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Changes in the Proteome of *Candida albicans* in Response to Azole, Polyene, and Echinocandin Antifungal Agents[▽]

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The yeast *Candida albicans* is an opportunistic human fungal pathogen and the cause of superficial and systemic infections in immunocompromised patients. The classes of antifungal agents most commonly used to treat *Candida* infections are the azoles, polyenes, and echinocandins. In the present study, we identified changes in *C. albicans* protein abundance using two-dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption ionization–time of flight mass spectroscopy following exposure to representatives of the azole (ketoconazole), polyene (amphotericin B), and echinocandin (caspofungin) antifungals in an effort to elucidate the adaptive responses to these classes of antifungal agents. We identified 39 proteins whose abundance changed in response to ketoconazole exposure. Some of these proteins are involved in ergosterol biosynthesis and are associated with azole resistance. Exposure to amphotericin B altered the abundance of 43 proteins, including those associated with oxidative stress and osmotic tolerance. We identified 50 proteins whose abundance changed after exposure to caspofungin, including enzymes involved in cell wall biosynthesis and integrity, as well as the regulator of β-1,3-glucan synthase activity, Rho1p. Exposure to caspofungin also increased the abundance of the proteins involved in oxidative and osmotic stress. The common adaptive responses shared by all three antifungal agents included proteins involved in carbohydrate metabolism. Some of these antifungal-responsive proteins may represent potential targets for the development of novel therapeutics that could enhance the antifungal activities of these drugs.

Candida albicans is an opportunistic yeast and a harmless commensal organism within the endogenous microflora of many healthy individuals; however, in immunocompromised patients, this fungus is a major cause of infections ranging from superficial mycoses to invasive, deep-seated systemic candidiasis. *C. albicans* is considered the most common human fungal pathogen, and overall, *Candida* species are the fourth leading cause of bloodstream infections in the United States (10, 33). The most effective classes of antifungal agents used to treat *Candida* infections are the azoles, polyenes, and echinocandins. The azole antifungals, such as ketoconazole, fluconazole, and itraconazole, inhibit the biosynthesis of ergosterol, which is the major sterol within the fungal membrane. The azoles exert their activity by specifically binding to the heme group in the active site of the cytochrome P450 enzyme 14α-lanosterol demethylase (Erg11p). The inhibition of Erg11p results in insufficient amounts of ergosterol within the fungal membrane and the accumulation of the toxic sterol 14α-methylergosta-8, 24-(28)-dien-3β,6α-diol, which also inhibits fungal growth (1, 18). The polyenes, including amphotericin B, natamycin, and nystatin, are amphipathic antimycotics that target ergosterol within the fungal cell membrane. After the binding of ergosterol, these agents expand the lipid bilayer, forming channels through the membrane, which lead to the leakage of intracellular potassium and other molecules, resulting in fungal death. The echinocandins, such as caspofungin, anidulafungin, and micafungin, are acylated cyclic hexapeptides that exert their fungicidal activity by noncompetitively inhibiting β-1,3-glucan synthase, an enzyme required for cell wall biosynthesis. Inhibition of this protein results in the absence of normally required β-glucans in the fungal cell wall, causing cell death.

These classes of antifungal agents represent a limited arsenal of drugs used to combat *Candida* infections; moreover, these agents also have significant limitations that restrict their routine clinical use. The azole antifungals are fungistatic, are associated with drug-drug interactions, and are hindered by the emergence of azole-resistant species of *C. albicans* (46, 51). The polyene agents have adverse side effects, such as nephrotoxicity, renal failure, and infusion-related toxicity (2, 3, 47). The echinocandin antifungals are somewhat costly and have been associated with fever, thrombophlebitis, and hepatic toxicity (17, 34, 36). Although the echinocandins are effective against fluconazole-resistant strains of *Candida*, there have been cases of resistance to this class of drugs in *C. albicans* (26, 28). In the face of these limitations, the identification of novel antifungal targets and the development of new antifungal agents are warranted for the effective treatment of *Candida* infections.

In a previous study, we used genome-wide gene expression profiling by microarray analysis to identify the changes in *C. albicans* gene expression in response to clinically available representatives of the polyene, azole, echinocandin, and pyrimidine classes of antifungal agents (24). That study revealed that

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82, 256, and 480 genes were differentially expressed following exposure to ketoconazole, amphotericin B, and caspofungin, respectively. Briefly, ketoconazole exposure induced the expression of genes associated with ergosterol biosynthesis and azole resistance, while amphotericin B caused alterations in the expression of genes involved in small-molecule transport and oxidative stress response. Exposure to caspofungin was observed to induce the expression of genes associated with cell wall biosynthesis and maintenance, including the gene encoding the (1,3)-β-glucan synthase, *GSL1*.

Proteomic analysis has been applied to the study of many aspects of *C. albicans*, including its adaptive responses to salt, cadmium, peroxide, oxidative stress, and macrophage exposure (11, 21, 50). Proteomic analysis has also been used to study the adaptive response of *C. albicans* to fluconazole, itraconazole, and mulundocandin (5, 49) and to identify changes in protein abundance in matched sets of azole-susceptible and -resistant clinical isolates of *C. albicans* (15, 20). In the present study, we used the combination of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and matrix-assisted laser desorption ionization–time of flight mass spectroscopy (MALDI-TOF MS) to identify the changes in protein abundance in a wild-type strain of *C. albicans* after exposure to ketoconazole, amphotericin B, and caspofungin. We used the same conditions that we used in our previous microarray study, which allow the direct comparison of the transcriptome and the proteome representing these adaptive responses (22). Our study also allows a comparative analysis of these three commonly used classes of antifungal agents. We report here the changes in protein abundance associated with both specific and nonspecific adaptive responses to these antifungal agents in this pathogenic fungus.

MATERIALS AND METHODS

Antifungal agents and chemicals. Ketoconazole was purchased from Sigma Aldrich Chemical Company (St. Louis, MO), and amphotericin B was obtained from MP Biomedicals (Aurora, OH). A preparation of caspofungin acetate for injection (Cancidas; Merck) was obtained from the pharmacy at Le Bonheur Children's Medical Center. Stock solutions (10 mg/ml) of ketoconazole and amphotericin B were prepared in dimethyl sulfoxide (DMSO; Sigma), while stocks of caspofungin were made in sterile water. All antifungal stock solutions were prepared fresh before all experiments, and all other chemicals and reagents were obtained from Bio-Rad Laboratories (Hercules, CA), unless otherwise specified.

Organism and culture conditions. *C. albicans* wild-type strain SC5314 was cultured overnight in 10 ml of synthetic dextrose (SD) minimal medium containing 165 mM morpholinepropanesulfonic acid, 0.67% (wt/vol) yeast nitrogen base without amino acids, and 2% (wt/vol) dextrose at pH 7.0 in a temperature-controlled incubator at 30°C. For each sample, cells were diluted into 500 ml of fresh SD medium to an optical density (OD) of 0.005 and were subsequently grown at 30°C until an OD of 1.0 was reached. The cultures were then diluted with fresh SD medium to an OD of 0.1. After growth to an OD of 0.2, the antifungal agents were added to the cultures at concentrations equivalent to their 50% inhibitory concentrations (ketoconazole, 19.13 μg/ml; amphotericin B, 0.029 μg/ml; caspofungin, 0.0075 μg/ml) and the cultures were incubated at 30°C with shaking at 250 rpm in a temperature-controlled incubator for 6 h. Due to the photosensitivity of amphotericin B, all cultures with amphotericin B were incubated in the dark. After the 6-h incubation, the ODs were measured to ensure that the antifungals used in the drug-treated cultures inhibited at least half of the fungal growth compared to the growth achieved by the DMSO-treated controls. All drug exposure experiments were conducted in triplicate.

Preparation of protein extracts. *C. albicans* cells were collected by centrifugation. The pelleted cells were washed three times with ultrapure water and were broken in cold 50 mM Tris buffer (pH 7.4) containing 1 mM EDTA with 0.5-mm-diameter glass beads with a Mini-Bead Beater 8 apparatus (BioSpec Products, Inc., Bartlesville, OK). Briefly, an equal volume of glass beads was added to the cell pellet in 2-ml conical bottom tubes and the samples were beaten for six 20-s bursts. Between bursts, the samples were incubated on ice for 1 min. After disruption, the samples were centrifuged at 20,000 × *g* for 15 min at 4°C to remove the glass beads, unbroken cells, and particulate debris from the homogenate. After centrifugation, the soluble protein fraction was collected, the protein content was measured by the bicinchoninic acid (BCA) method (41), and the proteins were stored at −80.0°C for further analysis.

2-D PAGE. For isoelectric focusing (IEF), samples containing 300 μg of protein were dissolved in 300 μl rehydration buffer consisting of 7 M urea, 2 M thiourea, 4% (wt/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 50 mM dithiothreitol (DTT), 0.2% (vol/vol) Biolyte 3-10 ampholytes, and 0.001% (wt/vol) bromophenol blue. The samples were then added to 17-cm ReadyStrip immobilized pH gradient (IPG) strips (Bio-Rad) and run on a Protean IEF cell (Bio-Rad) at 20.0°C. The protein samples were subjected to passive rehydration for 12 h and then run at 250 V for the first 15 min, followed by a linear increase in voltage to a maximum of 10,000 V, at which point the strips were run for an additional 5 h. After IEF, the IPG strips were removed and reduced in equilibration solution consisting of 1.5 M Tris-HCl (pH 8.8), 7 M urea, 2% (wt/vol) sodium dodecyl sulfate (SDS), 20% (vol/vol) glycerol, and 1% (wt/vol) DTT for 10 min at 25°C. The IPG strips were then alkylated in equilibration buffer containing 1.5 M Tris-HCl (pH 8.8), 7 M urea, 2% (wt/vol) SDS, 20% (vol/vol) glycerol, and 1.5% (wt/vol) iodoacetamide for 10 min at 25°C. The proteins were subsequently separated in the second dimension on the basis of their molecular masses by using 12% polyacrylamide gels containing 2.6% *N*,*N*′-methylenebisacrylamide and run at a constant 200 V for 6 to 7 h at 15°C in a Protean Plus Dodeca cell (Bio-Rad). After electrophoresis, the gels were fixed and stained with SYPRO ruby.

Gel imaging and analysis software. The 2-D gels were imaged on an FX imager (Bio-Rad) at the medium SYPRO ruby intensity setting at a resolution of 300 dots per inch. Spot comparison of 2-D gel images was performed with PDQuest software (version 7.1; Bio-Rad) to measure the differences in spot intensity. Protein spots between gel images were compared by using an automated matching program, and any mismatched spots were corrected by use of a manual matching feature in the software. After removal of the background, the individual spot volume for each resolved protein spot was normalized against the total spot volume and area in the gel. Protein spots that were consistently differentially expressed in the mean normalized spot volume by at least 1.5-fold in three independent experiments were selected for excision and subsequent identification. Statistical analysis was performed by Student's *t* test.

Protein trypsinization and extraction. Protein spots were excised from the gels and transferred to 1.5-ml microcentrifuge tubes prewashed three times with 50% methanol–10% glacial acetic acid. The proteins were then destained twice in 200 μl of 50% acetonitrile at 23°C and vortexed for 30 min. The samples were placed in a 96-well Millipore polytetrafluoroethylene 0.45-μm-pore-size filter plate; the wells had been pretreated three times with 20% methanol–0.1% trifluoroacetic acid (TFA). The protein samples were washed with acetonitrile; subsequently digested in a 100-μl aliquot of fresh digestion buffer containing 12.5 ng/μl ultrapure sequencing-grade trypsin (Promega, Madison, WI), 25 mM ammonium bicarbonate, and 10% acetonitrile; and incubated at 37°C overnight. The tryptic polypeptides were extracted in a 25-μl aliquot of fresh extraction solution containing 50% acetonitrile and 5% TFA.

Protein identification and database search. Protein identification was carried out by the methods described by Cummings et al. (7). Briefly, MS spectra were recorded on an Ultraflex matrix-assisted laser desorption ionization reflecting time of flight (MALDI-ToF/ToF) mass spectrometer (Bruker Daltonics, Bremen, Germany). The autolytic trypsin fragment peaks of *m*/*z* 842.509 and *m*/*z* 2,211.104 were used for internal calibration of the peptide mass spectra with mass resolutions of between *m*/*z* 2,500 and 8,000. External calibration of this instrument was carried out with a 2:1 mixture of des-*R*-bradykinin and adrenocorticotropin (Sigma). Mass spectra parameter settings were 20 kV for the extraction voltage and 11.8 kV for the reflector voltage. Both FlexControl software (version 2.0) and FlexAnalysis software (version 2.0) were used to record the mass spectra data and sample analysis, respectively. For each sample, the signal-to-noise ratio was calculated by using the SNAP algorithm and the default settings within the FlexAnalysis software. For each protein identified, MALDI-TOF mass fingerprint data were used to perform a BLAST search of the sequences against those in a custom database (http://genolist.pasteur.fr/CandidaDB/) containing known *C. albicans* open reading frame (ORF) DNA sequences by using PROWL software. For each protein identified, a *Z*-score value was calculated from the software that measured the significance of the identification. *Z*-score values of 1.20 and 1.65 ranked the mass data search to 90

and 95% levels of confidence of nonrandom matches to the specific ORF, respectively.

RESULTS

Changes in protein abundance in response to ketoconazole exposure. A total of 398 proteins were resolved by 2-D PAGE after exposure to ketoconazole. Among them, 116 proteins were found to be changed in abundance and 39 were identified. Of those 39 proteins, the abundance of 32 proteins was increased, while the abundance of 7 was decreased compared to the abundance for the DMSO-treated controls. The ketoconazole-responsive proteins are shown in Fig. 1. Descriptions of these proteins, including their putative biological function, molecular properties, and changes in relative levels of expressions, are displayed in Table 1. The proteins in the largest category that responded to ketoconazole exposure are involved in carbohydrate metabolism and included enzymes that function in the tricarboxylic acid (TCA) cycle (Aco1p, Cit1p, Idh2p, Mdh1p, and Mdh11p), glycolysis and gluconeogenesis (Cdc19p, Gpm1p, Pgm2p), the pentose phosphate pathway (Gnd1p), and pyruvate transformation (Pdb1p and Pdc11p). Other proteins shown to be responsive to this drug are involved in amino acid biosynthesis (Bat22p, Gdh3p, and His1p), cell stress and heat shock (Snz1p, Ssa1p, and Hsp90p), and protein biosynthesis (Tif5p and Tuf1p). Of particular interest are the enzymes involved in ergosterol biosynthesis, including Erg6p, Erg10p, and Erg13p, as well as proteins that have previously been identified as being associated with azole resistance, such as Csh1p and Orf19.251.

Changes in protein abundance in response to amphotericin B exposure. A total of 391 proteins were resolved by 2-D PAGE after exposure to amphotericin B. Among them, 60 were found to be changed in abundance and 43 were identified. Of those 43 proteins, the abundance of 39 proteins was increased, while the expression of 4 was decreased. These amphotericin B-responsive proteins are displayed in Fig. 1; and descriptions of these proteins, including their putative biological function, molecular properties, and changes in relative levels of expression, are shown in Table 2. The proteins in the largest class responsive to amphotericin B are involved in cell stress and heat shock (Snz1p, Ssa1p, Ssa4p, Ssb1p, Ssc1p, Hsp60p, and Hsp90p). Other proteins responsive to this drug are involved in the TCA cycle (Aco1p, Idh2p, Fum12p, Lsc2p, Mdh1p, and Mdh11p), amino acid biosynthesis (Aat1p, Met6p, Sam2p, Thr4p, and Cys3p), and glycolysis and gluconeogenesis (Pdb1p, Pdc11p, and Gnd1p); and some of these proteins had unknown functions (Ipf1364p, Ipf8762p, and Ipf19154p). Also identified were enzymes involved in oxidative stress (Cip1p, Trr1p, Trx2p, Tsa1p, and Yhb1p) and other proteins associated with osmotic tolerance (Asr2p and Rhr2p).

Changes in protein abundance in response to caspofungin exposure. A total of 264 proteins were resolved by 2-D PAGE after exposure to caspofungin. Among them, 78 were found to be changed in abundance and 50 were identified. Of those 50 proteins, the abundance of 32 proteins was increased, while the abundance of 18 was downregulated. The caspofungin-responsive proteins are labeled in Fig. 1; and descriptions of these proteins, including their putative biological functions, molecular properties, and changes in relative levels of expression are provided in Table 3. The proteins in the largest class responding to caspofungin exposure are involved in glycolysis and gluconeogenesis (Cdc19p, Gnd1p, Pdb1p, Pgk1p, Pgm2p, Pdc11p, Fba1p, and Hxk2p), while other proteins shown to be responsive to this drug are involved in cell stress and heat shock (Snz1p, Ssa1p, Ssa4p, Ssb1p, Ssc1p, and Hsp90p), the TCA cycle (Aco1p, Cit1p, Idh2p, Mdh1p and Mdh11p) and amino acid biosynthesis (Bat22p, Hom2p, Gdh3p, and Sah1p). Also identified were the enzymes involved in cell wall biosynthesis, such as Gal10p, Psa1p, and the regulator of β-1,3-glucan synthase activity, Rho1p.

DISCUSSION

In the present study, we used a combination of 2-D PAGE and MALDI-TOF mass spectroscopy to elucidate the specific and nonspecific adaptive responses to ketoconazole, amphotericin B, and caspofungin in *C. albicans*. Using this approach, we identified a total of 132 proteins corresponding to 126 genes whose abundance changed upon exposure to these antifungal agents. This work expands on our previous study in which we used microarray analysis to identify the genes in *C. albicans* that respond to ketoconazole, amphotericin B, and caspofungin exposures (24).

We identified 39 proteins that were differentially expressed following exposure to ketoconazole. Not surprisingly, several proteins that increased in abundance (Erg6p, Erg10p, and Erg13p) are directly involved in ergosterol biosynthesis. Sterol transmethylase (Erg6p) catalyzes the formation of zymosterol to fecosterol by using (*S*)-adenosyl-L-methionine as a cosubstrate. Acetyl-coenzyme A (acetyl-CoA) acetyltransferase (Erg10p) catalyzes the thiolytic cleavage of acetoacetyl-CoA to acetyl-CoA, which is the first step in the formation of mevalonate, a precursor required for the biosynthesis of ergosterol. The enzyme 3-hydroxymethylglutaryl-CoA synthase (Erg13p) catalyzes the production of hydroxymethylglutaryl-CoA from acetyl-CoA and acetoacetyl-CoA and functions in the second step in mevalonate biosynthesis. Our previous microarray analysis of *C. albicans* under these conditions found *ERG6* to be upregulated. Moreover, in response to a longer exposure to itraconazole, De Backer et al. found *ERG6*, *ERG10*, and *ERG13* to be upregulated (7a). Our data demonstrate that increased protein abundance accompanies the increased transcription of these genes in response to azole treatment. Interestingly, the increased abundance of both Erg6p and Erg10p has also been observed in azole-resistant clinical isolates of *C. albicans* (14, 15) as well as a laboratory-derived fluconazole-resistant strain of *C. albicans* produced after exposure to fluconazole (49). In one isolate, the increased abundance of these proteins was due to a G648D amino acid substitution in the transcription factor Upc2p. The upregulation of Erg6p, Erg10p, and Erg13p upon azole exposure is likely in response to the activation of Upc2p upon the depletion of ergosterol, which is consistent with the mechanism of action of this class of antifungals.

We also observed that ketoconazole exposure induced the expression of Csh1p and Orf19.251, which are proteins whose genes were previously shown to be associated with azole resistance (30). Csh1p is a putative aldo-keto reductase and is homologous to aryl-alcohol dehydrogenases in *Saccharomyces*

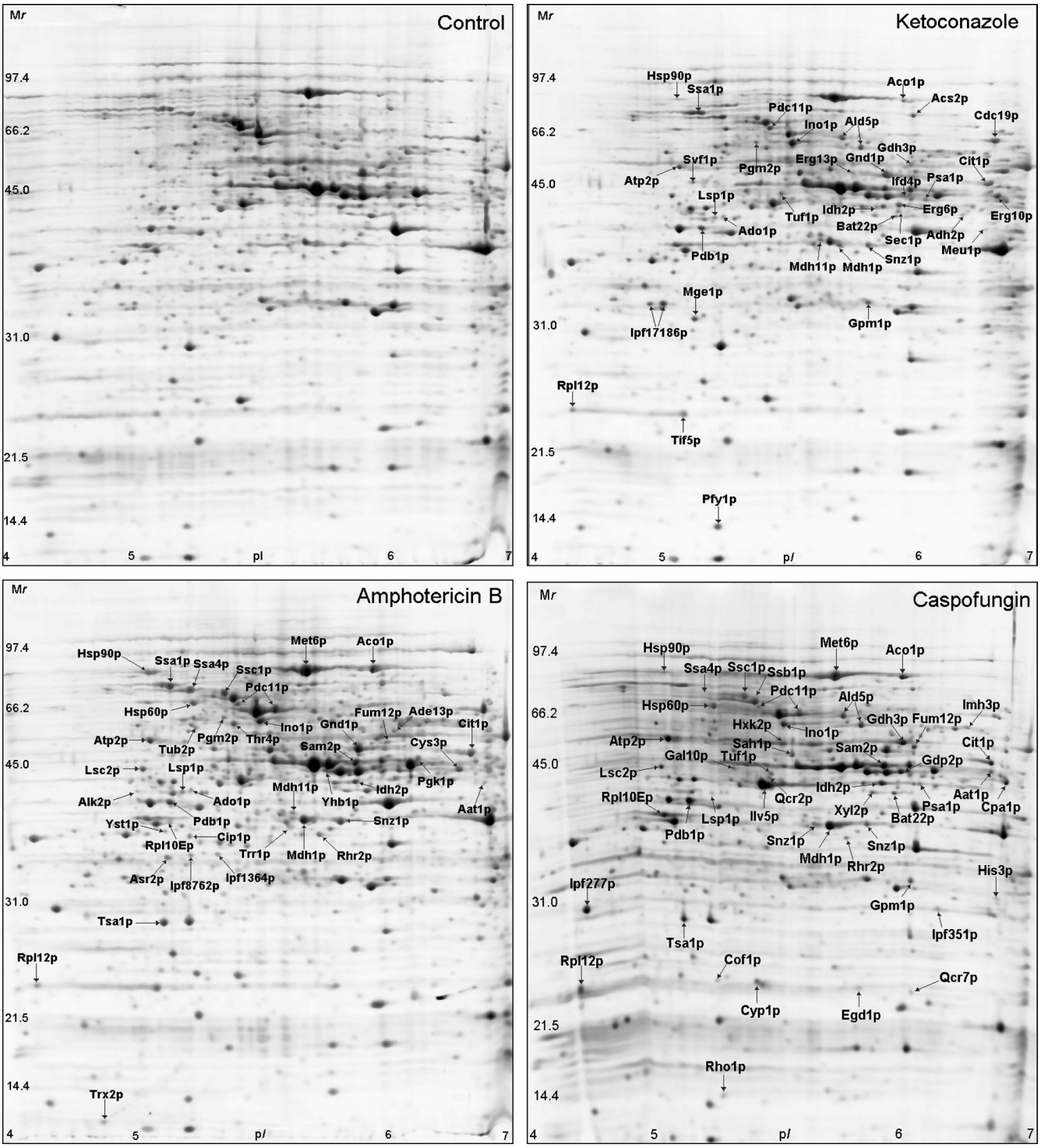

FIG. 1. 2-D expression profiles of the soluble proteins from *C. albicans* wild-type strain SC5314: untreated control, ketoconazole-treated, amphotericin B-treated, and caspofungin-treated cells after staining of the gels with SYPRO ruby.

cerevisiae (38). This protein has been shown to function in cell surface hydrophobicity (39). Another protein associated with azole resistance that was found to be responsive to ketoconazole was Orf19.251, a heat shock protein of the ThiJ/PfpI protein family (4). In *S. cerevisiae*, the ortholog of Orf19.251 is Hsp31p, and the expression of *HSP31* is regulated by the transcription factor Yap1p (40). Mutants lacking *orf19.251* are hypersensitive to reactive species of oxygen, which suggests that this gene functions to protect the cell against oxidative stress (40). In *C. albicans*, both Csh1p and Orf19.251 are

TABLE 1. Description of the ketoconazole-responsive proteins identified by MALDI-TOF mass spectroscopy

Protein	CGD[b] systematic name	Description	pI	M_r (10^3)	*Z* score	Protein coverage (%)	No. of matched peptides	Fold change in expression[c]	*t* test
Aco1p	Orf19.6385	Aconitate hydrotase	6.0	84.2	1.7	21	33	2.5 ± 1.4	0.02
Acs2p	Orf19.1064	Acetyl-CoA synthetase	5.7	73.8	1.6	11	6	1.6 ± 0.1	0.04
Adh2p[a]	Orf19.5113	Aldehyde dehydrogenase I	6.3	36.8	2.3	26	8	3.8 ± 0.1	0.00
Ado1p[a]	Orf19.5591	Adenosine kinase	5	38.2	2.4	47	14	2.7 ± 1.1	0.04
Ald5p	Orf19.5806	Aldehyde dehydrogenase	5.5	54.0	2.3	49	16	1.9 ± 0.8	0.02
Ald5p	Orf19.5806	Aldehyde dehydrogenase	5.5	54.0	2.4	30	11	1.6 ± 0.2	0.02
Atp2p	Orf19.5653	F1F0 ATPase complex, β subunit	4.9	55.7	2.4	48	14	1.5 ± 0	0.00
Cdc19p	Orf19.3575	Pyruvate kinase	6.5	55.4	2.3	26	10	1.9 ± 0.5	0.02
Cit1p	Orf19.4393	Citrate synthase	6.3	48.7	2.4	30	12	2 ± 0.3	0.00
Erg6p[a]	Orf19.1631	Sterol transmethylase	5.7	43.1	2.3	24	8	4.3 ± 0.4	0.00
Erg10p[a]	Orf19.1591	Acetyl-CoA acetyltransferase	6.5	41.9	1.7	14	5	2.4 ± 0.7	0.00
Erg13p	Orf19.7312	3-Hydroxy-3-methylglutaryl-CoA synthase	5.7	49.7	2.4	42	16	1.6 ± 0.2	0.03
Gnd1p	Orf19.5024	6-Phosphogluconate dehydrogenase	6.1	56.9	2.3	27	11	1.6 ± 0.5	0.10
Idh2p	Orf19.5791	Isocitrate dehydrogenase	6.4	39.3	2.3	45	12	1.5 ± 0	0.00
Ino1p	Orf19.7585	Myoinositol-1-phosphate synthase	5.3	57.8	2.3	44	15	2.6 ± 0.4	0.00
Csh1p[a]	Orf19.4477	Putative aryl-alcohol dehydrogenase	6.0	38.3	2.0	31	10	2.4 ± 0.9	0.08
Svf1p	Orf19.6068	Unknown function	4.9	42.9	2.3	20	7	1.6 ± 0.2	0.02
Ipf17186p	Orf19.251	Heat shock protein 31 of DJ-1/Pfpl family	4.7	25.8	2.3	30	7	1.9 ± 0.1	0.00
Ipf17186p	Orf19.251	Heat shock protein 31 of DJ-1/Pfpl family	4.7	25.8	2.2	21	7	3.1 ± 0.3	0.00
Lsp1p	Orf19.3149	Unknown function	4.9	35.6	1.4	33	7	2.2 ± 0.2	0.02
Meu1p	Orf19.6938	Regulator of *ADH2* expression	7.2	37.6	1.9	15	5	2.9 ± 0.7	0.02
Mdh11p	Orf19.7481	Malate dehydrogenase	5.4	36.0	1.5	24	5	3.2 ± 1.0	0.01
Mdh1p[a]	Orf19.7481	Mitochondrial malate dehydrogenase precursor	5.7	34.7	2.3	37	8	1.6 ± 0.3	0.03
Mge1p	Orf19.2524	Heat shock protein	5.6	27.2	2.4	31	9	3.8 ± 0.1	0.00
Pdb1p	Orf19.5294	Pyruvate dehydrogenase	5.4	41.3	2.3	30	8	1.7 ± 0.1	0.01
Pfy1p	Orf19.5076	Actin-binding protein	5.3	13.8	2.4	51	6	1.8 ± 0.2	0.02
Pgm2p	Orf19.2841	Phosphoglucomutase	5.3	61.8	2.4	29	13	1.9 ± 0.5	0.06
Rpl12p	Orf19.1635	Ribosomal protein	9.5	17.7	2.0	30	6	1.7 ± 0.1	0.03
Sec1p	Orf19.13833	Transport protein	8.1	89.6	1.3	17	8	2.6 ± 0.5	0.03
Snz1p	Orf19.2947	Stationary-phase protein	5.8	31.8	1.7	15	6	1.5 ± 0	0.00
Tif5p	Orf19.4261	Translation initiation factor (eIF-5A)	5.0	16.4	1.9	33	5	1.6 ± 0.4	0.08
Tuf1p[a]	Orf19.6047	Translation elongation factor TU	5.7	46.7	2.0	24	7	1.5 ± 0.2	0.03
Bat22p	Orf19.6994	Branched amino acid aminotransferase	5.9	40.8	2.1	27	10	0.4 ± 0	0.01
Gdh3p	Orf19.4716	NADP-glutamate dehydrogenase	5.7	49.6	2.4	23	9	0.3 ± 0	0.01
Gpm1p	Orf19.903	Phosphoglycerate mutase	5.8	27.4	2.3	40	7	0.4 ± 0	0.00
Hsp90p	Orf19.6515	Heat shock protein	4.8	80.8	1.8	21	14	0.3 ± 0.1	0.05
Pdc11p	Orf19.2877	Pyruvate decarboxylase	5.4	62.4	2.3	30	14	0.5 ± 0.1	0.00
Psa1p	Orf19.4943	GDP-mannose pyrophosphorylase	5.9	40.0	2.3	25	8	0.2 ± 0	0.00
Ssa1p	Orf19.1065	Heat shock protein of HSP family	4.9	70.1	2.4	21	12	0.5 ± 0.2	0.08

[a] Proteins whose genes were previously shown to be differentially expressed by microarray analysis.
[b] CGD, Candida Genome Database.
[c] Change in expression between treated cells and control cells.

known targets of the multidrug resistance regulator Mrr1p, which is a transcription factor that regulates the expression of the major facilitator superfamily drug efflux pump gene *MDR1* (30). Indeed, the abundance of both proteins was increased in an azole-resistant clinical isolates and was due to gain-of-function mutations in Mrr1p. Moreover, Csh1p was among the genes that we previously found to be differentially expressed in response to ketoconazole (24). These data suggest the possibility that Mrr1p is also activated upon azole treatment. The physiological roles of Csh1p and Ipf17186 both in azole resistance and in response to ketoconazole exposure have not yet been determined.

In response to amphotericin B exposure we found several proteins to be increased in abundance, including Yhb1p, Cip1p, Trx2p, Trr1p, and Tsa1p, all of which function in response to oxidative stress. Yhb1p is a putative flavohemoglobin that functions as a dioxygenase in nitric oxide detoxification (44). In *S. cerevisiae*, mutants lacking *YHB1* had levels of protein nitrosylation 10-fold higher than those observed in wild-type cells after exposure to donors of nitrous oxide, and the growth of the mutants was inhibited by nitrosative challenge (23). The cadmium-induced protein (Cip1p) is an enzyme with significant similarity to plant isoflavone reductases. The expression of this protein is regulated by the transcription factor Cap1p, a known regulator of oxidative stress (45, 52). This protein was also induced in the yeast-to-hypha transition and upon macrophage interaction (25, 31). Two additional amphotericin B-responsive enzymes were thioredoxin (Trx2p) and thioredoxin reductase (Trr1p), both of which are involved in the thioredoxin system. The thioredoxin system functions to maintain the cellular redox state and defend the cell against oxidative stress. Trx2p is an enzyme that reduces proteins by catalyzing thiol-disulfide reactions with two cysteine residues that shift from the reduced form [$(SH)_2$] to the oxidized form (S_2). The oxidized form returns to its reduced form in a reaction catalyzed by Trr1p by using NADPH as an electron

TABLE 2. Description of the amphotericin B-responsive proteins identified by MALDI-TOF mass spectroscopy

Protein	CGD[b] systematic name	Description of gene product	pI	M_r (10^3)	*Z* score	Protein coverage (%)	No. of matched peptides	Fold change in expression[c]	*t* test
Aco1p[a]	Orf19.6385	Aconitate hydratase	6.3	84.2	2.4	19	10	2.9 ± 0.7	0.04
Ado1p	Orf19.5591	Adenosine kinase	5.0	38.2	2.3	35	8	4.5 ± 3.8	0.06
Alk2p	Orf19.7513	*N*-Alkane inducible cytochrome P450	6.4	59.8	1.3	18	7	2.0 ± 0.2	0.01
Asr2p	Orf19.7284	Unknown function	5.4	27.8	2.2	29	8	7.6 ± 4.1	0.00
Atp2p	Orf19.5653	F1F0 ATPase complex, β subunit	4.9	55.7	2.3	45	16	2.7 ± 1.2	0.07
Cip1p	Orf19.113	Cadmium-induced protein	5.1	33.0	1.9	20	5	4.7 ± 2.0	0.00
Cit1p	Orf19.4393	Citrate synthase	6.3	48.7	1.4	17	6	2.1 ± 0.5	0.01
Cys3p[a]	Orf19.6402	Cystathionine gamma-lyase	6.2	42.9	1.7	17	4	5.4 ± 4.7	0.00
Fum12p	Orf19.6724	Fumarate hydratase, 5′ end	7.8	29.6	2.2	35	8	5.9 ± 4.9	0.01
Gnd1p	Orf19.5024	6-Phosphogluconate dehydrogenase	6.1	56.9	2.3	27	13	1.9 ± 0.2	0.01
Hsp60p[a]	Orf19.717	Heat shock protein 60	5.2	60.1	2.3	25	14	1.6 ± 0.2	0.00
Hsp90p	Orf19.6515	Heat shock protein	4.8	80.8	1.8	21	14	1.8 ± 0.2	0.10
Idh2p	Orf19.5791	Isocitrate dehydrogenase	6.4	39.3	2.2	38	8	1.8 ± 0.5	0.01
Ino1p	Orf19.7585	Myoinositol-1-phosphate synthase	5.3	57.8	2.4	44	15	2.8 ± 1.7	0.00
Ipf1364p	Orf19.6435	Unknown function	10.1	14.7	1.5	45	5	2.3 ± 0.2	0.01
Ipf8762p	Orf19.822	Unknown function	5.2	21.5	1.4	19	5	2.7 ± 0.9	0.00
Lsc2p	Orf19.1860	Succinate-CoA ligase, β subunit 3′ end	4.8	22.1	1.3	27	5	2.1 ± 0.2	0.01
Lsp1p	Orf19.3149	Unknown function	4.9	35.6	1.4	33	7	2.1 ± 0.5	0.00
Mdh1p	Orf19.7481	Mitochondrial malate dehydrogenase precursor	5.7	34.7	2.2	33	8	1.8 ± 0.3	0.00
Mdh11p	Orf19.7481	Malate dehydrogenase	5.4	36.0	1.5	24	5	1.8 ± 0.8	0.00
Pdb1p	Orf19.5294	Pyruvate dehydrogenase	5.4	41.3	2.3	30	8	1.7 ± 0.1	0.05
Pdc11p	Orf19.2877	Pyruvate decarboxylase	5.4	62.4	2.3	28	10	1.6 ± 0.9	0.18
Pgk1p	Orf19.3651	Phosphoglycerate kinase	6.1	45.2	2.4	51	20	3.9 ± 3.4	0.00
Pgm2p	Orf19.2841	Phosphoglucomutase	5.3	61.8	2.3	28	13	1.7 ± 0.3	0.03
Rhr2p	Orf19.5437	DL-Glycerol phosphatase	5.5	28.1	1.8	25	5	3.6 ± 1.1	0.03
Rpl10e1p	Orf19.7015	Ribosomal protein L10	4.7	33.3	2.4	36	12	1.7 ± 0.2	0.02
Rpl12p	Orf19.1635	Ribosomal protein	9.5	17.7	2.0	30	6	1.6 ± 0.5	0.03
Sam2p	Orf19.657	*S*-Adenosylmethionine synthetase	5.6	42.2	1.7	23	8	1.7 ± 0.8	0.17
Ssa1p	Orf19.1065	Heat shock protein of HSP family	4.9	70.1	2.4	18	10	1.6 ± 0.5	0.05
Ssa4p[a]	Orf19.4980	HSP70 mRNA for heat shock protein	5.1	70.3	2.4	27	16	1.5 ± 0.3	0.25
Ssc1p	Orf19.1896	Mitochondrial heat shock protein 70	5.5	69.7	2.4	29	19	1.8 ± 0.1	0.03
Snz1p[a]	Orf19.2947	Stationary-phase protein	5.8	31.8	2.0	15	5	2.3 ± 0.5	0.00
Thr4p	Orf19.4233	Threonine synthase	5.2	57.7	2.4	28	10	1.6 ± 0.2	0.02
Trr1p	Orf19.4290	Thioredoxin reductase	5.4	34.7	2.1	31	9	1.6 ± 0.1	0.00
Trx2p	Orf19.1976	Thioredoxin	4.9	13.71	1.2	33	3	1.7 ± 0.2	0.00
Tsa1p	Orf19.7417	Thioredoxin peroxidase	5.0	21.8	2.4	33	6	2.5 ± 0.1	0.00
Tub2p	Orf19.6034	β-Tubulin	4.6	48.77	2.3	26	12	4.0 ± 2.8	0.01
Yhb1p[a]	Orf19.3707	Flavohemoglobin	5.6	45.93	1.5	31	10	2.8 ± 1.4	0.10
Yst1p	Orf19.6975	Ribosomal protein, exon 2	4.8	25.6	2.1	25	6	5.2 ± 2.8	0.01
Aat1p	Orf19.3554	Aspartate aminotransferase	8.7	48.9	2.0	22	7	0.4 ± 0.4	0.00
Ade13p	Orf19.3870	Adenylosuccinate lyase	5.9	54.4	2.0	21	8	0.5 ± 0.1	0.00
Met6p[a]	Orf19.2551	5-Methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	5.4	85.7	2.4	29	13	0.5 ± 0.2	0.02
Pdc11p	Orf19.2877	Pyruvate decarboxylase	5.4	62.4	2.4	43	21	0.1 ± 0.0	0.01

[a] Proteins whose genes were previously shown to be differentially expressed by microarray analysis.
[b] CGD, Candida Genome Database.
[c] Change in expression between treated cells and control cells.

source. Trr1p functions as a stress-induced reductase and is the key regulatory enzyme that determines the redox state of this system. In *S. cerevisiae*, the transcriptional regulators Yap1p and Skn7p mediate the expression of both *TRX2* and *TRR1* in response to hydrogen peroxide (19, 29). Another amphotericin B-responsive protein involved in oxidative stress was thioredoxin peroxidase (Tsa1p), which belongs to a family of antioxidant enzymes capable of reducing reactive forms of oxygen, nitrogen, and sulfur using Trx2p as a reducing source (16). In *C. albicans*, Tsa1p was found to be increased in abundance in hyphae compared to its abundance in the yeast form and has been implicated in the function of hyphal cell wall biosynthesis (6). In *S. cerevisiae*, the transcriptional regulators Yap1p and Skn1p are required for the H_2O_2-mediated induction of both *TSA1* and *TSA2* (22), and the disruption of *TSA1* renders cells more sensitive to hydrogen peroxide (9). In the present study, it is not surprising that in response to amphotericin B we observed increases in the abundance of multiple proteins involved in oxidative stress and in the thioredoxin system, as this antifungal agent is known to cause significant oxidative damage in *C. albicans* (42, 43).

C. albicans exposure to amphotericin B also resulted in the increased abundance of Rhr2p and Asr2p, which are proteins involved in osmotic stress. In yeast, the response to osmotic stress is through the increased production of glycerol, which is regulated, in part, by a signaling system called the high-osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway. One amphotericin B-responsive protein

TABLE 3. Description of the caspofungin-responsive proteins identified by MALDI-TOF mass spectroscopy

Protein	CGD[b] Systematic name	Description	pI	M_r (10^3)	*Z* score	Protein coverage (%)	No. of matched peptides	Fold change in expression[c]	*t* test
Aco1p[a]	Orf19.6385	Aconitate hydratase	6.0	84.2	1.7	19	10	3.5 ± 2.4	0.04
Ald5p	Orf19.5806	Aldehyde dehydrogenase	5.5	54.0	2.3	38	14	1.5 ± 0.3	0.04
Ald5p	Orf19.5806	Aldehyde dehydrogenase	5.5	54.0	2.3	29	10	1.6 ± 0.2	0.04
Atp2p	Orf19.5653	F1F0 ATPase complex, β subunit	4.9	55.7	2.4	32	10	4.0 ± 0.2	0.02
Cit1p	Orf19.4393	Citrate synthase	6.3	48.7	2.2	17	7	1.8 ± 0.5	0.05
Cpa1p	Orf19.4630	Arginine-specific carbamoylphosphate synthase, small chain	6.5	47.3	1.3	12	4	2.5 ± 0.4	0.01
Fum12p	Orf19.6724	Fumarate hydratase, 5′ end	7.8	29.6	2.2	35	8	3.2 ± 1.5	0.01
Gpd2p	Orf19.691	Glycerol 3-phosphate dehydrogenase	5.2	40.8	1.3	19	5	1.7 ± 0.3	0.02
Gdh3p	Orf19.4716	NADP-glutamate dehydrogenase	5.7	49.6	2.4	23	9	1.7 ± 0.4	0.01
Hsp60p	Orf19.717	Heat shock protein 60	5.2	60.1	2.0	24	11	2.4 ± 0.2	0.03
Idh2p	Orf19.5791	Isocitrate dehydrogenase	6.4	39.3	2.4	57	16	1.9 ± 0.7	0.09
Ilv5p	Orf19.88	Keto acid reducto-isomerase	6.2	44.8	2.4	45	16	1.5 ± 0.8	0.19
Ino1p	Orf19.7585	Myoinositol-1-phosphate synthase	5.3	57.8	2.3	44	15	1.6 ± 0.2	0.02
Ipf277p	Orf19.3268	Human IgE-dependent histamine-releasing factor homolog	4.3	18.5	2.1	33	5	1.6 ± 0.3	0.01
Ipf351p	Orf19.7531	Unknown function	7.9	25.2	2.4	39	8	2.5 ± 1.3	0.10
Lsc2p	Orf19.1860	Succinate-CoA ligase, β subunit 3′ end	4.8	22.1	1.5	29	4	1.5 ± 0.1	0.01
Mdh1p	Orf19.7481	Mitochondrial malate dehydrogenase precursor	5.7	34.7	1.8	20	5	1.7 ± 0.6	0.11
Pdb1p	Orf19.5294	Pyruvate dehydrogenase	5.4	41.3	2.3	32	9	2.7 ± 0.3	0.01
Pdc11p[a]	Orf19.2877	Pyruvate decarboxylase	5.4	62.4	2.3	28	10	2.1 ± 0.3	0.00
Qcr2p	Orf19.2644	Ubiquinol cytochrome *c* reductase, chain II	5.5	39.5	2.1	29	6	2.3 ± 0.4	0.03
Qcr7p	Orf19.5629	Ubiquinol cytochrome *c* reductase, subunit 7	5.7	14.4	2.3	38	7	28.3 ± 6.7	0.01
Rho1p	Orf19.2843	GTP-binding protein of the RHO subfamily	5.3	9.3	2.4	24	4	1.5 ± 0.6	0.22
Rhr2p	Orf19.5437	DL-Glycerol phosphatase	5.5	28.1	1.3	26	4	1.9 ± 0.1	0.00
Rpl10ep	Orf19.7015	Ribosomal protein L10	4.7	33.3	2.3	19	5	1.8 ± 0.4	0.01
Rpl12p	Orf19.1635	Ribosomal protein	9.5	17.7	2.0	30	6	5.4 ± 0.7	0.02
Sam2p	Orf19.657	*S*-Adenosylmethionine synthetase	5.6	42.2	1.5	29	8	1.6 ± 0.6	0.12
Snz1p	Orf19.2947	Stationary-phase protein	5.8	31.8	1.7	15	6	2.9 ± 0.7	0.05
Snz1p	Orf19.2947	Stationary-phase protein	5.8	31.8	2.3	24	7	1.7 ± 0.5	0.02
Ssc1p	Orf19.1896	Mitochondrial heat shock protein 70	5.5	69.7	2.4	41	28	2.7 ± 0.8	0.04
Tsa1p	Orf19.7417	Thioredoxin peroxidase	5.0	21.8	2.3	40	7	2.6 ± 0.9	0.05
Tuf1p	Orf19.6047	Translation elongation factor TU	5.7	46.7	2.0	29	9	2.3 ± 0.4	0.03
Xyl2p	Orf19.7676	D-Xylulose reductase	5.6	38.8	1.8	17	5	1.6 ± 0.1	0.00
Aat1p[a]	Orf19.3554	Aspartate aminotransferase	8.7	48.87	2.4	24	11	0.3 ± 0.1	0.00
Bat22p[a]	Orf19.6994	Branched amino acid aminotransferase	5.9	40.8	1.8	20	6	0.5 ± 0.1	0.00
Cof1p	Orf19.953.1	Cofilin	4.9	16.3	2.2	65	8	0.4 ± 0	0.00
Cyp1p[a]	Orf19.6472	Cyclophilin peptidylprolyl isomerase	7.9	17.7	1.8	28	4	0.4 ± 0	0.00
Egd1p[a]	Orf19.1154	GAL4 DNA-binding enhancer protein	5.5	17.0	2.4	66	7	0.4 ± 0.3	0.03
Gpm1p[a]	Orf19.903	Phosphoglycerate mutase	5.8	27.4	1.7	28	5	0.3 ± 0.1	0.00
His3p	Orf19.183	Imidazole glycerol phosphate	6.3	24.1	2.0	15	5	0.2 ± 0.1	0.00
Hsp90p[a]	Orf19.6515	Heat shock protein	4.8	80.8	1.8	21	14	0.1 ± 0.1	0.01
Hxk2p	Orf19.542	Hexokinase II, 3′ end	5.1	47.5	2.3	18	10	0.3 ± 0.1	0.06

[a] Proteins whose genes were previously shown to be differentially expressed by microarray analysis.
[b] CGD, Candida Genome Database.
[c] Change in expression between treated cells and control cells.

regulated by this pathway is Rhr2p, a DL-glycerol-3-phosphatase that functions in glycerol biosynthesis. Previous studies with *S. cerevisiae* have demonstrated that the expression of *RHR2* is significantly induced under conditions of hyperosmotic stress (32). Mutants lacking this gene have a low intracellular glycerol content, do not have glycerol-3-phosphatase activity, and are hypersensitive to osmotic stress. Another protein found to be responsive to amphotericin B and involved in osmotic stress is Asr2p. A previous study showed that *ASR2* had significantly increased expression under conditions of osmotic stress and is regulated by cyclic AMP, and thus, the gene was designated *ASR2*, for adenylyl cyclase stress responsive (12). As amphotericin B is believed to compromise the structural integrity of the fungal membrane by forming pores through the membrane, it is reasonable to assume that *C. albicans* cells treated with amphotericin B undergo significant changes in intracellular solute concentrations, resulting in the increased abundance of proteins that function in response to cellular osmolarity.

The fungal cell wall is a dynamic structure composed of interconnecting mannoproteins, chitin, and β-glucans and is responsible for maintaining osmotic stability. Among these components, the β-1,3-glucan is the primary cell wall constituent (19, 20). The echinocandins, such as caspofungin, inhibit the activity of β-1,3-glucan synthase, which is an important enzyme required for the synthesis of β-1,3-glucans. Among the proteins that increased in abundance in response to caspofungin treatment was Rho1p, which is a low-molecular-weight GTPase that regulates the activity of β-1,3-glucan synthase in both *C. albicans* and *S. cerevisiae*. Rho1p in *C. albicans* is

essential for yeast cell viability and functions in actin cytoskeleton reorganization and cell wall biosynthesis (48). As caspofungin noncompetitively inhibits the production of β-1,3-glucans, it is not surprising that we observed an increase in the abundance of its regulator, Rho1p, in response to this antifungal drug. Other caspofungin-responsive proteins associated with cell wall biosynthesis included GDP-mannose pyrophosphorylase (Psa1p) and UDP-glucose-4-epimerase (Gal10p), both of which were decreased in abundance in response to caspofungin exposure, and xylitol dehydrogenase (Xyl2p), which was increased in abundance in response to caspofungin exposure. Briefly, Psa1p catalyzes the formation of GDP-mannose from mannose-1-phosphate and GTP for cell wall biosynthesis. This protein is required for the incorporation of mannose into more complex N-linked and O-linked glycoproteins. In *S. cerevisiae*, mutants lacking *PSA1* are unable to survive, and hypomorphic mutants display defects in cell wall biosynthesis and are sensitive to hyperosmolarity (13). Additionally, the Gal10p enzyme functions in galactose metabolism and catalyzes the epimerization of UDP-galactose to UDP-glucose. The absence of *GAL10* in *C. albicans* affects the cell wall organization, the oxidative stress response, filamentation, and the formation of biofilms (37). Therefore, in addition to galactose metabolism, Gal10p appears to be required for cell wall integrity, normal hyphal growth, and resistance to oxidative stress. Furthermore, Xyl2p is a zinc-containing protein and a member of the medium-chain dehydrogenase superfamily that functions in xylulose metabolism. In *S. cerevisiae*, the expression of *XYL2* is induced by xylose, and mutants lacking this gene have defects in their cell walls (8, 35). Although Xyl2p has been implicated in the function of cell wall biosynthesis, its specific function is unknown. The alterations in Rho1p, Psa1p, Gal10p, and Xly2p expression are characteristic of compromised cell wall integrity and are consistent with the mechanism of action of this antifungal agent.

A previous study used 2-D PAGE and MALDI-TOF MS to identify the alterations in *C. albicans* protein expression following exposure to the echinocandin antifungals mulundocandin and the mulundocandin derivative HMR3270 (5). That study revealed that 41 proteins were identified as being increased in abundance and 38 proteins were identified as being decreased in abundance upon exposures to mulundocandin and HMR3270. The caspofungin-influenced proteins identified in the present study that were also found to be similarly increased in abundance in response to mulundocandin and HMR3270 exposures were Cit1p, Gdh3p, Idh2p, Ino1p, Qcr2p, Sam2p, and Tsa1p. The increased abundance of these proteins in response to these two echinocandins appears to represent a common stress response to this class of antifungal agents.

The abundance of nine proteins increased in response to all three antifungals: Atp2p, Aco1p, Cit1p, Idh2p, Mdh1p, Ino1p, Pdb1p, Rpl12p, and Snz1p. Among them, Aco1p, Cit1p, Idh2p, and Mdh1p function in the TCA cycle. The TCA cycle is a central metabolic process in the mitochondria that converts acetyl-CoA to carbon dioxide and water to produce chemical energy in the form of ATP and NADH (after oxidative phosphorylation). It is also important to note that the intermediates of this process are used as precursors in the biosynthesis of amino acids, lipids, and heme. Three of these proteins have also been observed to increase in abundance in response to cadmium or upon interaction with macrophages, suggesting that they may be part of a general adaptation to environmental stress (27, 50). The increased abundance of these proteins in response to all three antifungal agents suggests an increase in energy demand during the adaptive response to these stresses.

As this proteomic analysis used conditions identical to those that we used in our previous microarray study of adaptive responses to antifungal agents, it affords a unique opportunity to compare the similarities and differences in these two types of data. Changes in protein abundance were in agreement with changes in gene expression for 6 of 39 proteins (Erg6p, Adh2p, Ado1p, Csh1p, Mdh1p, and Tuf1p) in response to ketoconazole, 7 of 43 proteins (Cys3p, Aco1p, Yhb1p, Snz1p, Hsp60p, Ssa4p, and Met6p) in response to amphotericin B, and 11 of 50 proteins (Aco1p, Sah1p, Bat22p, Met6p, Ssb1p, Egd1p, Cyp1p, Aat1p, Gpm1p, Pdc11p, and Hsp90p) in response to caspofungin. Given the limitations inherent to the methods used in our proteomic analysis, the resolution of each protein representing every gene found to be differentially expressed in our microarray analysis would not be expected. Our proteomic analysis is limited to the most abundant soluble proteins that can be resolved and visualized by 2-D PAGE that could also be successfully identified by MALDI-TOF MS. Likewise, limitations of the early-generation arrays used in our microarray analysis may have resulted in some differentially expressed genes not being detected. Moreover, changes in gene expression would be expected to precede changes in protein abundance temporally. The measurement of both gene expression and protein abundance at the same time point in these two studies may account for some of the discordance between these data. Finally, it is likely that some of the changes in protein abundance observed in response to antifungal exposure are not transcriptionally regulated.

In conclusion, our findings reveal specific and nonspecific antifungal-induced changes in protein abundance in *C. albicans*. This proteomic analysis expands on our previous microarray analysis, as this study provides insight into the adaptive responses to these antifungal agents at the translational level. It is possible that some of these antifungal-responsive proteins may represent potential targets for the development of novel therapeutic agents and could enhance the activities of these drugs against this pathogenic fungus.

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