516	--	-1.2	1.1	1.3	-1.6	-1.7	-1.9	-1.3	-1.1	-1.2
<i>IPF12577</i>	orf19.5238	--	-1.7	-1.6	-1.5	-1.7	1.0	1.3	-1.0	-1.1	1.2
<i>IPF12579</i>	orf19.5239	--	-1.1	-1.9	1.0	-1.7	-1.0	-1.2	-1.9	-1.6	-5.3
<i>IPF12758.5f</i>	orf19.334	--	-1.2	1.4	-1.3	-1.9	-1.7	-1.7	-1.8	-1.4	-1.5
<i>IPF12887</i>	orf19.4780	--	-2.0	-1.8	-2.4	-1.8	-1.1	-1.6	-1.1	1.1	1.3
<i>IPF1292</i>	orf19.7381	Transcription factor activity	1.3	-1.2	1.4	-4.6	-3.0	-3.5	1.2	1.1	1.0
<i>IPF13131.3</i>	orf19.1587	Glucose transporter activity	-2.0	-1.2	-3.4	-1.9	-1.7	-2.0	-2.9	-3.8	-7.4
<i>IPF13377</i>	orf19.2350	--	-2.1	-1.5	-2.3	-1.6	1.2	1.1	-1.8	1.0	1.2
<i>IPF13493</i>	orf19.5063	--	-4.3	-3.0	-1.5	-1.3	-1.1	-1.1	-1.7	-1.9	-2.5
<i>IPF13769</i>	orf19.6209	Monocarboxylic acid transporter activity	-1.1	-1.3	-1.6	-1.5	-1.8	-2.5	-1.6	-1.6	-1.9
<i>IPF1384</i>	orf19.6445	Dodecenoyl-CoA delta-isomerase activity	-1.1	1.3	-1.7	1.0	-2.1	1.5	-1.8	-1.8	-1.8
<i>IPF13879</i>	orf19.1120	--	2.2	3.5	1.3	-2.0	-1.5	-1.5	1.2	1.3	3.9
<i>IPF1390</i>	orf19.6449	--	-1.5	2.1	-1.4	-1.3	-1.0	-2.0	-4.3	-1.8	-2.0
<i>IPF13921</i>	orf19.4749	--	-1.9	-1.7	-1.5	-3.6	-2.9	-2.2	-1.2	-1.0	1.0
<i>IPF13971</i>	orf19.5204	--	-1.3	1.0	-2.6	-2.2	-1.5	-2.8	-1.5	-1.2	-3.1
<i>IPF1401</i>	orf19.6066	--	-1.7	-1.0	-1.7	-1.5	-1.5	-1.5	-1.7	-1.0	-1.3
<i>IPF14501.5f</i>	orf19.2455	--	1.3	1.6	-1.1	-1.6	-1.5	-2.0	-1.1	-1.3	-1.2
<i>IPF1471</i>	orf19.304	--	-1.5	-1.5	-1.2	-1.6	-1.5	-1.7	1.1	1.1	-1.3
<i>IPF14744</i>	orf19.5125	--	-1.3	2.5	-1.7	1.2	1.1	-1.4	-3.5	-2.7	-1.5
<i>IPF14773.5f</i>	orf19.3586	--	-1.1	1.4	1.3	1.4	-1.7	-5.5	-1.6	-1.7	-2.0
<i>IPF1505</i>	orf19.348	--	-2.9	-1.3	-2.1	-3.2	-1.5	-1.7	-1.0	-1.2	-8.9
<i>IPF15051</i>	orf19.1258	--	-1.4	-2.7	1.2	-2.2	1.2	1.9	-2.5	-1.6	-2.7
<i>IPF15153</i>	orf19.5867	--	1.0	1.2	1.2	-1.9	-1.7	-2.6	1.1	1.2	1.4
<i>IPF15177</i>	orf19.750	--	-1.0	1.1	-1.0	-1.7	-1.6	-2.0	1.1	1.1	-1.5
<i>IPF15575</i>	orf19.1247	--	-1.7	-1.5	-1.5	-1.2	-1.2	-1.3	-1.5	1.1	-1.2
<i>IPF15632</i>	orf19.3469	--	-1.7	-1.2	-1.8	-2.2	-1.6	-1.5	-1.4	-1.1	-1.2

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF16067</i>	orf19.3190	Specific RNA polymerase II transcription factor activity	-1.1	-1.2	-1.9	-1.6	-1.8	-2.4	-2.4	-1.4	-1.7
<i>IPF1615.3eoc</i>	orf19.980	--	-1.1	-1.0	-1.8	-1.2	-1.2	-1.8	-1.8	-1.5	-2.4
<i>IPF16253</i>	orf19.3932	--	-1.5	-1.3	-1.5	-2.1	-3.3	-1.7	-2.8	-1.7	1.4
<i>IPF16752</i>	orf19.2711	RNA polymerase II transcription elongation factor activity	-1.3	-1.6	1.1	-1.6	-1.5	-1.7	1.1	1.0	-1.2
<i>IPF17050</i>	orf19.1049	--	-2.7	-8.2	-5.6	-11.4	-2.3	-17.6	-1.9	-2.4	1.1
<i>IPF17074</i>	orf19.2792	--	-1.8	-1.3	-1.4	-2.3	-1.5	-1.9	-1.0	-1.1	-1.7
<i>IPF17094</i>	orf19.2143	--	-1.2	-1.1	-1.3	-1.5	-1.6	-2.7	-1.2	1.2	1.1
<i>IPF17190</i>	orf19.3869	--	-1.5	-1.1	-1.3	-2.2	-2.0	-1.5	-1.0	1.3	-1.1
<i>IPF17430.5eoc</i>	orf19.73	--	1.2	-9.5	3.6	-1.5	-1.6	-6.1	1.1	-1.0	1.2
<i>IPF17706</i>	--	--	1.1	1.2	-1.0	-3.5	-11.2	-4.7	-1.6	-1.1	-1.9
<i>IPF18474</i>	orf19.6610	Structural constituent of cytoskeleton	1.1	-1.1	-1.7	-1.3	-1.3	-1.6	-1.8	-1.7	-1.8
<i>IPF18732</i>	orf19.711	--	1.4	1.5	-1.2	-1.3	1.1	1.0	-2.4	-1.7	-1.9
<i>IPF19749</i>	orf19.94	--	-1.5	1.0	-1.4	-1.4	-2.0	1.1	-2.6	-1.9	-3.0
<i>IPF19772</i>	orf19.5602	--	-1.1	-1.5	-1.2	-1.3	-1.1	1.1	-1.5	-1.8	-2.0
<i>IPF19814</i>	orf19.7106	--	-1.7	-1.7	-1.8	1.1	1.6	1.7	1.1	-1.3	1.0
<i>IPF19859</i>	orf19.3375	--	-1.3	1.8	-1.6	-1.9	-1.7	-1.6	-1.0	1.2	-1.1
<i>IPF20135</i>	orf19.993	--	-2.4	-1.7	-1.6	-1.8	-1.7	-2.0	1.2	1.2	-1.3
<i>IPF2053</i>	orf19.5267	--	-5.5	-5.3	-6.8	-1.1	1.0	-1.3	-1.0	1.3	-1.5
<i>IPF2275.5eoc</i>	orf19.6654	--	-2.2	-1.9	-2.0	-1.9	-2.0	-4.1	-1.1	1.2	1.1
<i>IPF2283</i>	orf19.6660	--	-2.6	-2.4	-2.4	-2.8	-1.5	-1.7	1.2	-1.4	1.9
<i>IPF2319</i>	orf19.6680	Transcription factor activity	1.0	-1.0	1.0	-1.5	-1.7	-1.6	1.1	1.1	1.8
<i>IPF2374</i>	orf19.6805	--	-1.7	-2.4	2.0	-2.1	-2.1	-2.3	-1.5	-1.5	1.1
<i>IPF2383</i>	orf19.6808	--	-1.7	-2.4	-2.2	-1.2	1.1	1.1	-1.1	-1.4	-1.9
<i>IPF2522</i>	orf19.7460	--	-1.7	-1.5	-2.1	1.2	1.1	1.2	-1.4	-1.0	1.0
<i>IPF2542</i>	orf19.6629	Phospholipase C activity	-1.3	-1.6	1.1	-1.6	-1.5	-2.0	-1.0	-1.1	1.0
<i>IPF2645</i>	orf19.7405	--	-1.8	-1.5	-1.9	-1.2	-1.3	-1.4	-1.1	1.2	-1.7
<i>IPF2795</i>	orf19.6763	--	-1.8	-1.6	-1.8	-1.4	-1.5	-1.3	1.2	1.2	-1.1
<i>IPF2822</i>	orf19.6781	Transcription factor activity	-2.4	-2.4	-1.1	-2.2	-3.1	-2.2	1.5	-1.4	-1.4
<i>IPF2839</i>	orf19.7296	--	-2.1	-1.6	-3.7	-1.7	-1.5	-1.7	-1.9	-1.8	-5.4
<i>IPF3092</i>	orf19.4445	--	1.1	1.6	1.0	-1.2	-1.9	-1.4	-2.4	-2.0	-1.5
<i>IPF3506</i>	orf19.6741	--	-1.6	-1.3	-1.1	-6.4	-3.6	-5.0	1.1	1.5	2.8
<i>IPF3539</i>	orf19.2813	--	-2.1	7.3	-1.1	-1.2	-1.4	-7.6	-2.1	-2.2	-1.6
<i>IPF3624</i>	orf19.6713	--	1.2	1.4	-1.5	-1.9	-1.9	-2.8	-2.4	-1.5	-2.1
<i>IPF3631</i>	orf19.6718	--	-1.3	-1.7	-1.1	-12.6	-4.5	-6.2	1.8	-1.3	1.2
<i>IPF3634</i>	orf19.6720	--	-3.9	-3.5	-5.8	-4.2	-3.8	-6.0	-3.0	-2.7	-3.2
<i>IPF3661</i>	orf19.899	--	-2.5	-1.6	-1.9	-1.3	1.1	-1.0	-1.4	-1.1	-1.8
<i>IPF3937</i>	orf19.868	--	-1.0	1.0	-1.3	-3.0	-2.0	-2.2	-1.2	-1.1	-1.3
<i>IPF4065</i>	orf19.1862	--	-2.9	-1.6	-2.1	-8.3	-1.9	-1.9	1.3	1.5	-1.1
<i>IPF407</i>	orf19.7504	--	1.2	1.5	1.1	1.2	1.2	2.3	-1.5	-1.7	-2.4
<i>IPF409</i>	orf19.7502	--	-7.2	-2.8	-4.0	-3.3	-2.7	-2.1	-2.4	-2.7	-3.8
<i>IPF4430</i>	orf19.2959.1	--	-1.6	-1.0	-2.4	-5.0	-3.0	-4.6	-2.2	-1.5	1.7

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
IPF4537	orf19.905	--	-2.2	-1.7	-1.6	-2.5	-1.1	-1.3	-1.0	-1.0	1.4
IPF4667	orf19.1847	Carboxy-lyase activity	-2.2	-1.6	-1.8	-3.6	-3.3	-2.9	-1.0	-1.2	3.2
IPF4674	orf19.1852	--	-1.4	-1.1	-1.4	-1.5	-1.5	-1.9	-1.4	1.0	-1.1
IPF4704	orf19.5278	--	-1.6	1.1	-1.2	-1.1	-1.1	-1.1	-1.7	-1.6	-2.0
IPF4928	orf19.7660	--	-1.1	1.0	-1.5	-1.7	-1.6	-1.5	-1.4	-1.6	-1.2
IPF4991	orf19.2531	--	-1.5	1.1	-2.1	-5.6	-6.5	-8.3	-2.3	-1.8	1.1
IPF4999	orf19.5843	--	-1.4	-2.2	1.0	-6.6	-6.7	-7.8	-1.2	-1.1	1.7
IPF5282	orf19.5663	--	-1.5	-1.6	-2.6	-2.1	1.1	-1.3	-1.0	1.1	1.3
IPF5330	orf19.3448	--	-1.9	-6.0	-1.8	-4.1	-1.7	1.9	2.1	-2.1	1.7
IPF5353	orf19.3003	--	-1.3	-1.2	-1.4	-1.7	-1.7	-1.9	-1.1	1.1	-1.1
IPF5360	orf19.3007.2	--	1.1	1.0	-2.0	-2.9	-2.0	-3.1	-2.2	-1.9	-2.0
IPF5453	orf19.5692	--	1.1	1.1	-1.3	-2.3	-1.8	-1.9	1.1	1.1	1.1
IPF5806	orf19.815	--	-1.4	1.0	1.2	-1.7	-1.8	-2.1	-1.1	1.2	1.2
IPF5849	orf19.5496	--	-2.1	-1.6	-1.7	-2.1	-1.1	-1.2	1.2	1.1	2.0
IPF5966	orf19.6793	--	-1.2	-1.1	-2.0	-1.7	-2.0	-1.7	-1.5	1.4	2.4
IPF5972	orf19.7314	--	-1.3	1.0	-1.3	-1.6	-1.7	-2.2	-1.5	-1.1	-1.8
IPF5988	orf19.7305	--	-3.1	-2.9	-3.3	-8.4	-5.1	-5.0	-1.8	-1.7	1.1
IPF6003	orf19.1490	--	1.0	1.2	-1.0	-1.7	-1.5	-1.8	1.2	1.2	2.3
IPF6108	orf19.4811	--	-1.8	-2.4	-1.5	1.0	-1.1	-1.2	1.0	1.0	1.2
IPF6181	orf19.4174	Nucleoside transporter activity	-1.8	-1.6	-2.2	-2.7	-1.2	1.1	-1.0	-1.1	-1.3
IPF6274	orf19.6316	--	-1.8	-1.2	-1.5	-2.5	-2.0	-3.0	1.1	-1.0	1.7
IPF6339	orf19.2724	--	-1.0	1.0	-1.8	-3.3	-3.8	-4.3	-3.2	-2.3	-2.4
IPF6459	orf19.1890	--	-1.5	-1.1	-1.3	-2.0	-1.7	-1.7	-1.1	1.0	-1.1
IPF6572	orf19.6874	--	-1.2	-1.1	-1.4	-1.4	-1.3	-2.6	-1.9	-1.6	-1.5
IPF6679	orf19.1306	--	-1.6	-1.3	-1.4	-1.1	-1.2	-1.1	-2.4	-2.1	-1.7
IPF6881	orf19.4246	--	-1.2	1.1	1.1	-2.6	-1.8	-1.9	-1.5	-1.1	-1.4
IPF6960	orf19.6654	--	-1.9	-1.8	-2.0	-2.6	-1.3	-1.2	-1.1	1.1	1.2
IPF7109	orf19.7350	--	1.0	1.1	-1.3	-1.9	-1.6	-2.3	-1.5	-1.2	-1.8
IPF7405	orf19.4666	--	-2.0	-1.5	1.0	-2.8	-2.2	-1.9	-1.0	1.0	1.9
IPF7452	orf19.2045	--	-2.1	-2.2	-1.6	-1.6	-1.5	-1.3	-1.0	-1.0	2.1
IPF7530	orf19.4531	ATP binding	-1.3	-1.6	1.1	-2.4	-2.5	-2.1	-1.4	-1.0	-1.1
IPF7616	orf19.1159	Homoserine O-acetyltransferase activity	1.3	1.6	-1.2	-2.3	-2.5	-2.0	-1.4	-1.3	-1.6
IPF7681	orf19.1397	Electron carrier activity	-1.2	1.0	1.3	-1.8	-1.8	-1.9	1.1	1.0	1.4
IPF7711	orf19.681	--	-1.1	-1.6	-2.3	-1.4	-1.3	-2.6	-1.5	-1.5	-1.9
IPF7715.3eoc	orf19.2296	--	-1.4	1.3	-2.1	-2.4	-1.7	-2.2	-1.6	-1.3	-3.3
IPF7721	orf19.431	Transcription factor activity	-1.4	-1.1	-1.5	-1.7	-1.6	-2.2	-1.1	-1.1	-1.3
IPF7733	orf19.4855	--	-1.7	-1.5	-2.0	-1.1	-1.1	1.0	1.3	-1.4	1.6
IPF7785	orf19.2962	--	-2.4	-3.6	1.5	-2.0	-1.1	1.2	-1.5	-1.5	-2.0
IPF7999	orf19.4190	--	-1.3	-1.5	-1.2	-1.9	-2.5	-2.1	1.3	1.0	1.1
IPF8302	orf19.6007	--	-2.3	-2.0	1.2	-2.0	-1.6	-1.5	-1.0	-1.2	1.6
IPF8307	orf19.6005	Glucose transporter activity	-1.2	1.1	-1.4	-2.2	-1.5	-2.4	-1.4	-1.4	-3.2

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>IPF8369</i>	orf19.6077	--	-2.5	-1.5	-3.2	-3.5	-1.9	-2.2	-2.1	-1.4	1.0
<i>IPF8901</i>	orf19.3758	--	-6.2	-3.4	-4.1	-1.6	-1.5	-3.5	1.2	-2.1	-1.3
<i>IPF9057</i>	orf19.1189	DNA bending activity	-3.0	-2.3	-2.6	-1.9	1.0	1.8	-3.2	-1.9	-3.0
<i>IPF9126</i>	orf19.5537	--	1.1	-1.2	-1.6	-1.8	-1.6	-1.6	1.3	1.4	1.8
<i>IPF9132</i>	orf19.5541	--	-1.3	1.0	1.4	-2.3	-1.5	-2.3	-1.1	1.2	1.1
<i>IPF9145</i>	orf19.6245	--	-1.4	-1.3	-1.3	-2.3	-1.5	-1.7	-1.8	1.3	-3.3
<i>IPF9167</i>	orf19.2737	--	-1.5	1.3	-1.7	-3.1	-1.7	-1.8	-1.2	-1.2	-1.3
<i>IPF9167</i>	orf19.2737	--	-1.4	1.6	-1.8	-1.8	-1.8	-1.6	-1.3	-1.1	-2.0
<i>IPF9282</i>	orf19.1510	--	-1.3	-1.1	-1.4	-1.7	-1.5	-2.9	-1.6	-1.4	-2.4
<i>IPF9425.3</i>	orf19.6888	--	2.0	1.4	-1.3	-1.5	-1.7	-2.4	-1.2	-1.4	-1.3
<i>IPF946</i>	orf19.7561	--	1.6	1.2	-1.2	-2.1	-4.5	-5.5	1.1	1.2	-2.6
<i>IPF9483</i>	orf19.3395	--	-2.6	-1.6	-2.5	-2.7	-2.0	-2.3	-2.2	-2.0	-1.8
<i>IPF9605</i>	orf19.3071	Protein tyrosine phosphatase activity	-2.6	-1.9	-1.5	-1.9	-1.1	-1.1	1.3	-1.0	1.6
<i>IPF9703.3f</i>	orf19.4880	--	-1.1	-1.3	-1.6	-1.8	-1.2	1.0	-1.9	-2.1	-1.8
<i>IPF9706</i>	orf19.4883	--	-4.3	-1.9	-2.1	1.2	1.0	1.5	1.1	-1.2	1.2
<i>IPF9821.3f</i>	orf19.4142	--	-1.3	-1.7	-1.1	-2.5	-2.0	-3.0	1.0	1.6	-1.4
<i>IPF9898</i>	orf19.3406	--	1.0	-1.7	1.2	-1.2	-1.3	1.0	-1.6	-1.6	-1.9
<i>IPF993</i>	orf19.4595	--	-1.5	-1.6	-1.5	-1.9	1.3	1.6	1.5	1.1	-3.1
<i>IPT1.5f</i>	orf19.4769	Transferase activity	-1.4	-1.5	-1.0	-1.5	-1.8	-2.4	-1.2	1.2	-1.6
<i>KRE2.3f</i>	orf19.4377	--	-1.3	1.1	1.5	-1.8	-1.8	-2.5	1.1	1.8	-1.8
<i>KRE2.5f</i>	orf19.4349.6	--	-1.4	-1.2	1.9	-1.6	-1.6	-4.6	-1.0	1.7	1.5
<i>MAE1</i>	orf19.3419	Malate dehydrogenase activity	1.2	1.2	-1.2	-1.6	-1.5	-1.6	1.0	1.3	-1.4
<i>MAL31</i>	orf19.3981	Alpha-glucoside:hydrogen symporter activity	-1.2	24.1	5.6	-1.9	-3.3	-2.5	-2.8	-2.0	-2.3
<i>MEP3</i>	orf19.1614	Ammonium transporter activity	-1.4	-1.5	-3.1	-2.6	-1.1	-1.6	-2.1	-2.5	-1.6
<i>MET1</i>	orf19.5811	Uroporphyrin-III C-methyltransferase activity	-1.2	-1.1	-1.6	-2.9	-2.9	-2.2	-1.2	1.1	-1.0
<i>MET10</i>	orf19.4076	Sulfite reductase (NADPH) activity	-1.9	-2.3	-1.7	-1.7	-1.5	1.1	-1.3	1.1	1.3
<i>MET14</i>	orf19.946	Adenylylsulfate kinase activity	-1.8	-1.7	-1.2	-2.1	-2.8	-3.1	1.2	1.2	1.8
<i>MET15</i>	orf19.5645	O-acetylhomoserine aminocarboxypropyltransferase activity	-1.2	1.2	-1.5	-1.8	-1.7	-1.8	-1.1	1.0	-1.3
<i>MET2</i>	orf19.2618	Homoserine O-acetyltransferase activity	1.0	1.1	-1.9	-1.7	-1.6	-1.8	-1.6	-1.8	-2.1
<i>MET3</i>	orf19.5025	Sulfate adenylyltransferase (ATP) activity	-1.4	-1.3	-1.3	-7.8	-7.8	-8.1	-1.0	1.2	-1.7
<i>MLS1</i>	orf19.4833	Malate synthase activity	-1.5	1.1	-3.2	-1.5	1.2	1.1	-2.2	-1.7	-2.4
<i>MSC2</i>	orf19.3132	Cation:cation antiporter activity	-1.2	1.4	-1.5	-1.5	-1.5	-2.4	-1.1	-1.2	1.6
<i>MTD1</i>	orf19.3810	Methylenetetrahydrofolate dehydrogenase (NAD ⁺) activity	-1.4	-1.5	-2.3	-1.0	-1.5	-2.0	-3.2	-2.6	-3.3
<i>MUP1</i>	orf19.5280	L-methionine porter activity	-1.5	-2.0	-2.2	-2.3	-1.3	-2.1	-1.4	-1.1	1.8
<i>MUP3</i>	orf19.321	L-methionine porter activity	-2.3	-1.7	-1.8	-2.1	-1.1	-1.2	-1.6	-1.3	-1.3
<i>NTA1</i>	orf19.850	Protein N-terminal asparagine amidohydrolase activity	-1.0	-1.4	-1.3	-1.5	-1.6	-1.6	1.4	-1.1	-2.9
<i>OAC1</i>	orf19.7411	Oxaloacetate carrier activity	-1.0	-3.4	-5.1	-1.7	-2.7	-3.5	-2.0	-1.5	-6.3
<i>OPT1</i>	orf19.2602	Oligopeptide transporter activity	-1.6	1.1	-2.2	-4.5	-2.4	-4.4	1.8	2.4	3.0
<i>OSM2</i>	orf19.5005	Fumarate reductase (NADH) activity	1.2	3.1	1.5	-1.1	-1.3	1.1	-1.7	-1.7	-11.9
<i>PAD1</i>	orf19.5731	Carboxy-lyase activity	1.2	1.0	-1.1	-1.7	-2.0	-1.8	-1.2	1.0	-2.2
<i>PAP11</i>	orf19.3197	RNA binding	1.0	-1.0	-1.0	-114.9	-116.9	-119.7	1.1	1.2	2.1

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>PCK1</i>	orf19.7514	Phosphoenolpyruvate carboxykinase (ATP) activity	-1.4	1.2	-2.4	-4.8	-3.9	-2.9	-2.0	-1.4	-1.8
<i>PGA13</i>	orf19.6420	--	-2.6	-1.6	-2.3	-1.6	-1.7	-3.8	2.1	2.9	4.5
<i>PGA18</i>	orf19.301	--	-1.2	1.1	-1.5	-1.8	-1.5	-2.7	-1.3	1.0	-1.4
<i>PGA26</i>	orf19.2475	--	-3.3	-2.2	-1.8	2.8	6.5	3.2	1.5	1.1	1.5
<i>PGA31</i>	orf19.5302	--	1.4	2.3	-1.3	-1.1	-1.6	-1.4	-1.7	-2.8	-2.8
<i>PGA35</i>	orf19.4910	--	-2.2	-1.9	-2.1	-1.3	-1.0	-1.4	1.3	1.5	1.8
<i>PGA36</i>	orf19.5760	--	1.0	1.3	-1.6	-2.3	-1.9	-3.0	-2.0	-1.6	-2.2
<i>PGA49</i>	orf19.4404	--	-6.6	2.2	1.7	-2.5	-1.9	-2.3	-1.0	1.1	-12.8
<i>PHO87</i>	orf19.2454	Inorganic phosphate transporter activity	-1.8	-1.9	-1.6	-2.5	-1.5	-1.9	-1.2	1.2	2.0
<i>PIK1</i>	orf19.3199	1-phosphatidylinositol 4-kinase activity	1.1	-1.2	-1.4	-19.1	-30.5	-11.5	-1.0	-1.0	1.4
<i>PMT2</i>	orf19.6812	Dolichyl-phosphate-mannose-protein mannosyltransferase activity	-1.5	-1.9	-1.6	-1.9	1.0	-1.1	-1.1	1.1	1.4
<i>POL21.3f</i>	orf19.2669	Di-, tri-valent inorganic cation transporter activity	-1.8	-1.7	-1.5	-2.4	1.0	-1.1	-3.1	-1.5	1.6
<i>POL21.5f</i>	orf19.2668	--	-1.7	-1.8	-1.7	-3.6	-1.5	2.0	1.1	-1.9	-2.0
<i>POT12</i>	orf19.1704	Acetyl-CoA C-acyltransferase activity	-1.2	1.9	2.0	1.5	-1.3	-2.3	-1.8	-1.6	-2.1
<i>POX18.3f</i>	orf19.3330.3	--	-1.1	-2.7	-1.9	-1.6	1.3	1.0	-1.6	-2.0	-6.6
<i>PRC3</i>	orf19.2474	--	-1.9	-1.5	-1.5	-1.9	1.1	1.5	1.4	1.7	-1.4
<i>PRP9</i>	orf19.3178	--	-1.6	1.1	1.3	-3.2	-2.2	-1.7	-1.1	-1.0	-2.4
<i>PST2</i>	orf19.5285	--	-1.9	-1.2	-2.2	-1.7	-2.0	-1.8	-1.3	-1.7	-1.3
<i>PUT2</i>	orf19.3974	1-Pyrroline-5-carboxylate dehydrogenase activity	-1.4	1.5	1.1	-1.5	-1.7	-1.8	1.1	-1.0	-1.8
<i>RBT2</i>	orf19.1415	Ferric-chelate reductase activity	-2.0	-2.2	-2.8	-2.8	-3.1	-2.6	-4.3	-3.2	-3.2
<i>RDH54</i>	orf19.5367	DNA topoisomerase activity	-1.5	-1.5	-1.7	-2.0	-1.3	1.3	1.6	1.0	1.1
<i>RNR2</i>	orf19.5845	Ribonucleoside-diphosphate reductase activity	-2.5	-1.4	-2.0	1.3	-1.2	-2.0	-2.3	-1.5	-3.8
<i>RNR22</i>	orf19.1868	Ribonucleoside-diphosphate reductase activity	-3.0	-2.2	-3.3	-2.2	1.4	-1.3	2.8	1.1	-1.2
<i>ROM2</i>	orf19.906	Rho-guanyl-nucleotide exchange factor activity	1.1	1.4	-1.3	-1.5	-1.6	-2.1	-1.0	-1.1	-2.2
<i>RTA2</i>	orf19.24	Phospholipid-translocating ATPase activity	-2.0	-1.9	-1.7	-1.2	-1.1	-1.1	-2.6	-1.6	-1.3
<i>RTA3</i>	orf19.23	Phospholipid-translocating ATPase activity	-3.0	-2.1	-1.5	1.0	1.4	2.3	-2.1	-1.5	-1.5
<i>SAP7</i>	orf19.756	Aspartic-type endopeptidase activity	-1.0	-1.3	-1.1	-3.0	-2.2	-1.8	-2.1	-1.6	-2.4
<i>SEO2</i>	orf19.700	Transporter activity	-1.9	-2.0	-1.6	-2.3	-2.8	-2.7	-2.1	-2.5	-2.8
<i>SER2</i>	orf19.5838	Phosphoserine phosphatase activity	-2.0	-2.3	-1.7	1.1	1.0	-1.0	-2.1	-1.6	-3.4
<i>SFT2</i>	orf19.2342	--	-1.8	-1.6	-1.7	-3.1	-1.3	1.1	-1.0	-1.3	1.2
<i>SHM2</i>	orf19.5750	Glycine hydroxymethyltransferase activity	-2.1	-2.0	-1.8	-1.8	-1.2	-1.1	-2.2	-1.5	1.1
<i>SKN1.3</i>	orf19.7362	--	1.4	-1.1	1.5	-2.0	-2.2	-2.6	-1.2	-1.1	-1.9
<i>SMF3</i>	orf19.5022	Manganese ion transporter activity	-1.3	-1.4	-1.6	-1.5	-1.5	-2.1	-1.1	-1.2	1.2
<i>SMP3</i>	orf19.5792	Alpha-1,2-mannosyltransferase activity	-1.3	-1.3	1.1	-1.7	-1.5	-2.1	-1.0	1.2	1.3
<i>SN42</i>	orf19.4132	--	-1.9	-1.3	-1.0	-2.5	-2.1	-1.7	-1.2	1.0	-1.2
<i>SOU1</i>	orf19.2896	2,4-dienoyl-CoA reductase (NADPH) activity	-3.2	-2.3	-2.4	-9.2	-6.6	-8.5	-2.1	-1.2	-1.2
<i>SSU1</i>	orf19.7313	Sulfite transporter activity	-1.1	1.1	1.1	-1.5	-1.5	-1.4	-1.7	-1.7	-1.5
<i>SSY1</i>	orf19.814	Amino acid binding	-2.0	-1.5	-2.1	-2.1	1.2	1.6	-1.1	-1.3	-1.8
<i>STL1</i>	orf19.5753	Glucose transporter activity	-1.7	-1.6	-1.7	-4.5	-8.7	-10.8	-1.7	-1.1	1.4
<i>SUL1</i>	orf19.2738	Sulfate transporter activity	-2.5	-1.8	-2.8	-17.9	-6.0	-8.1	-1.4	-1.0	-1.5

Table B-2 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5 vs. F1			G5 vs. G1			6692 vs. 5833		
			1	2	3	1	2	3	1	2	3
<i>SUN42</i>	orf19.5032	--	-1.5	-1.5	-1.6	-1.9	-1.3	-1.9	-1.2	1.1	1.1
<i>TAF25</i>	orf19.3242	General RNA polymerase II transcription factor activity	-1.1	-1.1	-1.3	-1.5	-1.6	-1.6	-1.1	-1.2	-1.9
<i>Tca5b</i>	orf19.2428.2	--	-2.3	4.1	1.0	-1.6	1.8	-1.7	-2.2	-1.7	-1.7
<i>TEC1</i>	orf19.5908	Specific RNA polymerase II transcription factor activity	-1.1	-1.0	-1.6	-2.6	-3.5	-2.9	-1.5	-1.3	-2.1
<i>TES12</i>	orf19.4122	--	1.6	1.1	1.5	-2.2	-1.8	-1.9	1.0	-1.1	-1.3
<i>TPK2</i>	orf19.2277	cAMP-dependent protein kinase activity	-2.0	-1.5	-2.3	-1.5	-1.4	1.0	-1.2	-1.1	-1.5
<i>UGA12.3f</i>	orf19.802	4-aminobutyrate transaminase activity	-1.1	-1.8	-1.1	-1.6	-1.6	-1.7	-1.2	1.3	-1.9
<i>UGA12.5f</i>	orf19.802	4-aminobutyrate transaminase activity	-1.0	-1.1	-1.1	-1.6	-1.9	-2.8	-1.0	-1.0	-1.4
<i>VCX1</i>	orf19.405	Calcium ion transporter activity	-2.1	-1.7	-1.7	-2.1	-1.3	-1.2	-1.1	1.0	-1.8
<i>VPS184</i>	orf19.5543	Protein binding	5.1	1.5	-2.8	-1.8	1.1	-1.3	-1.6	-1.6	-2.3
<i>YHM1</i>	orf19.685	Transporter activity	-1.4	-1.2	-1.2	-1.8	-1.7	-2.0	-1.1	1.1	1.4
<i>YOR100</i>	orf19.2599	Carnitine:acyl carnitine antiporter activity	-1.4	-1.2	-1.0	-2.4	-1.8	-2.0	-1.3	-1.7	-1.4
<i>ZRT2</i>	orf19.1585	Low affinity zinc ion transporter activity	-2.0	-2.0	-2.3	-3.3	-1.2	-1.3	1.1	1.2	1.4

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b orf19 nomenclature according to the Assembly 19 version.

^c GO annotation found at CGD (<http://www.candidagenome.org/>).

Table B-3. Genes down-regulated in the absence of *MRR1*.

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5Δ <i>mrr1</i> vs. F5				G5Δ <i>mrr1</i> vs. G5			
			Strain A		Strain B		Strain A		Strain B	
			1	2	1	2	1	2	1	2
<i>AAF1</i>	orf19.7436	--	-3.5	-3.7	-2.2	-2.9	-5.6	-1.9	-4.3	-1.8
<i>ACO1</i>	orf19.6385	Aconitate hydratase activity	1.1	-1.8	1.0	-1.9	-2.7	-1.5	-2.0	-1.7
<i>AKL1</i>	orf19.5357	Protein serine/threonine kinase activity	-1.6	-1.8	-1.6	-1.7	-1.6	-1.9	-1.6	-1.5
<i>ALS7</i>	orf19.7400	Carbohydrate binding	-1.5	-2.1	-1.9	-1.8	1.2	-1.1	1.3	-1.1
<i>AMYG1</i>	orf19.4899	Alpha-glucosidase activity	-3.8	-1.9	-4.7	-1.7	1.1	-1.7	1.1	1.7
<i>ARD8</i>	orf19.6322	2,4-dienoyl-CoA reductase (NADPH) activity	-1.6	-3.3	-1.7	-2.4	-3.1	-2.3	-2.2	-1.7
<i>BFR2</i>	orf19.7624	--	1.6	-4.8	2.1	-2.5	-2.4	-1.7	-2.0	-2.2
<i>BMR1</i>	orf19.5604	Multidrug transporter activity	-21.4	-47.5	-35.0	-83.7	-168.9	-149.4	-143.0	-43.9
<i>CDC1</i>	orf19.3083	--	1.1	-2.1	-2.1	-1.8	-2.4	-1.6	-2.6	-2.4
<i>CDR11.5f</i>	orf19.918	ATP binding	-4.9	1.2	-2.0	-1.0	-1.9	-1.8	-2.6	-1.5
<i>CDR3</i>	orf19.1313	ATP binding	-3.3	-1.7	-2.0	-1.8	-1.0	1.3	-1.3	3.6
<i>CHO2</i>	orf19.169	Methylene-fatty-acyl-phospholipid synthase activity	-1.2	-1.5	-1.2	-1.8	-3.7	-1.5	-2.0	-3.2
<i>CHS3.5f</i>	orf19.4937	Chitin synthase activity	-25.6	-8.2	-2.3	-2.1	-4.7	5.6	-2.3	13.8
<i>CHS4</i>	orf19.7349	Enzyme activator activity	-2.0	-2.9	-1.8	-1.5	-1.7	1.0	-2.3	-1.5
<i>CLN21</i>	orf19.6028	Cyclin-dependent protein kinase regulator activity	-14.4	-8.8	-1.6	-4.6	-7.8	5.2	-1.2	3.6
<i>CNH1</i>	orf19.367	Cation:cation antiporter activity	-1.9	-2.0	-2.8	-1.8	-2.1	-1.2	-1.6	1.5
<i>CNH1</i>	orf19.367	Cation:cation antiporter activity	-3.3	-2.5	-10.7	-3.1	-2.6	-2.0	-1.9	1.6
<i>CPH1</i>	orf19.4433	Transcription factor activity	-2.0	-3.0	-1.8	-2.0	-3.4	-2.0	-2.6	1.6
<i>CTR1</i>	orf19.3646	Copper ion binding	-1.3	-2.2	-1.4	-1.7	-2.8	-1.6	-1.7	-1.7
<i>DHH1</i>	orf19.6197	Protein binding	-2.9	-1.8	-1.5	-1.6	-1.4	1.0	-1.7	1.4
<i>DLD3</i>	orf19.5805	D-lactate dehydrogenase (cytochrome) activity	-1.9	-4.1	-2.8	-2.5	1.3	-1.1	-1.1	1.1
<i>DNA2</i>	orf19.1192	ATP-dependent DNA helicase activity	-2.6	-2.2	-1.6	-1.5	-2.0	-1.1	-1.4	-1.2
<i>DRS1</i>	orf19.7635	ATP-dependent RNA helicase activity	1.0	-2.2	1.1	-2.6	-2.4	-1.8	-3.2	-2.4
<i>DRS23</i>	orf19.323	Phospholipid-translocating ATPase activity	-1.6	-2.8	-1.6	-2.1	-2.2	-1.1	-1.2	1.1
<i>EBP4</i>	orf19.3433	NADPH dehydrogenase activity	-2.6	-1.6	-1.6	-1.9	-1.3	-2.1	-1.2	-1.0
<i>EFG1</i>	orf19.610	Transcription factor activity	-2.2	-2.9	-1.9	-1.5	-3.8	-2.2	-2.3	1.3
<i>FAS2.53f</i>	orf19.5949	3-oxoacyl-[acyl-carrier-protein] reductase activity	-17.0	-1.2	-1.4	1.2	-2.9	-2.0	-2.7	-1.8
<i>FRE30.3f</i>	orf19.6139	Ferric-chelate reductase activity	-1.8	-5.5	-1.8	-3.8	-4.3	-1.9	-3.0	-2.5
<i>FRE30.53f</i>	orf19.6140	Ferric-chelate reductase activity	-5.0	-4.3	-2.1	-2.5	-4.3	-1.7	-3.4	-1.7
<i>FRE7</i>	orf19.7077	Ferric-chelate reductase activity	-1.5	-2.2	-1.8	-3.3	-3.0	-1.6	-2.4	-1.7
<i>GAC1</i>	orf19.7053	Protein phosphatase type 1 activity	-1.5	-3.8	-1.5	-3.3	-3.9	-1.5	-4.8	-1.2
<i>GAL1</i>	orf19.3670	Galactokinase activity	-1.5	-1.5	-20.4	-4.4	-1.0	1.4	-1.3	-1.3
<i>GAP6</i>	orf19.6659	Amino acid permease activity	-1.8	-2.4	-1.6	-2.4	-2.0	-1.9	-1.5	-1.5
<i>GDH2</i>	orf19.2192	Glutamate dehydrogenase activity	-1.5	-2.6	-1.8	-2.9	-1.1	1.0	1.0	-1.2
<i>GPX1</i>	orf19.86	Glutathione peroxidase activity	-2.8	-1.1	-1.6	-1.4	-1.8	-2.0	-1.6	-1.7
<i>GRP2</i>	orf19.4309	Oxidoreductase activity	-2.1	-1.9	-1.8	-1.8	-3.7	-3.7	-3.5	-2.9
<i>GTS1</i>	orf19.6393	Specific RNA polymerase II transcription factor activity	-2.4	-1.7	-1.9	-1.7	-1.5	1.1	-1.4	1.2
<i>GUT1</i>	orf19.558	Glycerol kinase activity	-2.0	-3.5	-1.6	-3.8	-4.9	-1.9	-3.0	1.6
<i>HEM12</i>	orf19.5369	Uroporphyrinogen decarboxylase activity	-2.0	-1.7	-1.7	-1.6	-1.6	-1.2	-1.2	1.4
<i>HGT11</i>	orf19.4527	Glucose transporter activity	-4.1	-9.5	-3.9	-2.7	-8.5	-3.1	-5.7	-6.3

Table B-3 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5Δmrr1 vs. F5				G5Δmrr1 vs. G5			
			Strain A		Strain B		Strain A		Strain B	
			1	2	1	2	1	2	1	2
<i>HGT12</i>	orf19.3668	Glucose transporter activity	-4.2	-2.3	-4.4	-2.2	-12.4	-5.6	-6.5	-4.2
<i>HIK1</i>	orf19.5181	Osmosensor activity	-8.1	-2.2	-2.1	-1.8	-3.7	-1.5	-2.5	1.9
<i>HSP78.5f</i>	orf19.882	ATP-dependent peptidase activity	-1.4	1.3	-1.4	1.4	-1.5	-1.5	-1.7	-1.6
<i>IFA17.5f</i>	orf19.4511	--	-3.8	-2.7	-2.0	-1.7	-2.2	1.9	-1.8	1.3
<i>IFA18.53f</i>	orf19.4508	--	-3.9	-2.2	-1.8	-1.7	2.0	1.2	2.9	-2.3
<i>IFD2</i>	orf19.771	Aryl-alcohol dehydrogenase activity	-4.2	-2.3	-3.8	-2.0	-2.0	-4.1	-1.6	-3.4
<i>IFD4</i>	orf19.4477	Aryl-alcohol dehydrogenase activity	-2.7	-4.6	-2.4	-1.9	-4.0	-4.4	-3.4	-2.9
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	-3.3	-2.1	-2.6	-2.6	-6.1	-5.2	-4.5	-2.9
<i>IFD5</i>	orf19.1048	Aryl-alcohol dehydrogenase activity	-15.0	-12.2	-15.8	-9.6	-4.9	-4.2	-4.1	-2.7
<i>IFD6</i>	orf19.4476	Aryl-alcohol dehydrogenase activity	-31.2	-41.8	-27.0	-21.3	-12.8	-22.1	-12.6	-78.1
<i>IFD7</i>	orf19.629	Aryl-alcohol dehydrogenase activity	-10.5	-4.3	-5.8	-6.0	-8.0	-8.2	-9.6	-6.5
<i>IFK3.3f</i>	orf19.857	--	-1.8	-8.8	-2.1	-5.6	-2.1	-1.9	-2.0	-5.9
<i>IMP2</i>	orf19.1981	Peptidase activity	1.0	1.3	-1.4	-1.0	-2.4	-1.6	-1.8	-2.1
<i>IPF10062</i>	orf19.1086	--	-1.9	-1.9	-1.5	-1.6	-2.0	-1.3	-1.5	1.4
<i>IPF11006</i>	orf19.4658	--	-1.5	2.1	1.2	1.5	-2.1	-1.6	-3.0	-2.3
<i>IPF11090.5f</i>	orf19.4622	--	-8.4	-1.3	-1.2	1.1	-2.4	-1.5	-2.1	-1.6
<i>IPF11569</i>	orf19.9	--	-1.3	-6.0	1.2	-1.1	-1.9	-1.9	-2.2	-2.6
<i>IPF11610</i>	orf19.2146	H3/H4 histone acetyltransferase activity	-1.6	-1.7	-1.8	-2.1	-1.6	-1.3	-1.9	-1.2
<i>IPF11694</i>	orf19.4355	Nicotinamide mononucleotide permase activity	-2.2	-1.6	-2.6	-3.0	-2.4	-1.9	-3.5	2.6
<i>IPF12105</i>	orf19.5633	--	-1.8	-2.3	-1.6	-1.8	-2.3	-2.1	-2.6	1.4
<i>IPF12316</i>	orf19.5069	--	-2.5	1.8	-1.7	-1.1	-2.4	-2.1	-3.0	-2.2
<i>IPF12513</i>	orf19.5605	--	-2.7	-3.2	-1.7	-2.7	-1.5	-1.2	-1.2	1.1
<i>IPF12606.5eoc</i>	orf19.3288	--	-1.5	-3.7	-1.7	-4.4	-3.6	-1.4	-3.4	-1.2
<i>IPF1264</i>	orf19.7371	Transcription factor activity	-1.8	-3.4	-1.5	-2.0	-6.1	-1.4	-8.2	1.1
<i>IPF1292</i>	orf19.7381	Transcription factor activity	-1.8	-2.2	-1.9	-2.0	-3.5	-1.2	-5.3	-1.2
<i>IPF13042</i>	orf19.2410	--	-1.4	1.1	1.7	1.6	-2.1	-1.6	-3.5	-2.0
<i>IPF13116</i>	orf19.3463	--	1.2	-3.3	1.3	-2.4	-3.5	-1.6	-2.2	-2.7
<i>IPF13202</i>	orf19.215	--	-1.7	-1.6	-1.5	-1.9	-2.3	-1.1	-2.9	1.6
<i>IPF13361</i>	orf19.2330	snoRNA binding	1.5	-1.3	1.7	-1.2	-5.3	-1.8	-4.1	-1.7
<i>IPF13383.53eoc</i>	orf19.1840	--	-3.9	-1.8	-1.6	-1.5	-1.4	-1.1	-1.5	1.5
<i>IPF13565</i>	orf19.1609	--	-1.7	-1.9	-2.6	-1.8	3.4	2.8	3.3	3.2
<i>IPF13756.5f</i>	orf19.4479	--	-3.5	-1.3	1.6	-1.7	-6.6	-1.5	-6.5	-2.4
<i>IPF1382</i>	orf19.6444	--	-1.0	-1.3	-1.2	-2.0	-2.3	-2.0	-1.6	-1.8
<i>IPF14888</i>	orf19.4339	--	-1.1	-1.2	1.2	-1.2	-5.2	-2.3	-4.1	-1.6
<i>IPF14895</i>	orf19.93	--	1.2	-1.6	-1.2	-1.8	-2.6	-1.6	-2.5	-29.4
<i>IPF15013</i>	orf19.5626	--	-1.7	-2.0	-1.5	-2.3	-1.9	-1.4	-2.4	-2.8
<i>IPF15153</i>	orf19.5867	--	-2.0	-1.8	-1.6	-1.5	-1.1	1.5	-1.2	1.3
<i>IPF15183</i>	orf19.1806	--	-2.3	-2.0	-2.0	-1.5	-1.2	-1.2	-1.2	1.6
<i>IPF15273</i>	orf19.1822	Transcription factor activity	-3.3	-12.7	-1.6	-3.3	-1.4	-1.2	-1.1	-4.1
<i>IPF1588</i>	orf19.972	--	-1.5	-1.8	-2.5	-2.1	-1.7	-2.9	-1.1	-1.7

Table B-3 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5Δmrr1 vs. F5				G5Δmrr1 vs. G5			
			Strain A		Strain B		Strain A		Strain B	
			1	2	1	2	1	2	1	2
IPF16	orf19.5984	--	1.0	-1.0	-1.6	-1.3	-2.4	-1.9	-1.8	-1.6
IPF16189.5f	orf19.5576	--	-1.1	1.1	1.2	1.1	-3.2	-1.6	-1.6	-1.7
IPF1634	orf19.6578	Inorganic phosphate transporter activity	-1.5	-2.4	-2.0	-3.6	-5.0	-1.5	-4.2	-2.6
IPF16981	orf19.2458	Transcription factor activity	-1.9	-1.7	-1.9	-1.6	1.7	-1.0	-1.1	-1.4
IPF17186	orf19.251	--	-10.9	-11.0	-8.7	-6.2	-5.5	-9.5	-5.6	-7.4
IPF17914.3eoc	orf19.5124	--	-2.1	-1.5	-2.2	-1.9	-1.6	-2.0	1.1	1.3
IPF17998	orf19.3147	--	-1.6	-2.6	-2.5	-2.3	2.4	1.0	3.1	1.0
IPF18316	orf19.5145	--	-1.9	-1.8	-2.1	-1.7	-1.7	-2.1	1.2	-1.0
IPF1837	orf19.5877	Alcohol O-acetyltransferase activity	-1.5	-1.6	-2.1	-1.9	-1.6	-2.6	-1.3	1.0
IPF18586	orf19.761	--	-1.6	-2.1	-1.7	-2.5	-7.9	-2.6	-3.2	1.3
IPF18690.5eoc	orf19.6621	Structural constituent of cytoskeleton	-136.5	-2.1	-1.5	-1.5	-1.2	1.0	1.2	-1.0
IPF1922	orf19.7148	--	-1.7	-1.8	-2.3	-2.8	-3.2	-1.7	-1.8	2.0
IPF19538.3f	orf19.6957.3	--	-1.5	-2.0	-2.1	-2.1	-7.6	-6.8	-4.1	-3.1
IPF1956	orf19.7159	--	-1.6	1.8	-2.2	-1.5	-2.9	-1.5	-1.5	-1.7
IPF19664	orf19.2510	Chaperone binding	-7.4	-9.0	-1.5	-1.7	1.1	1.1	-1.2	-1.0
IPF19700	orf19.1600	--	-1.5	-1.6	-1.6	-1.5	-2.0	1.1	-1.8	1.0
IPF19810	orf19.1430	--	-2.4	-1.9	-2.1	-2.0	1.3	-1.1	-1.1	12.4
IPF2050	orf19.5265	Structural constituent of cytoskeleton	-2.3	-2.0	-1.7	-1.5	-1.1	-1.1	1.3	1.1
IPF2111	orf19.4106	--	-2.3	1.4	-1.2	1.1	-1.6	-2.2	-1.9	-3.0
IPF2130	orf19.7204	--	-2.8	1.2	-2.4	-1.5	-2.1	-4.4	-1.5	-3.6
IPF2142	orf19.7197	--	2.4	1.4	7.7	-12.7	-11.8	-1.6	-36.7	-1.7
IPF2186	orf19.7166	--	-2.9	-3.5	-3.7	-3.4	-7.0	-5.1	-4.8	-2.7
IPF3080	orf19.7042	--	-1.5	-1.2	-1.2	-2.3	-10.3	-6.3	-9.3	-2.6
IPF3092	orf19.4445	--	-1.7	-3.1	-1.7	-12.5	-3.8	-1.4	-2.7	-3.2
IPF3239	orf19.2875	Signal recognition particle binding	1.9	2.4	2.0	2.0	-1.5	-2.1	-1.6	-2.0
IPF3277	orf19.4384	Fructose transporter activity	-3.5	-4.2	-2.5	-2.9	-2.6	-1.4	-3.5	1.8
IPF3329	orf19.7388	MAP kinase kinase activity	-1.6	-3.4	-1.5	-2.3	-2.2	1.2	-2.3	1.4
IPF3426	orf19.6173	--	-1.3	-3.7	-2.0	-3.0	-2.7	-1.6	-2.5	-1.6
IPF3448	orf19.6185	--	-4.4	-18.6	-2.8	-2.1	-2.5	-2.9	-2.3	-1.2
IPF3642	orf19.6726	DNA binding	-1.7	-1.5	-2.0	-2.5	-1.7	1.2	-1.4	-1.8
IPF3687	orf19.886	Cytoskeletal adaptor activity	-1.6	-1.9	-2.3	-1.7	-2.8	-1.6	-2.2	1.4
IPF3912	orf19.6514	Specific RNA polymerase II transcription factor activity	-1.2	-1.4	-1.2	-1.3	-2.2	-1.8	-1.9	-2.5
IPF3967	orf19.673	NADPH dehydrogenase activity	-1.6	-1.2	-3.2	-1.5	-1.5	-1.5	-1.7	-3.0
IPF4160	orf19.5459	--	-1.4	-3.5	-2.1	-1.3	-2.6	-1.6	-1.7	-1.5
IPF4253	orf19.1630	--	-1.9	-1.7	-3.5	-2.5	-3.2	-1.5	-3.1	-1.3
IPF4290	orf19.541	--	1.4	-2.1	-1.0	-2.0	-1.8	-1.6	-2.3	-2.0
IPF4799	orf19.3342	--	-1.6	-1.5	-2.0	-1.9	-1.2	-1.1	-1.0	-1.0
IPF486	orf19.7065	--	-1.1	1.5	1.2	1.6	-2.2	-1.8	-1.9	-2.0
IPF5064	orf19.804	Transporter activity	-1.5	-1.8	-1.7	-1.5	-1.9	-1.0	-2.3	1.5
IPF5149	orf19.6530	Signal recognition particle binding	-1.4	1.1	-1.4	-1.3	-4.1	-2.2	-2.1	-2.3

Table B-3 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5Δmrr1 vs. F5				G5Δmrr1 vs. G5			
			Strain A		Strain B		Strain A		Strain B	
			1	2	1	2	1	2	1	2
IPF5217	orf19.7270	--	-1.2	-3.0	-2.1	-1.9	-3.2	-2.3	-1.6	-1.7
IPF5228.5	orf19.7265	--	-1.9	-2.7	-2.4	-2.3	-1.2	-1.7	1.1	-1.1
IPF525	orf19.7085	--	-2.9	-17.2	-2.4	-2.4	-1.7	-2.6	-1.7	-3.4
IPF5291	orf19.3674	UDP-glucose 4-epimerase activity	-1.3	-2.1	-1.5	1.1	-2.7	-1.9	-2.6	-3.9
IPF564	orf19.7104	--	-1.5	-1.5	-1.7	-1.8	-1.7	-1.6	-1.6	-1.1
IPF5987	orf19.7306	--	-7.4	-6.4	-9.0	-6.9	-8.6	-12.5	-7.4	-10.5
IPF6011	orf19.1486	--	-2.4	-10.4	-1.8	-2.1	-5.7	-1.9	-3.8	1.2
IPF6067	orf19.6734	General transcriptional repressor activity	-2.0	-1.6	-1.9	-1.8	-3.7	-1.6	-4.4	1.4
IPF6298	orf19.1964	--	3.0	-1.1	1.5	1.3	-3.7	-1.8	-2.1	-1.8
IPF6700	orf19.2459	--	-1.5	-2.5	-2.2	-1.8	-1.1	1.2	-1.0	1.3
IPF690.5f	orf19.7590	--	-4.3	-1.6	-1.6	-2.5	-1.6	1.6	-2.3	-1.2
IPF708	orf19.5370	--	-1.5	-1.6	-2.3	-1.5	-2.6	-1.6	-1.1	1.8
IPF7493	orf19.4682	Glucose transporter activity	-1.5	-2.3	-6.0	-2.4	-7.5	-3.4	-9.1	-2.6
IPF7666	orf19.4056	Transcription factor activity	-4.8	-8.1	-2.0	-1.7	-8.2	1.0	-8.5	2.6
IPF7685	orf19.1396	ARF GTPase activator activity	-1.8	-1.8	-1.9	-1.7	-2.0	-1.9	-1.7	1.1
IPF7686	orf19.1395	Inorganic phosphate transporter activity	-1.7	-2.0	-1.7	-1.5	1.6	1.3	1.3	2.7
IPF776	orf19.5338	Transcription factor activity	-1.7	-2.8	-1.8	-3.0	-11.2	-1.0	-11.9	1.2
IPF7817	orf19.3131	NADPH dehydrogenase activity	-3.8	-4.7	-5.5	-3.6	-3.6	-2.8	-3.3	-2.1
IPF7863	orf19.1449	--	-16.9	-6.9	-15.5	-8.0	-2.9	-4.5	-3.0	-4.3
IPF7998	orf19.4191	--	-1.4	-2.1	1.4	-1.7	-3.2	-1.6	-4.7	-3.2
IPF823	orf19.7245	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase activity	1.4	-1.1	1.1	-1.7	-2.2	-1.9	-2.0	-1.5
IPF8405	orf19.1721	Carbonate dehydratase activity	1.5	1.0	1.2	-1.3	-6.1	-1.6	-4.1	-1.6
IPF8464	orf19.6855	--	-1.9	-9.7	-2.7	-3.2	-2.4	-1.1	-3.9	-7.5
IPF8627	orf19.3969	Specific transcriptional repressor activity	-1.9	-4.0	-1.8	-1.5	-2.2	1.1	-2.0	1.3
IPF9063	orf19.203	--	1.0	-1.8	-1.9	-1.5	-2.4	-1.8	-1.8	-1.5
IPF9238	orf19.6143	--	-1.7	-2.5	-18.5	-2.7	-1.6	-1.9	-1.2	-3.6
IPF9538	orf19.271	2,4-dienoyl-CoA reductase (NADPH) activity	-164.3	-548.1	-280.0	-137.2	-15.3	-16.6	-25.5	-13.3
IPF9560	orf19.1942	--	-1.8	-1.6	-1.7	-1.8	-1.4	-1.0	-1.5	1.4
LAT1	orf19.6561	Dihydrolipoyllysine-residue acetyltransferase activity	-1.0	-3.1	-1.4	-2.2	-3.3	-2.7	-1.6	-1.7
LEU42	orf19.1375	2-isopropylmalate synthase activity	-2.0	-1.6	-2.0	-2.2	1.5	-1.1	1.8	1.5
LSC2.3eoc	orf19.1860	Succinate-CoA ligase (ADP-forming) activity	-1.0	-2.1	-1.3	-2.1	-2.6	-1.5	-1.7	-2.2
MDL2.3f	orf19.5599	ATP binding	1.1	1.2	-1.1	-1.5	-1.9	-1.7	-1.9	-1.5
MET3	orf19.5025	Sulfate adenyltransferase (ATP) activity	2.7	2.0	1.1	1.2	-2.3	-1.5	-2.0	-2.0
MRP1.5f	orf19.1662	Structural constituent of ribosome	2.0	1.5	-1.2	-1.8	-2.0	-1.5	-1.7	-3.8
MRR1	orf19.7372	RNA polymerase II transcription factor activity	-74.6	-35.6	-17.1	-75.6	-69.9	-19.6	-83.5	-5.9
MSB1.5eoc	orf19.1133	--	-1.9	-2.4	-1.6	-2.8	-2.5	-1.1	-2.2	1.8
NDH1	orf19.339	NADH dehydrogenase activity	-1.2	-1.6	-1.1	-1.8	-1.8	-1.5	-1.5	-2.1
NOP4	orf19.5198	RNA binding	-1.3	-2.4	1.5	-1.2	-3.5	-1.5	-4.4	-2.4
NUC2	orf19.6531	--	1.1	-1.6	-1.2	-1.6	-2.8	-1.8	-1.6	-1.6
OGG1	orf19.7190	Oxidized purine base lesion DNA N-glycosylase activity	-2.2	-3.9	-1.4	-1.9	-4.3	-2.2	-5.4	-2.2

Table B-3 (continued).

CandidaDB Name ^a	orf19 Name ^b	GO Annotation (Molecular Function) ^c	F5Δmrr1 vs. F5				G5Δmrr1 vs. G5			
			Strain A		Strain B		Strain A		Strain B	
			1	2	1	2	1	2	1	2
<i>ORM1</i>	orf19.5751	--	-1.3	-2.2	-1.3	-1.5	-2.3	-1.5	-1.6	-1.6
<i>PEX13</i>	orf19.7282	Protein binding	-1.5	-2.5	-2.0	-1.9	1.1	-1.3	1.1	-1.9
<i>PGA9</i>	orf19.2108	Copper, zinc superoxide dismutase activity	-1.2	1.1	-1.9	1.3	-2.3	-1.7	-2.5	-1.6
<i>PHR1</i>	orf19.3829	1,3-beta-glucanosyltransferase activity	-2.8	-7.8	-2.3	-1.7	-2.0	-1.3	-2.0	1.6
<i>PRP12</i>	orf19.4379	Exonuclease activity	-1.7	-1.8	-1.9	-1.7	1.3	-1.4	1.1	1.1
<i>PRP5</i>	orf19.6831	ATP-dependent RNA helicase activity	-1.1	1.5	-1.1	1.3	-2.3	-1.5	-1.8	-1.6
<i>PRY2</i>	orf19.7218	--	-1.8	-1.6	-2.6	-2.7	-4.0	-7.0	-1.6	1.4
<i>RBT4</i>	orf19.6202	--	-1.9	-2.5	-3.2	-1.6	-1.4	-1.2	-1.2	1.9
<i>RIP1</i>	orf19.5893	Ubiquinol-cytochrome-c reductase activity	1.1	-1.4	1.1	-1.6	-1.6	-1.5	-1.5	-1.6
<i>RKII</i>	orf19.1701	Ribose-5-phosphate isomerase activity	-1.7	-1.6	-1.6	-1.5	-1.7	1.2	-1.8	-1.6
<i>RPC31</i>	orf19.2831	DNA-directed RNA polymerase activity	1.2	-4.1	2.3	-1.6	-3.4	-1.6	-2.4	-3.3
<i>RPN4</i>	orf19.1069	Transcription factor activity	-1.7	1.2	-1.6	-1.3	-2.0	-1.8	-2.3	-2.1
<i>SEC31</i>	orf19.6217	--	-2.0	-1.8	-1.8	-1.6	-2.8	-1.6	-2.3	1.5
<i>SEN1</i>	orf19.5938	ATP-dependent RNA helicase activity	-2.1	-3.9	-1.5	-2.1	1.3	-1.9	3.1	2.5
<i>SEN2</i>	orf19.2735	tRNA-intron endonuclease activity	-1.5	-1.5	-1.6	-2.1	-5.1	-1.6	-3.8	3.2
<i>SIS2</i>	orf19.7378	Protein phosphatase inhibitor activity	-1.8	-1.9	-1.8	-2.1	1.1	-1.7	-1.2	-1.5
<i>SNF3</i>	orf19.5962	Glucose transporter activity	-1.7	-3.2	-2.2	-2.1	-3.0	-2.0	-2.0	-1.0
<i>SOD21</i>	orf19.7111	Manganese superoxide dismutase activity	-1.9	-5.2	-1.5	-1.5	-1.8	-2.0	1.5	-1.0
<i>SOU3</i>	orf19.732	2,4-dienoyl-CoA reductase (NADPH) activity	-1.6	1.1	-2.0	1.1	-1.7	-1.8	-1.6	-1.7
<i>TAF90</i>	orf19.536	General RNA polymerase II transcription factor activity	-1.2	-1.2	-1.2	-1.5	-2.2	-1.8	-1.5	-1.6
<i>TAO3.5f</i>	orf19.1693	Transcription factor activity	-59.3	-1.7	-1.6	-1.7	-2.4	-1.2	-2.0	1.6
<i>TFB2</i>	orf19.5846	General RNA polymerase II transcription factor activity	-1.5	-2.0	-1.5	-1.8	-1.2	-1.2	-1.5	-1.2
<i>TIM22</i>	orf19.1352	Protein transporter activity	-1.1	-3.0	1.0	-2.0	-2.2	-2.1	-2.3	-3.1
<i>TOF1.5f</i>	orf19.4136	--	-1.7	1.0	-1.7	1.2	-2.4	-1.5	-1.7	-1.9
<i>TRK1.5f</i>	orf19.600	Chloride transporter activity	-1.8	-2.6	-1.7	-2.4	-2.1	1.4	-2.1	1.6
<i>UGA2</i>	orf19.345	Succinate-semialdehyde dehydrogenase activity	-1.8	-2.7	-3.0	-2.4	-1.4	1.2	-1.1	1.5
<i>WHI3</i>	orf19.6494	RNA binding	-1.7	-1.9	-1.6	-2.0	-3.0	-1.6	-2.4	1.3
<i>YAE1</i>	orf19.7037	--	-1.4	2.3	1.1	1.2	-1.9	-2.1	-1.5	-1.6
<i>YNK1</i>	orf19.4311	Nucleoside diphosphate kinase activity	-1.1	-1.0	1.3	-1.7	-2.8	-1.7	-1.6	-1.5

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b orf19 nomenclature according to the Assembly 19 version.

^c GO annotation found at CGD (<http://www.candidagenome.org/>).

APPENDIX C: *UPC2* SUPPLEMENTAL DATA

Table C-1. Genes up-regulated in either resistant isolate S2 or gain-of-function S2 allele in SC5314.

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
				S2vS1		Strain A	
				1	2	1	2
<i>BMRI</i>	<i>MDR1</i>	orf19.5604	Multidrug transporter activity	2.1	3.7	5.4	2.5
<i>DDR48</i>	<i>DDR48</i>	orf19.4082	--	7.1	8.0	2.4	2.4
<i>ECM3</i>	<i>ECM3</i>	orf19.1563	--	1.6	1.5	1.7	2.9
<i>ERG1</i>	<i>ERG1</i>	orf19.406	Squalene monooxygenase activity	2.4	1.6	1.5	1.6
<i>ERG24</i>	<i>ERG24</i>	orf19.1598	Delta 14-sterol reductase activity	2.3	2.0	3.2	3.5
<i>ERG26</i>	<i>ERG26</i>	orf19.2909	C-3 sterol dehydrogenase (C-4 sterol decarboxylase) activity	2.0	1.6	2.0	1.9
<i>ERG27</i>	<i>ERG27</i>	orf19.3240	3-keto sterol reductase activity	2.3	2.1	2.2	3.0
<i>FEN2</i>	--	orf19.5535	Pantothenate transporter activity	2.2	2.2	2.3	2.7
<i>FTH1</i>	<i>FTH1</i>	orf19.4802	--	1.5	1.5	1.8	2.4
<i>IDP2</i>	<i>IDP2</i>	orf19.3733	Isocitrate dehydrogenase (NADP+) activity	3.6	2.1	1.8	1.8
<i>IFA24.3</i>	<i>FGR51</i>	orf19.156	--	1.8	1.9	1.7	1.6
<i>NCP1</i>	<i>NCP1</i>	orf19.2672	NADPH-hemoprotein reductase activity	2.5	1.5	1.9	1.7
<i>QRI8</i>	--	orf19.7329	Ubiquitin-protein ligase activity	1.5	2.0	1.6	1.5
<i>TEF41</i>	<i>TEF4</i>	orf19.2652	Translation elongation factor activity	4.1	3.4	18.8	19.0
<i>IPF12101</i>	<i>PGA7</i>	orf19.5635	--	1.6	2.7	5.4	6.0
<i>IPF1218</i>	<i>SOD4</i>	orf19.2062	Copper, zinc superoxide dismutase activity	2.3	2.3	2.2	1.8
<i>IPF14119</i>	<i>DAG7</i>	orf19.4688	--	1.8	1.6	1.7	1.7
<i>IPF14652</i>	--	orf19.2653	--	4.3	1.6	1.7	4.3
<i>IPF18080</i>	--	orf19.7357	--	1.9	1.6	1.5	1.6
<i>IPF20010</i>	--	orf19.5799	--	2.0	2.2	2.2	1.6
<i>IPF20169</i>	<i>PGA10</i>	orf19.5674	Heme binding	4.1	10.8	5.6	7.0
<i>IPF2903</i>	--	orf19.7456	--	1.8	2.3	2.0	2.2
<i>IPF3352</i>	--	orf19.4013	--	2.3	2.3	2.0	1.9
<i>IPF4059</i>	--	orf19.1865	--	1.6	2.1	2.7	1.9
<i>IPF4721</i>	--	orf19.3737	--	1.5	1.6	2.3	1.8
<i>IPF6298</i>	--	orf19.1964	--	3.0	1.6	1.7	2.3
<i>IPF6518</i>	--	orf19.1691	--	3.3	5.3	1.7	2.0
<i>IPF7289</i>	<i>UPC2</i>	orf19.391	Transcription factor activity	1.6	2.4	2.1	2.4
<i>IPF7397</i>	--	orf19.1800	--	2.9	2.6	1.8	1.6
<i>IPF7432</i>	--	orf19.6219	--	2.0	2.3	1.9	2.1
<i>IPF867</i>	<i>SET3</i>	orf19.7221	NAD-dependent histone deacetylase activity	2.1	1.7	3.6	3.2
<i>IPF8970</i>	--	orf19.496	--	1.9	2.5	2.2	1.6
<i>ALK3</i>	--	orf19.7512	Catalytic activity	1.5	1.7	1.0	-1.1
<i>ARG5.6</i>	<i>ARG5.6</i>	orf19.4788	N-acetyl-gamma-glutamyl-phosphate reductase activity	7.4	12.7	1.6	-1.8
<i>ARP6</i>	--	orf19.4904	--	1.7	1.6	1.1	-1.3
<i>BET5</i>	--	orf19.302	--	1.8	1.5	-1.1	1.1

Table C-1 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
				S2vS1		Strain A		Strain B	
				1	2	1	2	1	2
CAN2	CAN2	orf19.111	Basic amino acid transporter activity	1.5	1.9	-2.1	1.1	3.2	2.7
CBP1	CBP1	orf19.7323	Steroid binding	1.5	1.6	1.3	1.4	1.9	1.4
CDR12	CDR11	orf19.918	ATPase activity	1.8	3.1	1.4	-1.7	1.9	1.4
Cirt4a	CIRT4B	orf19.2839	--	1.7	1.6	-1.0	1.1	1.9	1.7
COQ4	COQ4	orf19.3008	--	1.5	1.5	1.9	1.4	1.6	1.4
CPA2	--	orf19.3221	Carbamoyl-phosphate synthase activity	3.4	2.2	1.6	-1.4	-1.2	-1.2
CTA1	CAT1	orf19.6229	Catalase activity	1.5	1.6	1.4	1.5	1.2	1.3
DAL51	DAL9	orf19.6956	Allantoate transporter activity	1.5	2.4	-1.1	1.2	2.1	1.6
DNL1	LIG4	orf19.5798	DNA ligase (ATP) activity	1.6	2.2	1.3	1.7	3.0	1.9
ECM18	ECM18	orf19.6958	--	2.1	1.5	-1.3	1.7	1.7	1.2
ECM21.3	ECM21	orf19.4887	--	1.5	2.1	-1.1	1.2	1.5	1.3
ECM42	ECM42	orf19.6500	Amino-acid N-acetyltransferase activity	1.7	2.9	1.2	-2.0	-1.1	-1.0
ERG3	ERG3	orf19.767	C-5 sterol desaturase activity	1.5	1.5	1.1	1.3	1.2	1.3
ERG4	ERG4	orf19.5379	--	1.5	1.6	1.3	1.7	1.3	1.7
ERG7	ERG7	orf19.1570	Lanosterol synthase activity	1.5	1.9	1.6	1.4	1.9	1.4
EXG1	XOG1	orf19.2990	Glucan 1,3-beta-glucosidase activity	2.1	1.6	1.2	-1.3	-1.0	-1.0
FET34.3eoc	FET34	orf19.4215	Ferroxidase activity	1.6	1.7	2.1	1.3	2.6	2.1
FRP1	--	orf19.2496	--	2.6	2.2	1.3	1.6	1.3	1.5
FTR1	FTR1	orf19.7219	Ferrous iron transporter activity	1.5	2.4	1.4	1.5	1.3	1.5
FUN34.5eoc	--	orf19.2496	--	2.7	1.7	1.4	2.3	1.7	1.5
GAL1	GAL1	orf19.3670	Galactokinase activity	2.0	1.6	-1.1	-1.3	-1.2	-1.3
GAL10	GAD1	orf19.1153	UDP-glucose 4-epimerase activity	1.6	1.7	-1.2	-1.5	-1.1	-1.3
GDH2	GDH2	orf19.2192	Glutamate dehydrogenase activity	2.5	1.6	1.1	-1.0	1.1	1.2
GLK1	GLK1	orf19.734	Glucokinase activity	1.6	1.8	1.1	1.2	1.5	1.4
IFA4	IFA4	orf19.4510	--	3.0	2.3	-2.4	1.4	-1.2	-1.2
IFC3	IFC3	orf19.3749	Oligopeptide transporter activity	1.5	2.2	-1.4	-1.2	2.1	1.5
IFK3	FMO2	orf19.857	--	1.8	2.5	-1.1	1.1	1.0	-1.3
IFR3	--	orf19.2908	--	1.7	2.1	1.1	1.7	2.1	1.1
ILV3	ILV3	orf19.4040	Dihydroxy-acid dehydratase activity	1.5	1.8	1.4	1.1	1.2	1.2
LSC2.3eoc1	LSC2	orf19.1860	Succinate-CoA ligase (ADP-forming) activity	1.6	1.5	1.3	1.2	1.1	1.2
LYP1	LYP1	orf19.651	Basic amino acid transmembrane transporter activity	1.5	2.5	-1.3	-1.3	1.4	1.5
MEP3	MEP1	orf19.1614	Ammonium transporter activity	2.9	3.0	-1.1	1.5	2.3	2.0
MSY1	--	orf19.109	Tyrosine-tRNA ligase activity	1.7	1.5	-1.4	-2.2	-1.5	-1.8
NAM2	NAM2	orf19.5705	Leucine-tRNA ligase activity	1.8	1.5	-1.8	-2.4	-1.7	-2.4
NEM1	--	orf19.4657	--	3.0	1.8	1.0	1.2	-1.0	-1.3
ORM1	--	orf19.5751	--	1.7	1.6	1.6	1.4	1.3	1.3
OSM2	OSM2	orf19.5005	Fumarate reductase (NADH) activity	1.6	1.5	-1.2	1.0	1.2	1.0
PHR1	PHR1	orf19.3829	1,3-beta-glucanosyltransferase activity	1.8	2.3	1.3	1.5	1.0	1.2
PLB5	PLB5	orf19.5102	Lysophospholipase activity	1.6	1.8	1.4	1.1	1.8	1.5
POT14	ERG10	orf19.1591	Acetyl-CoA C-acetyltransferase activity	1.8	1.8	1.2	1.3	1.3	1.3

Table C-1 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
				S2vS1		Strain A	
				1	2	1	2
<i>PRP6</i>	--	orf19.6356	RNA splicing factor activity, transesterification mechanism	2.0	1.5	-1.0	-1.2
<i>RAD1.3f</i>	<i>RAD1</i>	orf19.5318	Single-stranded DNA specific endodeoxyribonuclease activity	1.5	1.5	1.3	1.3
<i>RBT2</i>	<i>FRE10</i>	orf19.1415	Ferric-chelate reductase activity	2.1	2.9	1.1	-1.2
<i>RBT4</i>	<i>RBT4</i>	orf19.6202	--	1.6	1.9	1.3	1.6
<i>RNH1</i>	--	orf19.5614	Ribonuclease H activity	2.4	2.0	-1.0	-1.1
<i>SAP1</i>	<i>SAP1</i>	orf19.5714	Aspartic-type endopeptidase activity	1.7	1.9	1.3	-1.1
<i>SAP9</i>	<i>SAP9</i>	orf19.6928	Aspartic-type endopeptidase activity	1.6	1.8	1.3	1.2
<i>SCW11.3eoc</i>	<i>SCW11</i>	orf19.3893	Glucan 1,3-beta-glucosidase activity	1.7	1.6	-1.2	-1.7
<i>SSU1</i>	<i>SSU1</i>	orf19.7313	Sulfite transporter activity	1.8	1.6	1.5	1.1
<i>UGA12.3f</i>	<i>UGA1</i>	orf19.802	4-aminobutyrate transaminase activity	2.0	1.8	1.3	1.0
<i>UGA12.5f</i>	<i>UGA1</i>	orf19.802	4-aminobutyrate transaminase activity	1.8	1.6	1.5	1.4
<i>VPH2</i>	--	orf19.5237	--	1.8	2.0	-1.0	-1.1
<i>IPF10001</i>	--	orf19.6194	--	1.6	1.8	-1.3	-1.0
<i>IPF10431</i>	--	orf19.2888	--	1.5	2.1	-1.2	1.3
<i>IPF11105</i>	--	orf19.2262	--	1.6	1.5	1.2	1.1
<i>IPF11812</i>	<i>SEC3</i>	orf19.2911	--	1.7	1.5	1.2	-1.1
<i>IPF11849</i>	--	orf19.4459	--	3.8	3.3	2.9	1.2
<i>IPF1217</i>	--	orf19.2063	--	1.5	1.5	1.2	1.3
<i>IPF13131.3</i>	<i>HGT20</i>	orf19.1587	Glucose transporter activity	1.6	1.9	-1.2	1.8
<i>IPF13176.3</i>	<i>ARG3</i>	orf19.5610	Ornithine carbamoyltransferase activity	1.8	2.4	-1.2	-1.9
<i>IPF13423</i>	--	orf19.4128	--	1.8	2.9	1.1	-1.1
<i>IPF13815</i>	--	orf19.4553	--	1.8	1.7	1.3	1.1
<i>IPF13967</i>	--	orf19.5205	--	1.6	2.3	-1.0	1.3
<i>IPF14109</i>	<i>PGA45</i>	orf19.2451	--	5.1	3.9	1.4	1.8
<i>IPF14968</i>	--	orf19.3461	--	1.7	2.3	1.5	1.4
<i>IPF15423</i>	<i>SOD6</i>	orf19.2108	Copper, zinc superoxide dismutase activity	1.5	2.5	-1.3	1.2
<i>IPF16948</i>	--	orf19.3285	--	1.8	3.8	-1.2	-1.0
<i>IPF17054</i>	--	orf19.5037	--	3.2	2.1	1.2	1.2
<i>IPF17520.53eoc</i>	--	orf19.1021	--	5.9	2.6	--	--
<i>IPF18207</i>	<i>DAP1</i>	orf19.489	--	1.9	1.7	-1.0	1.7
<i>IPF18316.3f</i>	<i>SSP96</i>	orf19.5145	--	4.8	1.6	-1.4	-1.2
<i>IPF18418</i>	--	orf19.5620	--	2.0	2.6	1.4	1.3
<i>IPF19735</i>	--	orf19.3215	--	1.5	1.5	1.0	1.7
<i>IPF19772</i>	--	orf19.5602	--	1.7	1.7	-1.1	1.0
<i>IPF19908</i>	--	orf19.1344	--	2.2	3.0	2.0	1.2
<i>IPF19998</i>	--	orf19.1344	--	1.6	1.7	1.3	1.1
<i>IPF2065</i>	--	orf19.5274	--	1.7	2.5	1.4	1.2
<i>IPF227</i>	--	orf19.3244	--	2.2	1.5	-1.3	1.4
<i>IPF2561</i>	--	orf19.6624	--	1.5	2.2	1.1	-1.4
<i>IPF2884</i>	--	orf19.7449	--	5.8	1.6	1.4	1.2

Table C-1 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
				S2vS1		Strain A		Strain B	
				1	2	1	2	1	2
IPF3282	HXT5	orf19.4384	Fructose transporter activity	1.7	2.0	1.5	2.6	-1.0	-1.1
IPF3355	--	orf19.4014	--	2.0	2.0	1.7	1.1	1.5	1.0
IPF3535	--	orf19.6840	--	2.1	2.8	2.8	2.2	1.7	1.4
IPF3598	--	orf19.3047	Transcription cofactor activity	1.5	1.5	-1.0	-1.4	1.2	-1.2
IPF4729	--	orf19.3743	--	1.5	1.6	-1.4	1.5	1.7	1.1
IPF5723.exon1	--	orf19.6329	--	1.9	1.6	1.0	1.3	1.3	1.2
IPF5972	CDG1	orf19.7314	--	2.1	1.8	-1.2	-1.2	-1.0	-1.0
IPF6203	ZCF21	orf19.4166	--	1.7	1.7	1.6	1.4	1.7	1.0
IPF6375	--	orf19.520	--	1.9	1.5	1.1	-2.4	-1.5	-1.4
IPF6649	--	orf19.6704	--	1.8	1.5	-1.2	1.0	2.0	1.1
IPF6679	--	orf19.1306	--	1.7	2.0	-1.1	-1.2	1.2	-1.5
IPF6700	--	orf19.2459	--	1.6	1.5	1.2	-1.2	-1.3	-1.5
IPF721.3f	--	orf19.5365	--	1.5	1.6	1.8	-1.3	-1.3	-1.6
IPF7260	--	orf19.2790	Histone-lysine N-methyltransferase activity	1.5	1.5	1.1	-1.2	1.0	-1.2
IPF8311	--	orf19.6003	--	1.6	1.6	1.2	1.1	1.3	1.4
IPF8617	--	orf19.3705	Phosphoprotein phosphatase activity	1.8	1.7	1.1	1.0	1.6	1.2
IPF8796	PGA4	orf19.4035	--	1.6	1.6	-1.0	1.2	1.2	1.3
IPF9425.3	--	orf19.6888	--	1.8	1.6	1.2	-1.0	1.2	1.2
IPF9466	--	orf19.4066	--	2.0	2.0	-1.3	1.4	-1.3	-1.9
IPF9483	--	orf19.3395	--	1.6	1.5	1.1	1.3	1.6	1.4
IPF9995	--	orf19.2286	--	1.8	1.7	1.4	1.6	1.4	1.4
IPF9996	--	orf19.2285	--	1.8	1.8	1.1	1.0	1.9	1.8
ALS11.5f	ALS9	orf19.5742	--	-1.2	-1.0	1.5	4.9	2.7	1.9
ALS9.5eoc	ALS9	orf19.5742	--	-1.2	-1.1	1.5	5.2	2.9	2.1
APC11	--	orf19.7644	Protein binding	-1.2	-1.1	1.6	2.6	1.5	1.6
CDR11.5f	CDR11	orf19.918	ATP binding	-1.4	1.3	1.6	3.5	1.6	1.5
CHT1	CHT1	orf19.7517	Chitinase activity	1.3	1.4	1.7	1.6	1.8	1.6
CLN2	CLN3	orf19.1960	Cyclin-dependent protein kinase regulator activity	2.0	-1.0	1.8	2.7	2.1	2.4
CNH1.3f	CNH1	orf19.367	Cation:cation antiporter activity	-1.2	-1.3	1.5	3.4	2.4	1.5
CNH1.5f	CNH1	orf19.367	Cation:cation antiporter activity	-1.1	-1.4	2.0	9.2	1.7	1.7
DAL1	DAL1	orf19.5454	Allantoinase activity	-1.9	1.0	2.2	2.3	1.5	2.0
DSK2	--	orf19.5345	Protein binding	1.1	1.6	1.7	1.5	1.5	1.5
ERG2	ERG2	orf19.6026	C-8 sterol isomerase activity	2.2	1.3	2.2	2.5	1.9	1.9
ERG5	ERG5	orf19.5178	C-22 sterol desaturase activity	1.3	1.3	1.7	1.8	1.7	1.9
ERG6	ERG6	orf19.1631	Sterol 24-C-methyltransferase activity	1.3	1.4	1.7	1.9	1.6	1.8
FCY24	FCY24	orf19.7331	Transporter activity	1.3	1.1	1.8	2.2	1.9	1.9
FET5	FET34	orf19.4215	Ferroxidase activity	1.7	1.3	2.3	2.4	2.7	2.4
FRE5	FRP1	orf19.5634	Ferric-chelate reductase activity	2.1	2.8	19.3	40.7	24.8	29.6
FRP4	--	orf19.6997	--	-1.5	-5.9	1.5	2.8	3.2	2.7
FRP7	--	orf19.6995	--	-2.6	1.2	1.6	1.7	2.2	1.5

Table C-1 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
<i>GDI1</i>	--	orf19.7261	Rab GDP-dissociation inhibitor activity	1.1	1.4	1.5	2.4	1.7	1.8
<i>IFH2</i>	--	orf19.6398	--	-1.4	1.1	1.6	1.6	1.7	1.9
<i>IFU5</i>	<i>IFU5</i>	orf19.2568	--	1.7	1.3	1.5	2.5	2.3	2.3
<i>PIF2</i>	--	orf19.7538	DNA helicase activity	1.1	1.2	1.5	1.9	1.9	1.6
<i>RBT5</i>	<i>RBT5</i>	orf19.5636	Heme binding	1.2	1.3	2.4	3.0	3.0	3.0
<i>SAP5</i>	<i>SAP5</i>	orf19.5585	Aspartic-type endopeptidase activity	1.1	1.1	2.0	2.0	2.5	3.0
<i>SEC16.53f.eoc</i>	--	orf19.4346	Structural molecule activity	-1.1	-1.8	1.5	2.2	2.0	1.5
<i>SIT1</i>	<i>SIT1</i>	orf19.2179	Ferrichrome transporter activity	-1.2	1.5	1.5	1.7	2.1	1.9
<i>WSC4</i>	<i>WSC4</i>	orf19.7251	--	1.2	1.3	1.6	1.6	1.8	2.2
<i>IPF11259</i>	--	orf19.5210	Transcription factor activity	3.6	-1.4	3.0	2.1	4.5	2.9
<i>IPF11503</i>	--	orf19.3902	--	-1.3	-1.4	1.9	1.5	2.5	2.3
<i>IPF11601</i>	<i>NAG6</i>	orf19.2151	GTP binding	1.3	1.3	1.5	1.5	1.6	1.6
<i>IPF11607</i>	--	orf19.2149	--	1.6	1.2	2.0	2.1	2.5	1.5
<i>IPF12082.3f</i>	--	orf19.6832	--	1.2	1.3	1.5	1.9	2.2	1.9
<i>IPF12082.5f</i>	--	orf19.6832	--	1.2	-1.4	1.7	5.9	1.6	1.9
<i>IPF12262</i>	<i>FGR23</i>	orf19.1616	--	1.1	-1.4	2.1	2.2	2.6	2.1
<i>IPF12963</i>	<i>BUL1</i>	orf19.5094	--	1.2	-1.1	2.7	2.5	3.1	2.9
<i>IPF13377</i>	--	orf19.2350	--	1.2	1.4	1.8	2.4	2.2	2.2
<i>IPF13723</i>	--	orf19.260	--	2.7	-1.0	2.9	2.7	3.3	3.7
<i>IPF14981</i>	--	orf19.3483	--	1.1	3.6	2.1	1.8	3.0	2.2
<i>IPF1514</i>	--	orf19.344	--	1.1	12.2	3.0	17.3	3.5	2.4
<i>IPF1899</i>	--	orf19.7131	--	2.0	-1.1	1.9	3.8	2.0	1.9
<i>IPF20157</i>	--	orf19.6696	--	-21.5	-1.5	1.5	4.0	3.0	1.8
<i>IPF20164</i>	--	orf19.5809	Arylformamidase activity	-1.3	-1.1	1.7	1.5	1.9	1.7
<i>IPF257.3</i>	--	orf19.3261	--	-1.1	-1.4	2.4	2.3	3.0	2.8
<i>IPF2702</i>	--	orf19.2163	--	-2.9	2.2	1.6	4.4	2.6	2.1
<i>IPF3274</i>	--	orf19.4383	--	-1.4	-1.1	3.0	1.5	2.1	1.9
<i>IPF3444.5f</i>	--	orf19.6184	--	-1.7	1.2	1.5	1.9	1.5	1.8
<i>IPF3446</i>	--	orf19.6186	--	-1.3	1.3	2.5	1.8	1.8	1.5
<i>IPF3468</i>	--	orf19.4055	--	-1.1	-1.2	1.5	3.8	2.3	1.9
<i>IPF3687</i>	<i>PAN1</i>	orf19.886	Cytoskeletal adaptor activity	-1.1	1.0	1.5	2.1	2.2	1.7
<i>IPF4055</i>	--	orf19.1867	Malate transmembrane transporter activity	-1.9	2.2	1.7	2.1	2.3	1.9
<i>IPF4160</i>	--	orf19.5459	--	-1.2	1.1	1.5	3.1	1.5	1.7
<i>IPF4649</i>	--	orf19.6102	Specific RNA polymerase II transcription factor activity	-1.2	1.4	1.7	2.0	2.0	1.5
<i>IPF4724</i>	<i>PGA23</i>	orf19.3740	--	1.3	-1.2	1.7	2.4	2.8	2.1
<i>IPF5217</i>	--	orf19.7270	--	-2.3	-1.2	1.6	2.7	2.0	1.7
<i>IPF5556</i>	<i>PHO100</i>	orf19.4424	Acid phosphatase activity	1.1	1.3	4.6	2.5	3.4	3.4
<i>IPF5661</i>	--	orf19.7125	--	-2.0	-2.2	1.8	252.0	2.3	1.7
<i>IPF6067</i>	<i>TCC1</i>	orf19.6734	General transcriptional repressor activity	-1.2	1.1	1.7	1.6	1.7	1.5
<i>IPF6678</i>	--	orf19.1307	--	-1.7	1.1	1.8	1.6	2.9	2.3

Table C-1 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
				S2vS1		Strain A		Strain B	
				1	2	1	2	1	2
<i>IPF708</i>	--	orf19.5370	--	1.1	-1.9	1.7	2.0	1.6	1.5
<i>IPF8904</i>	--	orf19.1363	--	-2.4	1.3	2.4	1.5	1.8	1.7
<i>IPF9370</i>	--	orf19.1424	--	-1.2	1.4	1.5	1.6	1.7	2.0

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to the Assembly 19 version.

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Table C-2. Genes down-regulated in either S2 or gain-of-function S2 allele in SC5314.

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
<i>COQ1</i>	--	orf19.7478	Trans-hexaprenyltransferase activity	-2.8	-2.9	-1.5	-2.5	-2.4	-3.8
<i>MAS2</i>	--	orf19.6295	Mitochondrial processing peptidase activity	-1.6	-1.7	-1.6	-2.3	-2.7	-2.9
<i>MET14</i>	<i>MET14</i>	orf19.946	Adenylylsulfate kinase activity	-5.6	-6.9	-2.3	-2.9	-3.5	-3.7
<i>MET3</i>	<i>MET3</i>	orf19.5025	Sulfate adenylyltransferase (ATP) activity	-4.2	-3.8	-3.3	-4.0	-3.5	-5.9
<i>PET127</i>	--	orf19.2309	RNA binding	-1.7	-1.8	-2.0	-4.4	-7.7	-5.9
<i>IPF11801</i>	--	orf19.2798	Helicase activity	-1.6	-1.7	-2.3	-2.2	-2.0	-1.8
<i>IPF14872</i>	--	orf19.4133	--	-2.0	-1.8	-1.8	-3.5	-2.3	-4.0
<i>IPF20118</i>	--	orf19.3846	--	-3.8	-4.4	-3.6	-3.1	-1.7	-1.6
<i>IPF525</i>	--	orf19.7085	--	-2.4	-2.2	-1.7	-3.1	-1.7	-1.7
<i>IPF6504</i>	--	orf19.1544	--	-2.1	-2.0	-1.8	-1.5	-1.7	-1.6
<i>IPF7334</i>	--	orf19.3724	--	-2.3	-2.2	-1.6	-3.1	-1.8	-3.4
<i>BUL1</i>	--	orf19.4629	--	-2.0	-1.7	-1.8	-1.1	-1.2	-1.1
<i>CDC11</i>	<i>CDC11</i>	orf19.5691	Structural constituent of cytoskeleton	-1.8	-1.8	-1.1	-2.0	-1.3	-1.9
<i>CDC62</i>	--	orf19.5242	ATPase activity	-1.6	-1.7	1.9	6.7	-1.3	1.6
<i>Cirt3</i>	--	orf19.4918	--	-2.5	-2.5	-1.2	-1.4	1.3	-1.2
<i>COS162</i>	--	orf19.4240	--	-1.6	-1.6	1.1	1.1	-1.3	-1.2
<i>CTF8</i>	<i>CTF8</i>	orf19.576	--	-1.8	-1.7	-1.8	1.3	-1.6	-1.1
<i>CVB1</i>	--	orf19.1970	--	-1.6	-1.7	-1.2	-1.3	1.3	-1.5
<i>DAP2</i>	<i>DAP2</i>	orf19.4322	Dipeptidyl-peptidase and tripeptidyl-peptidase activity	-1.8	-2.0	-1.1	-1.1	1.2	-1.1
<i>DCG1</i>	<i>DCG1</i>	orf19.244	--	-1.8	-1.8	-2.1	-1.1	-1.1	-1.9
<i>FIP1</i>	--	orf19.4258	--	-2.2	-2.4	1.3	4.5	-1.7	-1.1
<i>FOL2</i>	--	orf19.3957	GTP cyclohydrolase I activity	-1.6	-1.6	1.1	-1.4	-1.2	-1.3
<i>GCN2</i>	<i>GCN2</i>	orf19.6913	Eukaryotic translation initiation factor 2alpha kinase activity	-1.6	-1.6	-1.1	1.3	-1.2	-1.4
<i>GLR1</i>	<i>GLR1</i>	orf19.4147	Glutathione-disulfide reductase activity	-1.6	-1.6	-1.0	-1.1	-1.1	-1.2
<i>GNA1</i>	<i>GNA1</i>	orf19.837	Glucosamine 6-phosphate N-acetyltransferase activity	-1.7	-3.1	-1.0	1.6	-1.2	-1.0
<i>HNMT3</i>	<i>HNMT3</i>	orf19.2587	Choline transporter activity	-3.1	-2.3	1.1	1.0	-1.1	-1.1
<i>HSL1</i>	<i>HSL1</i>	orf19.4308	Protein kinase activity	-2.3	-1.8	1.1	-1.5	1.3	-1.4
<i>IFG4</i>	<i>DAO1</i>	orf19.3065	--	-1.6	-1.6	1.0	-1.0	1.2	-1.0
<i>IFN2</i>	<i>GIT4</i>	orf19.1980	Phospholipid transporter activity	-2.0	-2.4	-1.2	-1.7	2.2	1.7
<i>IFQ3</i>	<i>RHD1</i>	orf19.54	--	-1.8	-1.7	-1.2	-1.2	1.0	-1.2
<i>IMG1</i>	--	orf19.1967	Structural constituent of ribosome	-2.6	-1.8	-1.2	-1.0	-1.6	-2.1
<i>LEU3</i>	<i>LEU3</i>	orf19.4225	Transcription factor activity	-1.7	-2.1	1.4	-1.3	-1.1	-1.3
<i>MAC1</i>	<i>MAC1</i>	orf19.7068	Specific RNA polymerase II transcription factor activity	-1.8	-1.6	1.0	-1.4	-1.2	-1.2
<i>MAK31</i>	--	orf19.1968.1	Amino acid N-acetyltransferase activity	-3.0	-6.2	1.4	1.4	-1.2	-1.2
<i>MAL31</i>	<i>MAL31</i>	orf19.3981	Alpha-glucoside:hydrogen symporter activity	-2.1	-1.7	-1.9	-1.4	1.6	-1.4
<i>MIG1</i>	<i>MIG1</i>	orf19.4318	Transcription factor activity	-1.6	-1.7	-1.1	-1.4	-1.5	-1.8
<i>MNN22</i>	<i>MNN22</i>	orf19.3803	Alpha-1,2-mannosyltransferase activity	-2.2	-2.3	1.1	-1.8	1.5	1.0
<i>MRPL36</i>	--	orf19.3205	Structural constituent of ribosome	-2.1	-1.7	-1.3	-1.3	-2.1	-2.0
<i>MYO1</i>	--	orf19.6294	Microfilament motor activity	-1.6	-4.1	1.2	3.3	-1.0	1.0
<i>NMD2</i>	--	orf19.864	Protein binding	-2.0	-3.4	1.3	-3.8	-1.5	1.0

Table C-2 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
<i>NUP2.3eoc</i>	--	orf19.3583	Structural molecule activity	-2.0	-2.4	1.0	-1.6	1.2	-1.2
<i>NUP49</i>	<i>NUP49</i>	orf19.4987	Structural molecule activity	-1.6	-1.6	1.1	-1.1	-1.7	-1.1
<i>OGG1</i>	--	orf19.7190	Oxidized purine base lesion DNA N-glycosylase activity	-1.7	-1.6	-1.1	-2.0	-1.7	-2.2
<i>OPT1</i>	<i>OPT1</i>	orf19.2602	Oligopeptide transporter activity	-3.0	-2.2	-1.1	1.0	1.1	-1.1
<i>PAF1</i>	--	orf19.3613	RNA polymerase II transcription elongation factor activity	-1.6	-2.3	1.3	-1.0	-1.4	1.1
<i>PAP11</i>	<i>PAP1</i>	orf19.3197	RNA binding	-1.6	-2.9	1.1	-1.1	1.2	-1.1
<i>PEL1</i>	<i>PEL1</i>	orf19.7072	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase activity	-1.8	-2.2	1.2	-2.4	-2.0	-2.6
<i>PRC2</i>	<i>PRC2</i>	orf19.4135	Carboxypeptidase C activity	-3.0	-2.0	-1.3	-1.2	1.2	1.3
<i>PSD2.3f</i>	--	orf19.3954	Phosphatidylserine decarboxylase activity	-1.9	-2.5	1.2	-1.1	-1.2	-1.4
<i>PUS4</i>	<i>PUS4</i>	orf19.1954	Pseudouridylate synthase activity	-1.6	-2.2	1.2	-1.3	-1.2	-1.9
<i>PZF1</i>	--	orf19.4125	RNA polymerase III transcription factor activity	-1.8	-1.9	-1.2	1.4	-1.7	-2.4
<i>RAD51</i>	<i>RAD51</i>	orf19.3752	Recombinase activity	-1.6	-1.8	1.0	-1.3	-1.0	-1.2
<i>RET2</i>	<i>RET2</i>	orf19.4236	Protein binding	-1.8	-1.6	-1.1	-1.3	-1.0	-1.2
<i>RMT2</i>	<i>RMT2</i>	orf19.920	--	-1.6	-1.9	1.3	-1.6	-2.2	-2.3
<i>RPC82</i>	--	orf19.2847	DNA-directed RNA polymerase activity	-2.6	-4.3	1.3	-1.5	-1.7	-2.8
<i>RVS161</i>	--	orf19.7124	Cytoskeletal protein binding	-1.9	-2.0	-1.0	-1.3	-1.1	-1.3
<i>SEF11.5eoc</i>	<i>SEF2</i>	orf19.1926	--	-1.7	-3.3	1.1	-1.4	-1.3	-1.7
<i>SMC4</i>	--	orf19.964	ATPase activity	-2.5	-1.7	-1.0	-1.6	1.3	-1.5
<i>SPE2</i>	<i>SPE2</i>	orf19.568	Adenosylmethionine decarboxylase activity	-1.6	-1.6	1.1	-1.4	-2.0	-2.2
<i>SSA4</i>	<i>HSP70</i>	orf19.4980	Unfolded protein binding	-4.2	-4.8	-3.4	-1.3	1.5	-1.1
<i>SSP120</i>	--	orf19.3173	--	-1.6	-2.5	1.2	1.6	1.1	-1.3
<i>SUA5</i>	--	orf19.7088	--	-1.8	-2.1	-1.0	-1.4	-4.0	-3.4
<i>SUL1</i>	<i>SUL2</i>	orf19.2738	Sulfate transporter activity	-2.5	-5.8	-1.9	-1.3	1.1	-1.5
<i>TOF1</i>	<i>YBL053</i>	orf19.4136	--	-2.2	-2.9	1.1	-1.6	-1.2	-1.8
<i>TRX2</i>	--	orf19.1976	Thiol-disulfide exchange intermediate activity	-1.6	-2.3	-1.3	1.1	-1.8	-1.7
<i>VPS4</i>	<i>VPS4</i>	orf19.4339	ATPase activity	-2.9	-1.7	1.0	-1.2	1.1	1.1
<i>IPF1047</i>	--	orf19.4563	--	-1.6	-2.1	1.2	-1.2	-3.1	-3.0
<i>IPF10521</i>	--	orf19.4062	--	-1.6	-2.0	-1.2	-1.0	-1.5	-1.7
<i>IPF10711</i>	--	orf19.3694	--	-2.0	-1.7	1.3	-1.4	1.0	-1.0
<i>IPF10785.exon1</i>	--	orf19.5732	--	-1.8	-4.1	1.9	1.2	-1.5	-1.4
<i>IPF10795</i>	<i>CRZ2</i>	orf19.2356	Transcription factor activity	-1.6	-1.7	1.3	-2.2	-1.6	-3.3
<i>IPF11027</i>	--	orf19.5722	--	-1.9	-1.7	-1.1	-1.5	-2.1	-2.7
<i>IPF11090.exon1</i>	--	orf19.4622	--	-1.7	-1.7	1.3	3.1	1.2	-1.4
<i>IPF11161.3</i>	<i>MTLAI</i>	orf19.3201	Transcription factor activity	-2.4	-3.6	-1.4	1.1	-1.3	-1.8
<i>IPF11167</i>	<i>OBPA</i>	orf19.3198	Oxysterol binding	-1.6	-2.3	-1.1	-1.3	-1.1	-1.5
<i>IPF1129</i>	--	orf19.5431	--	-2.5	-1.8	-1.0	-1.2	-1.9	-3.1
<i>IPF11344</i>	--	orf19.4030	--	-2.9	-3.5	-1.4	-1.8	-1.3	-1.6
<i>IPF11434.5f</i>	--	orf19.6462	--	-2.1	-1.9	1.2	-1.0	1.3	1.0
<i>IPF11492</i>	--	orf19.2392	--	-1.6	-1.8	1.2	-1.1	-1.4	-1.6

Table C-2 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
IPF11756	--	orf19.7006	--	-2.1	-2.1	1.2	-1.8	1.4	1.2
IPF11987.3eoc	--	orf19.692	--	-3.9	-1.7	1.2	1.1	1.6	-1.4
IPF11989	--	orf19.3625	--	-1.7	-2.9	-1.2	-12.7	-1.1	-1.5
IPF12141	--	orf19.1952	--	-1.7	-1.7	-1.1	-2.2	-2.0	-2.1
IPF12790	--	orf19.3180	Inositol-polyphosphate 5-phosphatase activity	-1.7	-1.7	-1.2	-1.1	1.6	-1.0
IPF12802	--	orf19.2513	--	-1.6	-2.0	3.6	-3.0	1.5	-5.0
IPF13142	--	orf19.53	--	-2.0	-3.6	-1.5	-1.5	-1.0	-1.7
IPF13247	--	orf19.3376	--	-7.4	-1.6	-1.1	1.2	1.4	1.2
IPF13407	--	orf19.753	Alpha-1,3-mannosyltransferase activity	-1.6	-4.2	-1.1	1.4	1.2	-1.1
IPF13683	--	orf19.2547	--	-1.8	-1.7	1.3	-1.5	-6.6	-3.4
IPF14348.3	--	orf19.1207	--	-1.6	-3.3	1.4	1.0	1.0	-1.2
IPF14487	--	orf19.6346	--	-2.8	-2.8	-1.3	2.1	-1.7	-1.5
IPF14506	--	orf19.1961	--	-2.4	-3.4	1.0	-1.2	1.3	1.1
IPF14663	--	orf19.4316	--	-2.1	-2.0	1.1	-1.2	-1.2	-1.1
IPF14743	--	orf19.3425	--	-1.7	-1.8	1.2	1.0	-1.0	1.0
IPF14871	--	orf19.4132	--	-1.8	-2.1	-1.3	1.1	1.2	1.3
IPF1497	--	orf19.351	--	-1.7	-1.6	-1.2	1.4	1.0	-1.0
IPF14985	--	orf19.4134	--	-2.9	-2.2	-1.1	-1.0	1.3	1.0
IPF1557	--	orf19.954	--	-1.6	-1.6	1.5	1.2	-1.2	-1.1
IPF1567	--	orf19.961.2	Structural constituent of ribosome	-1.7	-1.6	-1.5	1.3	-1.0	-1.1
IPF15811	--	orf19.4280	--	-1.6	-2.5	-2.9	-1.3	-1.2	-1.1
IPF15834	--	orf19.4675	--	-2.2	-1.9	-1.2	-1.3	-2.7	-2.1
IPF16141	--	orf19.2260	Transcription factor activity	-2.2	-2.0	-1.0	-2.2	-1.0	-2.1
IPF16758	--	orf19.5043	--	-1.6	-2.5	--	--	--	--
IPF16947	--	orf19.3286	--	-2.9	-3.5	-1.2	1.2	1.6	-1.1
IPF16988	--	orf19.4312	--	-2.7	-8.7	--	--	--	--
IPF17031	--	orf19.2544	--	-2.0	-2.1	1.2	-1.5	-2.1	-1.7
IPF17050	--	orf19.1049	--	-1.5	-1.6	-3.1	-1.4	-1.7	-1.8
IPF17186	--	orf19.251	--	-1.8	-2.2	-1.7	-1.6	-1.3	-1.3
IPF17296	--	orf19.175	Transcription factor activity	-3.3	-1.8	-12.3	1.1	-7.3	1.0
IPF17998	--	orf19.3147	--	-1.8	-1.8	-1.1	-1.5	-1.6	-2.0
IPF18517.5f	--	orf19.4349	--	-3.7	-33.3	1.1	1.1	-1.8	-1.6
IPF19310.3f	--	--	--	-2.2	-14.6	-1.9	#DIV/0!	11.0	-1.9
IPF1956	--	orf19.7159	--	-2.5	-1.7	1.1	-1.3	-1.2	-2.4
IPF19622	--	orf19.115	--	-2.7	-1.7	-1.1	-2.2	-1.5	1.1
IPF19775	--	orf19.4282	--	-2.2	-1.9	1.4	1.2	-1.0	-1.5
IPF19814	--	orf19.7106	--	-1.6	-1.8	1.1	-1.0	1.8	1.2
IPF19924	--	orf19.4324	--	-1.6	-6.7	-1.1	1.6	-1.8	-1.3
IPF19937	--	orf19.4740	Aminoacyl-tRNA hydrolase activity	-1.9	-1.6	-1.3	1.2	1.2	-1.1
IPF2359	--	orf19.7022	--	-1.7	-2.1	-1.5	-1.7	-1.2	-1.8

Table C-2 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
IPF2522	--	orf19.7460	--	-1.7	-1.6	-1.1	-1.0	-1.2	1.3
IPF2929	--	orf19.4306	--	-1.6	-1.6	-1.1	-1.5	-1.4	-1.6
IPF2953	--	orf19.4294	--	-1.6	-2.4	1.0	-1.0	-1.9	-1.7
IPF2997	--	orf19.6983	--	-1.8	-1.8	1.1	1.1	1.3	-1.4
IPF3087	MET28	orf19.7046	Specific RNA polymerase II transcription factor activity	-1.9	-1.8	-1.5	-2.3	-2.0	-2.8
IPF3144	--	orf19.4220	--	-1.8	-1.6	-1.3	-1.2	-1.0	-1.3
IPF3147	RIX7	orf19.4219	Helicase activity	-1.7	-1.7	1.4	-1.1	-1.9	-1.7
IPF3161	--	orf19.3161	--	-2.0	-1.9	-1.1	-2.4	-2.3	-2.6
IPF3178	--	orf19.3170	--	-2.1	-1.8	1.2	-2.5	-2.1	-3.9
IPF3621	--	orf19.6712	--	-1.7	-1.6	-1.2	-1.4	-1.3	-1.5
IPF3903	--	orf19.760	Structural constituent of ribosome	-1.6	-1.6	-1.3	1.7	-1.5	-1.7
IPF4279	--	orf19.547	Flap endonuclease activity	-1.8	-1.9	1.1	-3.3	-2.3	-2.2
IPF4450	POL93	orf19.6078	--	-1.9	-1.7	-1.3	-1.2	-1.6	-2.4
IPF448	--	orf19.7051	--	-1.6	-1.7	-1.2	-1.0	-1.8	-1.7
IPF4531	--	orf19.6989	rRNA primary transcript binding	-3.0	-1.7	-2.7	-1.1	-1.8	1.1
IPF495	--	orf19.7069	--	-1.7	-1.6	1.2	-1.5	-1.3	-1.7
IPF511	--	orf19.7078	--	-1.7	-1.7	-1.4	-1.3	-1.4	-1.6
IPF5330	--	orf19.3448	--	-1.6	-1.9	-1.5	-2.0	-1.5	-1.4
IPF539	--	orf19.7092	--	-2.1	-1.6	1.3	-1.0	1.3	-1.0
IPF5466	--	orf19.5683	--	-3.1	-2.4	-1.1	1.6	1.4	1.3
IPF560	--	orf19.7102	--	-1.7	-2.8	-1.2	-1.1	-1.0	-1.7
IPF5615	--	orf19.7107	--	-1.7	-1.8	1.1	-1.7	-1.8	-1.6
IPF5621	--	orf19.7111	--	-1.8	-2.1	-1.3	-1.1	1.1	-1.3
IPF563	--	orf19.7103	--	-2.1	-1.6	1.1	-1.2	1.1	-1.2
IPF5644	--	orf19.7116	RNA polymerase II transcription elongation factor activity	-2.6	-2.4	1.0	-1.3	-1.3	-1.3
IPF5661	--	orf19.7125	--	-2.0	-2.2	1.8	252.0	2.3	1.7
IPF5818	--	orf19.3821	--	-1.6	-2.1	-1.3	-1.2	-1.1	-1.6
IPF6252	--	orf19.1458	--	-1.9	-2.0	1.7	1.8	-1.5	-1.9
IPF6296	--	orf19.1966	--	-2.8	-1.8	1.6	-4.6	-1.3	-1.9
IPF6396	--	orf19.580	--	-2.3	-2.4	-1.3	-2.3	-1.2	-1.5
IPF6617.repeat2	LDG3	orf19.6486	--	-37.3	-5.1	2.3	1.4	1.3	3.7
IPF662	--	orf19.7601	rRNA (uridine-2'-O-)-methyltransferase activity	-1.6	-1.8	-1.1	-1.5	-2.9	-2.6
IPF6712.5f	--	orf19.1414	--	-1.6	-2.2	1.2	-1.1	1.0	-1.0
IPF7088	--	orf19.486	Microtubule binding	-1.8	-1.6	1.1	-1.6	1.4	1.4
IPF7204	PGA26	orf19.2475	--	-4.6	-3.0	-1.3	-1.2	-1.2	-1.2
IPF7400	--	orf19.1802	--	-1.6	-1.6	-1.2	1.1	1.3	1.2
IPF7466	--	orf19.2051	--	-1.7	-2.5	1.5	-1.0	-1.4	-1.4
IPF7616	--	orf19.1159	Homoserine O-acetyltransferase activity	-2.3	-1.9	-1.0	-2.6	-1.9	-1.9
IPF7669.3	--	orf19.450	--	-3.5	-1.6	-1.6	1.1	1.1	1.3
IPF7968	--	orf19.2693	Transcription corepressor activity	-1.8	-1.8	-1.7	-1.4	1.3	-1.2

Table C-2 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
IPF8421	--	orf19.928	--	-1.7	-1.6	1.1	-1.4	1.4	-1.2
IPF8535	PGA31	orf19.5302	--	-4.0	-2.6	1.3	1.5	1.5	-1.1
IPF8741.5f	RAD9	orf19.4275	--	-1.9	-2.5	-1.1	-1.3	-1.1	1.0
IPF8901	--	orf19.3758	--	-2.5	-3.6	1.6	2.0	-1.4	1.3
IPF8996	--	orf19.4634	--	-1.9	-1.8	-1.0	1.1	-2.0	-2.1
IPF9417	CTA8	orf19.4775	Transcription factor activity	-1.7	-1.8	1.8	-1.7	-1.9	-1.4
IPF9522.53f	SPR28	orf19.4266	Structural constituent of cytoskeleton	-2.0	-1.9	-1.6	7.7	-1.0	-1.0
IPF9525	--	orf19.4268	snoRNA binding	-2.0	-2.4	1.1	-1.3	-2.7	-2.1
IPF9529	--	orf19.4270	Alpha-1,3-mannosyltransferase activity	-1.8	-1.7	1.1	-1.4	-1.3	-1.2
IPF9566	--	orf19.1939	--	-1.8	-1.6	1.3	1.9	-2.0	-1.0
IPF9568	--	orf19.1938	--	-1.7	-2.3	-1.4	-1.7	-1.7	1.2
IPF9580	--	orf19.687	--	-1.7	-2.1	1.1	1.3	-1.1	-1.6
IPF9841	--	orf19.3204	--	-1.8	-2.2	-2.3	-1.1	-3.0	-2.2
IPF9972.3eoc	--	orf19.4522	--	-1.6	-1.6	-1.9	1.6	-2.0	1.4
ALS10	ALS10	orf19.2355	Cell adhesion molecule binding	-1.1	-1.3	-2.4	-1.7	-1.7	-1.9
ALS3.3eoc	ALS10	orf19.2355	Cell adhesion molecule binding	1.5	-1.1	-2.3	-2.0	-2.2	-2.0
ATP11	--	orf19.6916	--	1.7	-2.1	-1.9	-2.0	-3.1	-2.1
CUS2	--	orf19.5767	RNA binding	-1.2	-1.2	-2.1	-2.0	-1.7	-1.6
ECE1	ECE1	orf19.3374	--	-1.2	-6.0	-7.0	-3.8	-4.6	-3.5
FRE7	--	orf19.7077	Ferric-chelate reductase activity	-2.1	-1.1	-1.6	-1.6	-2.3	-2.5
GCN5	--	orf19.705	Histone acetyltransferase activity	1.1	1.0	-1.7	-1.7	-1.7	-2.1
HGT11	HGT1	orf19.4527	Glucose transporter activity	-1.4	1.0	-3.2	-1.8	-3.0	-2.8
HGT12	HGT2	orf19.3668	Glucose transporter activity	-1.1	1.1	-2.0	-6.6	-3.0	-2.7
HWP1	HWP1	orf19.1321	--	1.0	-1.9	-4.8	-4.5	-3.2	-2.7
MRPL10.3	MRPL10	orf19.3532	Structural constituent of ribosome	1.5	-1.2	-1.5	-2.2	-2.1	-2.6
MRS2	MRS2	orf19.2597	Magnesium ion transporter activity	-1.1	-1.6	-1.8	-1.9	-1.8	-2.2
MTF1	--	orf19.1528	Transcription factor activity	-1.5	-1.4	-1.6	-4.0	-3.7	-4.9
NAM2	NAM2	orf19.5705	Leucine-tRNA ligase activity	1.8	1.5	-1.8	-2.4	-1.7	-2.4
NCB2	--	orf19.5825	Transcription corepressor activity	1.1	-1.6	-1.6	-1.9	-1.7	-4.9
PAC10.3	--	orf19.5985	--	1.0	-1.1	-1.6	-1.7	-2.3	-1.9
PHB2	--	orf19.5973	--	-1.2	-1.4	-1.7	-2.1	-1.8	-1.9
RRP45	--	orf19.4078	3'-5'-exoribonuclease activity	1.3	-3.3	-1.7	-1.9	-3.3	-2.3
SEC9	--	orf19.117	--	1.5	-1.5	-1.9	-2.2	-1.9	-1.8
SOU1	SOU1	orf19.2896	2,4-dienoyl-CoA reductase (NADPH) activity	-1.2	-1.4	-3.0	-2.2	-2.1	-2.2
TLG1	--	orf19.3898	t-SNARE activity	1.6	-2.0	-2.3	-5.7	-1.6	-3.3
IPF10399	--	orf19.3367	Structural constituent of ribosome	1.1	-1.6	-1.8	-1.9	-2.6	-3.0
IPF10455	--	orf19.104	--	-3.2	-1.6	-2.0	-1.8	-1.8	-1.9
IPF10716	--	orf19.3691	--	-1.2	-1.9	-1.8	-2.1	-2.4	-1.6
IPF12047	--	orf19.3471	--	-1.7	-1.3	-1.7	-2.1	-2.2	-3.0
IPF1209	--	orf19.2068	--	-1.5	1.2	-1.7	-4.4	-2.2	-2.6

Table C-2 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	S2vS1		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}		UPC2 ^{S2-1} vs. UPC2 ^{S1-1}	
						Strain A		Strain B	
				1	2	1	2	1	2
IPF12152	--	orf19.6236	--	-1.1	-5.0	-1.7	-2.0	-3.2	-3.3
IPF1242	--	orf19.7360	--	1.1	1.0	-1.6	-2.3	-2.3	-2.1
IPF12844	--	orf19.2362	--	1.1	-1.8	-1.8	-1.9	-3.8	-5.0
IPF13221	--	orf19.1697	--	1.7	-1.7	-1.8	-3.7	-2.5	-2.5
IPF13402	--	orf19.36	--	1.0	-1.9	-1.6	-1.6	-1.7	-2.0
IPF13552	--	orf19.1300	--	-1.2	-1.2	-1.6	-2.7	-2.3	-2.7
IPF13613	--	orf19.725	--	-1.1	-1.3	-1.8	-4.6	-2.4	-1.7
IPF13782	--	orf19.2314	--	1.9	-3.0	-2.0	-2.4	-3.4	-5.1
IPF14574	--	orf19.1083	--	1.4	-1.2	-1.7	-1.7	-1.9	-2.4
IPF14921	--	orf19.1527	--	3.4	-1.3	-1.8	-1.9	-2.1	-3.2
IPF2232	--	orf19.6899	--	3.9	-7.0	-1.5	-5.6	-1.9	-1.8
IPF2555	--	orf19.6628	Structural constituent of cytoskeleton	-1.2	-2.9	-1.9	-2.0	-1.8	-3.6
IPF2839	--	orf19.7296	--	-1.0	1.3	-2.0	-3.1	-3.3	-2.5
IPF2998	--	orf19.6982	--	-1.9	-1.4	-2.8	-8.0	-4.9	-9.4
IPF3015	MODF	orf19.5029	ATP binding	-1.1	-1.6	-1.8	-2.4	-1.8	-2.5
IPF312	--	orf19.3154	--	1.0	-1.1	-1.6	-2.4	-1.6	-2.0
IPF3857	--	orf19.25	--	1.1	-1.4	-1.6	-2.0	-2.7	-2.6
IPF4080	MRP8	orf19.3844	Structural constituent of ribosome	1.5	-1.7	-1.6	-1.6	-1.6	-1.7
IPF4671	--	orf19.1849	--	1.6	-1.7	-1.8	-2.2	-1.7	-1.7
IPF4880	--	orf19.6266	3'-5'-exoribonuclease activity	-1.1	-1.2	-1.7	-1.6	-2.2	-2.9
IPF4991	CSP37	orf19.2531	--	1.1	-1.5	-4.3	-2.4	-1.6	-1.9
IPF5149	--	orf19.6530	Signal recognition particle binding	-1.8	-1.9	-1.9	-1.8	-1.9	-3.5
IPF61	--	orf19.5959	snoRNA binding	6.6	-20.0	-2.4	-2.9	-1.7	-2.7
IPF6339	--	orf19.2724	--	1.3	-1.0	-1.6	-2.1	-1.7	-2.0
IPF7166	--	orf19.1202	--	-1.3	-1.4	-1.7	-1.8	-1.8	-2.2
IPF8114	--	orf19.2939	--	-1.2	-1.6	-1.6	-1.7	-2.2	-1.5
IPF8422	--	orf19.927	--	-1.2	-2.7	-1.9	-2.3	-2.6	-3.5
IPF8727	--	orf19.1794	--	-1.8	-3.2	-2.0	-11.5	-1.6	-9.8
IPF8854	--	orf19.4900	Alpha-1,3-mannosyltransferase activity	-1.3	-2.0	-1.6	-2.2	-1.6	-2.2

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to the Assembly 19 version.

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Table C-3. Genes down-regulated in the absence of *UPC2*.

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	<i>Δupc2</i> vs. SC5314				<i>UPC2</i> ^{S2-1} vs. <i>UPC2</i> ^{S1-1}			
				Strain A		Strain B		Strain A		Strain B	
				1	2	1	2	1	2	1	2
<i>CHAI2</i>	<i>CHAI1</i>	orf19.1996	L-serine ammonia-lyase activity	-1.7	-1.9	-1.5	-1.9	1.1	1.1	-1.7	-1.2
<i>FDH4.3f</i>	--	orf19.1774	Formate dehydrogenase activity	-1.7	-2.5	-1.7	-1.8	-1.1	-3.3	-6.4	-3.5
<i>IPF14026</i>	--	orf19.718	Transcription factor activity	-1.9	-2.3	-2.2	-2.2	-1.2	-1.7	1.6	-3.0
<i>IPF15335</i>	--	orf19.4970	--	-3.0	-2.0	-1.6	-2.0	-2.8	1.1	-1.4	2.4
<i>IPF7289</i>	<i>UPC2</i>	orf19.391	Transcription factor activity	-262.2	-196.2	-1573.0	-164.5	2.1	2.5	2.4	2.5

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to the Assembly 19 version.

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

Table C-4. Genes up-regulated in the absence of *UPC2*.

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	Aupc2 vs. SC5314				UPC2 ^{S2-1} vs. UPC2 ^{S1-1}			
				Strain A		Strain B		Strain A		Strain B	
				1	2	1	2	1	2	1	2
AAF1	AAF1	orf19.7436	--	1.6	1.5	1.7	1.7	1.4	-1.1	1.2	-1.6
ALS10	ALS10	orf19.2355	Cell adhesion molecule binding	1.6	1.8	1.8	2.7	-2.4	-1.7	-1.7	-1.9
ALS3.3eoc	ALS10	orf19.2355	Cell adhesion molecule binding	1.6	1.5	1.8	2.3	-2.3	-2.2	-2.0	-2.0
ALS3.5eoc	ALS3	orf19.1816	Cell adhesion molecule binding	1.5	1.7	1.9	2.6	-2.1	-1.8	-1.2	-1.4
ARG1	ARG1	orf19.7469	Argininosuccinate synthase activity	1.6	1.5	2.5	1.5	1.6	1.2	-1.1	1.1
CAN2	CAN2	orf19.111	Basic amino acid transporter activity	1.7	1.6	2.1	1.7	-2.1	3.2	1.1	2.7
DDR48	DDR48	orf19.4082	--	4.4	5.2	8.2	11.5	2.4	3.4	2.4	3.8
ECM331	ECM331	orf19.4255	--	5.0	4.5	13.0	10.9	1.2	1.3	-2.1	1.9
FRP2	--	orf19.6169	--	1.8	1.6	1.8	1.6	-1.1	-1.3	-1.6	-1.1
GLG2	GLG2	orf19.7434	Glycogenin glucosyltransferase activity	1.8	1.9	2.9	1.6	-1.8	1.4	-1.0	-2.0
HGT12	HGT2	orf19.3668	Glucose transporter activity	1.8	3.0	2.2	7.9	-2.0	-3.0	-6.6	-2.7
IFC4	OPT4	orf19.176	Oligopeptide transporter activity	1.5	1.5	1.7	1.5	-1.0	4.5	2.9	4.4
INO1	INO1	orf19.7585	Inositol-3-phosphate synthase activity	2.0	2.5	3.4	3.6	1.7	1.3	1.2	1.3
MIF2	MIF2	orf19.5551	Centromeric DNA binding	1.7	1.6	1.5	1.6	-1.1	-1.4	-2.4	-1.9
OSM2	OSM2	orf19.5005	Fumarate reductase (NADH) activity	1.6	1.6	1.8	1.6	-1.2	1.2	1.0	1.0
RIM8	RIM8	orf19.6091	--	1.8	1.6	1.8	1.9	1.1	2.5	1.3	-1.4
RTA4	RTA4	orf19.6595	Phospholipid-translocating ATPase activity	1.5	1.6	1.8	2.4	-1.5	1.2	1.1	1.1
SNF31	HGT12	orf19.7094	Fructose transmembrane transporter activity	15.4	2.4	12.0	2.9	1.0	3.4	1.2	-1.0
YOX1	--	orf19.7017	--	1.6	2.1	1.8	1.6	1.1	-2.8	-1.1	-1.8
IPF10171.exon3	HGT9	orf19.644	Glucose transmembrane transporter activity	41.5	1.8	21.0	1.5	1.1	1.8	-1.7	1.1
IPF11801	--	orf19.2798	Helicase activity	2.2	2.4	2.0	2.5	-2.3	-2.0	-2.2	-1.8
IPF11876	--	orf19.5295	--	2.0	1.8	1.7	1.5	1.4	-1.2	-1.3	1.5
IPF12312	--	orf19.5070	--	1.8	1.5	1.7	1.8	1.3	-1.0	1.1	-1.1
IPF13409	--	orf19.751	--	2.3	1.8	2.3	1.9	-1.9	-1.1	1.0	-2.3
IPF1341	PGA13	orf19.6420	--	1.9	2.5	2.8	4.1	1.0	1.2	-1.6	-1.1
IPF14119	DAG7	orf19.4688	--	1.8	1.9	2.1	2.7	1.7	1.6	1.7	1.8
IPF14618	--	orf19.6079	--	1.6	2.0	1.8	3.0	-1.3	-1.2	5.3	-1.1
IPF16253	--	orf19.3932	--	1.8	1.9	2.4	2.2	-1.5	-2.5	-1.6	-1.9
IPF16843	--	orf19.2397	--	1.8	1.6	1.8	1.8	-1.1	-1.2	-1.3	-1.8
IPF18732	--	orf19.711	--	1.9	2.8	2.7	4.5	-1.1	1.4	-1.5	1.8
IPF19749	--	orf19.94	--	1.5	1.6	1.5	1.6	-2.2	-1.5	-2.0	-1.4
IPF2839	--	orf19.7296	--	3.3	2.2	4.3	3.1	-2.0	-3.3	-3.1	-2.5
IPF300	--	orf19.3282	--	2.0	1.9	2.4	2.2	1.0	1.2	-1.2	1.0
IPF4443	--	orf19.4706	--	1.9	2.0	2.0	2.9	1.3	-1.6	1.2	-1.2
IPF4724	PGA23	orf19.3740	--	5.6	3.5	9.9	8.4	1.7	2.8	2.4	2.1
IPF7109	--	orf19.7350	--	1.5	1.6	1.6	1.7	-1.1	1.1	1.1	1.0
IPF743	--	orf19.5353	--	2.3	2.3	2.8	3.4	-2.1	-1.1	-1.1	-1.1
IPF7493	HGT17	orf19.4682	Glucose transmembrane transporter activity	2.0	2.1	2.3	2.7	1.3	-1.2	1.1	-1.1
IPF7666	GAT2	orf19.4056	Transcription factor activity	1.6	1.6	1.5	1.5	-1.2	-1.1	1.1	-1.4

Table C-4 (continued).

CandidaDB Name ^a	CGD Name ^b	orf19 Number ^c	GO Annotation (Molecular Function) ^d	Δupc2 vs. SC5314				UPC2 ^{S2-1} vs. UPC2 ^{S1-1}			
				Strain A		Strain B		Strain A		Strain B	
				1	2	1	2	1	2	1	2
IPF9955	--	orf19.3499	--	3.2	4.2	4.6	9.7	-1.3	3.0	1.5	1.9
IPF9996	--	orf19.2285	--	6.1	1.9	4.8	2.2	1.1	1.9	1.0	1.8

^a Gene name at CandidaDB (<http://genolist.pasteur.fr/CandidaDB/>).

^b Gene name at CGD (<http://www.candidagenome.org/>).

^c orf19 nomenclature according to the Assembly 19 version.

^d GO annotation found at CGD (<http://www.candidagenome.org/>).

VITA

Teresa T. Liu, daughter of Larry and Ginny Liu, was born in Taipei, Taiwan on March 24, 1980. She immigrated to the United States in 1983 and enrolled in the Plano Independent School District in Plano, Texas. After graduating from Plano Senior High School in 1998, she attended Westminster College in Fulton, Missouri, graduating with a Bachelors of Arts in Biology and Computer Science in 2002. She enrolled in the Interdisciplinary Program at the University of Tennessee Health Science Center in 2002. She anticipates completing her Doctor of Philosophy in May 2008.