Msn2- and Msn4-Like Transcription Factors Play No Obvious Roles in the Stress Responses of the Fungal Pathogen *Candida albicans*†

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In *Saccharomyces cerevisiae*, the (C2H2)2 zinc finger transcription factors Msn2 and Msn4 play central roles in responses to a range of stresses by activating gene transcription via the stress response element (STRE; CCCCT). The pathogen *Candida albicans* displays stress responses that are thought to help it survive adverse environmental conditions encountered within its human host. However, these responses differ from those in *S. cerevisiae*, and hence we predicted that the roles of Msn2- and Msn4-like proteins might have been functionally reassigned in *C. albicans*. *C. albicans* has two such proteins: CaMsn4 and Mnl1 (for Msn2- and Msn4-like). CaMSN4, but not MNL1, weakly complemented the inability of an *S. cerevisiae* msn2 msn4 mutant to activate a STRE-lacZ reporter. Also, the disruption of CaMsn4 and Mnl1 had no discernible effect upon the resistance of *C. albicans* to heat, osmotic, ethanol, nutrient, oxidative, or heavy-metal stress or upon the stress-activated transcriptome in *C. albicans*. Furthermore, although Cap1-dependent activation of a Yap response element-luciferase reporter was observed, a STRE reporter was not activated in response to stresses in *C. albicans*. Ectopic expression of CaMsn4 or Mnl1 did not affect the cellular or molecular responses of *C. albicans* to stress. Under the conditions tested, the putative activation and DNA binding domains of CaMsn4 did not appear to be functional. These data suggest that CaMsn4 and Mnl1 do not contribute significantly to stress responses in *C. albicans*. The data are consistent with the idea that stress signaling in this fungus has diverged significantly from that in budding yeast.

All living organisms have evolved mechanisms to detect and respond to adverse environmental conditions. In particular, pathogenic microbes must adapt efficiently to stresses imposed by their microenvironments during disease establishment and progression. For example, microbial pathogens must evade or counteract host immune defenses, and they must adapt to changes in pH or nutrient deprivation, depending upon the site of infection.

*Candida albicans* is the major systemic fungal pathogen of humans (6, 44, 45). This fungus is carried as a commensal in the oral and gastrointestinal tracts of many individuals but often causes oral and vaginal infections when fungus-host interactions are disturbed. *C. albicans* also causes systemic infections of internal organs in immunocompromised patients (44), sometimes escaping phagocytic killing, even following engulfment (33). The fact that *C. albicans* is relatively resistant to oxidative stresses (27) might contribute to this. It is likely that *C. albicans* has evolved to counter host defenses in a range of distinct niches within the host, and presumably this is dependent upon specific stress responses.

The relatively benign budding yeast *Saccharomyces cerevisiae* adapts to stress by using several distinct signaling pathways (36). Responses to oxidative and heavy-metal stresses are dependent upon the bZIP transcription factor Yap1 (56), which activates stress-responsive genes via sequences closely related to the Yap response element (YRE; TTA[G/C]TAA) (10). *C. albicans* Cap1, which is a functional homologue of Yap1, mediates responses to oxidative, heavy-metal, and drug-induced stresses (1, 68).

In *S. cerevisiae*, general responses to stresses, including mild heat shock, starvation, osmotic stress, alcohol, and weak acids, are dependent upon the closely related, functionally redundant (C2H2)2 zinc finger transcription factors Msn2 and Msn4 (17, 37). There is a third Msn2- and Msn4-like protein in *S. cerevisiae*, Yer130c, but its cellular function remains obscure (http://db.yeastgenome.org/cgi-bin/SGD). In response to stresses, Msn2 and Msn4 accumulate in the nucleus (23, 26). This leads to the transcriptional activation of stress-responsive genes via stress response elements (STRE; CCCCT) in their promoters (36, 38, 39). Msn2 and Msn4 appear to interact directly with the STRE element (38), and this interaction is thought to be enhanced by yeast glycogen synthase kinase 3 (25). Msn2- and Msn4-mediated stress responses are down-regulated by the Ras-cyclic AMP pathway (20). Activation of this pathway leads to the phosphorylation of Msn2 by protein kinase A, which causes cytoplasmic accumulation of Msn2 and hence inhibition of the general stress response (23, 24).

Transcript profiling has revealed that *C. albicans* does not display a general stress response under conditions that stimulate such a response in *S. cerevisiae* (15). Hence, our working hypothesis was that the functions of Msn2- and Msn4-like proteins have diverged in *C. albicans*. In this study, we tested this hypothesis by examining Msn2- and Msn4-like proteins in...
C. albicans using a range of approaches, including reverse genetics and genomics. We show that in contrast to S. cerevisiae, C. albicans Msn2- and Msn4-like proteins do not play significant roles in responses to heat, osmotic, ethanol or nutrient stress. This indicates that the functions of Msn2- and Msn4-like proteins have been lost in C. albicans or that they play differing roles in these yeasts. Our data reinforce the notion that stress responses in C. albicans and S. cerevisiae have diverged significantly.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The S. cerevisiae strains used were W303-1A (MATa Suc2 ade2 can1 his3 leu2 trp1 ura3 [62]) and the congenic strain W303-1A (MATa Suc2 ade2 can1 his3 leu2 trp1 ura3 [62]) and the congenic strain W303-1A (MATa Suc2 ade2 can1 his3 leu2 trp1 ura3 [62]). The C. albicans strains are listed in Table 1. The strains were grown in yeast-peptone-dextrose (YPD) medium (57), yeast-peptone medium containing 3% raffinose or 3% galactose, YPD medium containing 10% fetal calf serum (61), synthetic complete (SC) medium (29), or SD minimal medium (57). Stress phenotypes were assayed by plating 10-fold dilutions of C. albicans strains under the conditions specified.

**Strain construction.** The CaMSN4 and MLNL1 loci were disrupted by Ura transformation, as described previously (19), to generate homologous single mutants (MSC4 and MSC8) (19). The mlnl::HisG-URA3-HisG disruption cassette deleted codons 2 to 903 of the 906-codon CaMSN4 open reading frame (ORF), and the mlnl::HisG-URA3-HisG disruption cassette deleted codons 3 to 757 of the 759-codon MLNL1 ORF. The homologous mlnl::msnl msnl::msnl double mutant (MSC2) was created by disrupting the CaMSN4 locus in the mlnl::mlnl mutant.

The CaMSN4 and MLNL1 loci were PCR amplified and cloned into a URA3-containing integrating plasmid, pClp10 (40), to create pMSN4 and pMLNL1. These plasmids and the control, pClp10, were integrated at the RPS10 locus in MSC12 to create the strains MSC13 to MSC15 (Table 1). Finally, to generate the homologous mlnl::mlnl mlnl::mlnl double mutant (MSC12) using a previously described nrg1::HisG-URA3-hisG disruption cassette (41). At each stage of this process, the genotype of each strain was confirmed by both PCR diagnosis and Southern analysis (not shown).

To achieve ectopic expression of CaMSN4 and MLNL1 in C. albicans, these ORFs were PCR amplified using primers MSN4-F, MSN4-R, MLNL1-F, and MLNL1-R (Table 2); resequenced; and cloned between the CaACT1 promoter and the ScCYC1 terminator in pACT1 (63). The resultant plasmids, pACT1-MSN4, pACT1-MSN4, and pACT1-MLNL1, were integrated at the RPS10 locus in CaMSN4 (40) to create the strains MSC16 to MSC18 (Table 1). To perform the complementation tests in S. cerevisiae, the CaMSN4 and MLNL1 ORFs from pACT1-MSN4 and pACT1-MLNL1 were subcloned into the centromeric plasmid pRS315 (58) under the control of the S. cerevisiae GAL1 promoter. This generated the plasmids pGAL10-MSN4 and pGAL10-MLNL1, which were transformed separately into the S. cerevisiae msn2::msn4 triple mutant (SNC10), the CaMSN4 and MLNL1 loci were disrupted using the previously described nrg1::HisG-URA3-HisG disruption cassette (41). At each stage of this process, the genotype of each strain was confirmed by both PCR diagnosis and Southern analysis (not shown).

**TABLE 1. C. albicans strains used in this study**

| Strain | Genotype | Source |
|--------|----------|--------|
| SCS314 | Wild type | 22     |
| CAPE2  | URA3::ura3::imm434 | 19     |
| CA14   | URA3::ura3::imm434 | 19     |
| CA18   | URA3::ura3::imm434 | 19     |
| MMY301 | URA3::ura3::imm434 | 88     |
| MMC4   | URA3::ura3::imm434 | 41     |
| MSC1   | URA3::ura3::imm434 | This study |
| MSC2   | URA3::ura3::imm434 | This study |
| MSC3   | URA3::ura3::imm434 | This study |
| MSC4   | URA3::ura3::imm434 | This study |
| MSC5   | URA3::ura3::imm434 | This study |
| MSC6   | URA3::ura3::imm434 | This study |
| MSC7   | URA3::ura3::imm434 | This study |
| MSC8   | URA3::ura3::imm434 | This study |
| MSC9   | URA3::ura3::imm434 | This study |
| MSC10  | URA3::ura3::imm434 | This study |
| MSC11  | URA3::ura3::imm434 | This study |
| MSC12  | URA3::ura3::imm434 | This study |
| MSC13  | URA3::ura3::imm434 | This study |
| MSC14  | URA3::ura3::imm434 | This study |
| MSC15  | URA3::ura3::imm434 | This study |
| MSC16  | URA3::ura3::imm434 | This study |
| MSC17  | URA3::ura3::imm434 | This study |
| MSC18  | URA3::ura3::imm434 | This study |
| SN1    | URA3::ura3::imm434 | This study |
| SN2    | URA3::ura3::imm434 | This study |
| SN3    | URA3::ura3::imm434 | This study |
| SN4    | URA3::ura3::imm434 | This study |
| SN5    | URA3::ura3::imm434 | This study |
| SN6    | URA3::ura3::imm434 | This study |
| SN7    | URA3::ura3::imm434 | This study |
| SN8    | URA3::ura3::imm434 | This study |
| SN9    | URA3::ura3::imm434 | This study |
| SN10   | URA3::ura3::imm434 | This study |
| SN11   | URA3::ura3::imm434 | This study |
| SN12   | URA3::ura3::imm434 | This study |
| SN13   | URA3::ura3::imm434 | This study |
| SN14   | URA3::ura3::imm434 | This study |
| SN15   | URA3::ura3::imm434 | This study |

The CaMSN4 and MLNL1 loci were PCR amplified and cloned into a URA3-containing integrating plasmid, pClp10 (40), to create pMSN4 and pMLNL1. These plasmids and the control, pClp10, were integrated at the RPS10 locus in MSC12 to create the strains MSC13 to MSC15 (Table 1). Finally, to generate the homologous mlnl::mlnl mlnl::mlnl double mutant (MSC12) using a previously described nrg1::HisG-URA3-hisG disruption cassette (41). At each stage of this process, the genotype of each strain was confirmed by both PCR diagnosis and Southern analysis (not shown).

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To generate the Salex4-MSN4 fusion, the CaMSN4 ORF was PCR amplified and cloned into pClp-LexA (53) using the primers MSN4ex-F and MSN4ex-R (Table 2). The forward primer introduced a (Gly)5-Pro-(Gly)5 linker between the amino-terminal LexA domain and the carboxy-terminal CaMSN4 domain (Table 2). The pClp-LexA-MSN4 plasmid was then transformed into C. albicans CA8 (Table 1) via integration at the RPS10 locus, selecting for the plasmid-borne URA3 marker (53). To generate a synthetic, codon-optimized VP16 activation domain, oligonu-
| Name       | Sequence (5'→3')                                      | Use                                      |
|------------|-------------------------------------------------------|------------------------------------------|
| MSN4-F     | CCACCAAGTCGACCAAATATGG                                | Amplification of CaMSN4 ORF for pACT1 fusion |
| MSN4-R     | CCCAAAAACGCGTTGATATAG                                  | Amplification of CaMSN4 ORF for pACT1 fusion |
| MNL1-F     | CGAAGGAGGGTCGACCAAATATGG                              | Amplification of MNL1 ORF for pACT1 fusion |
| MNL1-R     | GCAATAGACGCGTGCCACAAG                                  | Amplification of MNL1 ORF for pACT1 fusion |
| VP16-1T    | GGGAGATCTATAATGGCTCCACCAACCGATGTTTCTTTGGGTGATGAGCTCCACTTGGATGGTGAAGATGTTGC | Construction of VP16 activation domain   |
| VP16-2B    | TGTGGGGTGAAACCGGGCCCTGGAGAATCACCATCACCCAACATATCCAAATCGAAATCATCCAAAGCATCAGCGTGAGCCATAGCAA | Construction of VP16 activation domain   |
| VP16-3T    | CTCCATACGGTGCTTTGGATATGGCTGATTTCGAATTCGAACAAATGTTCACCGATGCTTTGGGTATTGATGAATACGG | Construction of VP16 activation domain   |
| VP16-3B    | ATCAATACCCAAAGCATCGGTGAACATTTGTTCGAATTCGAAATCAGCCATATCCAAAGCACCGTATGGAGCAGAATCG | Construction of VP16 activation domain   |
| VP16-T     | GGGAGATCTATAATGGCTCCAAC                                | Amplification of VP16 activation domain   |
| VP16-B     | CCCCCTGCAGTATCTCGAGTTAGG                               | Amplification of VP16 activation domain   |
| CYC1-T     | GATCCATATATGTCGACGTCCCTATTTATTTTTTTATAGTTATGTTATGTTATTAAGAACGTTATTTATATTTCAAATCCATGGG | ScCYC1 transcriptional terminator         |
| CYC1-B     | TCGACCCATGGATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAAAAAAATAAATAGGGACGTCGACATATATG | ScCYC1 transcriptional terminator         |
| Linker-T   | CTAGCATATGGTACCATATGATATCATATACGCGTATATACTAGTTATACTCGAGATATG                         | Polylinker                               |
| Linker-B   | CTATATCGAATATAGCGGCCGCAACTTTTCTCTTTTTCTTTGGGSV40 T-antigen NLS | SV40 T-antigen NLS                       |
| MSNDBD-F   | TGATGGTACCAGCAATCC                                      | Amplification of CaMsn4 DBD              |
| MSNDBD-R   | CAGCAAGGGATCCAACAAAC                                    | Amplification of CaMsn4 DBD              |
| MNLDBD-F   | GCTGGTACCGCTAGTG                                        | Amplification of Mn11 DBD                |
| MNLDBD-R   | GTGGAGGATCCAACAA                                       | Amplification of Mn11 DBD                |
| MSN4lex-F  | GGTCCACGCGTTGGTGGAGGTCCAGGTGGATCTCAAGAATTCCAACCTTTATTTGAAAC                          | Amplification of CaMsn4 for lexA fusion   |
| MSN4lex-R  | CAAAATCTGCAGGGTAAACACCATACA                             | Amplification of CaMsn4 for lexA fusion   |
| STRE-T     | GTCACCACCCCTAACAGCCCCTGTATACCCCTGGATCCCCCCTGTAAGCCCCTA | Introduction of STRE into reporter       |
| STRE-B     | CTAGTTTACTAACGCTATTACTAATCCGATTACTAAGGATCCTTACTAAGGCTCTTACTAAG | Introduction of YRE into reporter        |

**Table 2** Oligonucleotides used in this study
cleotides VP16-1T to VP16-4B were sequentially annealed and ligated together (Table 2). Codon optimization was performed using published tables of preferred C. albicans codons (5). The full-length VP16 fragment was then PCR amplified using primers VP16-T and VP16-B; the product was cloned into pGEM-T EASY (Promega, Southampton, United Kingdom) and sequenced to create pGEM-VP16. The S. cerevisiae CYC1 transcriptional terminator was then cloned downstream of the VP16 domain by inserting the annealed oligonucleotides CYC1-T and CYC1-B into the XhoI site of pGEM-VP16. The resultant clone was transformed into C. albicans strain CAI8, selecting for the URA3 marker (40).

To express VP16-CaMsn4DBD and VP16-Mnl1DBD fusions in C. albicans, primers VP16-T and VP16-B; the product was cloned into pCaEXPa (7). A new linker was then inserted between the VP16 and CYC1 sequences to introduce additional restriction sites (oligonucleotides Linker-T and Linker-B [Table 2]), and the simian virus 40 (SV40) T-antigen nuclear localization signal (NLS) was inserted between the new Nhel and Kpnl sites (oligonucleotides NLS-T and NLS-B [Table 2]). This generated the control VP16 expression plasmid, pMET3-VP16 (see Fig. 9).

Transcript profiling. Transcript profiling of the congeneric C. albicans strains CAI8, MSC12, MSC16, MSC17, and MSC18 was performed on cells growing exponentially in YPD medium. The cells were exposed to the appropriate stress (mild heat shock [23 to 37°C], osmotic stress [0.3 M NaCl], or oxidative stress [0.4 mM H2O2]) and analyzed 0, 10, 30, and 60 min thereafter. At each time point, RNA was isolated, Cy3 and Cy5-labeled cDNAs were prepared, and the probes were hybridized with arrays comprising ~95% of C. albicans ORFs (43). Slides were scanned at 10-μm resolution with a ScanArray 5000 scanner (version 2.11; Packard Bioscience) and quantified using QuantaArray software (version 2.0; Packard Bioscience), and data normalization and analysis were performed using GeneSpring software (Silicon Genetics, Redwood City, Calif.) and significance analysis of microarrays (64). Data from at least three independent experiments were used in the analysis. The data are accessible at http://www.ebr.mc.ca/genetics/stress/.

FIG. 1. Comparison of Msn2- and Msn4-like sequences in C. albicans and S. cerevisiae. (A) Cartoon of S. cerevisiae Msn2 (ScMsn2) illustrating the coordinates of the (C2H2)2 DNA binding domain (Zn finger), the nuclear export signal (NES), the NLS, and the repression of the NLS by protein kinase A (PKA) (24). (B) Sequence alignments for the (C2H2)2 regions of the Msn2- and Msn4-like proteins in C. albicans and S. cerevisiae. Residues conserved in all proteins, black; conservative substitutions, grey. (C) Percentage sequence similarities (identities) in these regions. IPF9113 and IPF9939 are most similar to the S. cerevisiae proteins ScYer130c and ScMsn4, respectively.

Sequence analyses. DNA sequences were analyzed using CandidaDB (http://genolist.pasteur.fr/CandidaDB) and the Stanford Genome Database (http://genome-www.stanford.edu/).

Southern, Northern, and Western analyses. Published methods were used for RNA and DNA preparation, Southern blotting, and Northern analysis (41). Western blotting was performed as described previously (11).

RESULTS

C. albicans has two MSN2- and MSN4-like loci. Our first aim was to identify MSN2- and MSN4-like loci in C. albicans. Detailed searches of the genome sequence (http://www-sequence.stanford.edu/group/candida) and the C. albicans genome database (http://genolist.pasteur.fr/CandidaDB) revealed two loci with significant sequence similarity to S. cerevisiae MSN2, MSN4, or YER130c: IPF9113 (orf19.6121) and IPF9939 (orf19.4752). These C. albicans proteins display significant sequence similarity in their putative DNA binding domains to each other, as well as to S. cerevisiae Msn2, Msn4, and Yer130c (Fig. 1). The sequence similarities between the C. albicans and S. cerevisiae proteins were limited to the putative DNA binding domains. This is also the case for other functionally related
transcription factors in these fungi, such as Gcn4, Nrg1, and Rox1/Rfg1 (2, 28, 41, 63).

IPF9113 is related to Msn2 but is most similar to Yer130c. However, C. albicans genes should not be named using the formal gene names of S. cerevisiae orthologues. Hence, IPF9113 was provisionally named MNL1 (for Msn2- and Msn4-like protein). IPF9939 is most similar to Msn4 and therefore was provisionally named CaMsn4. To avoid confusion, we use the prefixes "Ca" and "Sc" to distinguish C. albicans and S. cerevisiae orthologues.

CaMSN4 weakly complements an S. cerevisiae msn2 msn4 double mutation. The next objective was to test whether CaMsn4 and Mnl1 are functional homologues of ScMsn2, ScMsn4, or ScYer130c. Unfortunately, S. cerevisiae yer130c mutants display no obvious phenotype (http://db.yeastgenome.org/cgi-bin/SGD), and therefore it was not possible to perform a complementation test in such strains. However, we were able to exploit the transcriptional defect of S. cerevisiae msn2 msn4 cells with respect to STRE-lacZ activation (38).

GAL1-MSN4 and GAL1-MNL1 fusions were constructed to drive the expression of these C. albicans ORFs in S. cerevisiae using the ScGAL1 promoter. These centromeric plasmids, and the empty pGAL10 control, were transformed into S. cerevisiae wild-type and msn2 msn4 cells. These cells were exposed to osmotic stress during exponential growth on glucose (to repress the ScGAL1 promoter), raffinose (to derepress the ScGAL1 promoter), or galactose (to activate the ScGAL1 promoter). As expected (38), wild-type S. cerevisiae cells displayed STRE-lacZ induction in response to the stress, and the S. cerevisiae msn2 msn4 cells containing the empty pGAL10 plasmid showed no significant induction (Fig. 2). The STRE-lacZ reporter was not induced in cells containing pGAL10-MNL1, indicating that MNL1 is unable to complement the double msn2 msn4 mutation in S. cerevisiae. This was consistent with the idea that IPF9113 is most closely related to S. cerevisiae Yer130c (Fig. 1). In contrast, cells containing pGAL10-MSN4 displayed weak STRE-lacZ activation during growth on galactose. This activation was not apparent during growth on raffinose. This suggested that CaMSN4 is able to complement the double msn2 msn4 mutation, but only weakly.

Cellular phenotypes of C. albicans msn4 and mnl1 mutants. Isogenic homozygous null mutants were generated to examine the roles of CaMsn4 and Mnl1 in C. albicans (Table 1). This involved the sequential deletion of both alleles for each locus in this diploid fungus using standard Ura-blasting procedures (19). Each CaMSN4 allele was inactivated by deleting essentially all of the 759-codon ORF and inserting the Ura blaster cassette (see Materials and Methods). Similarly, MNL1 was disrupted by replacing all of the 906-codon ORF with the Ura blaster cassette. A double mutant was then created by disrupt-
ing the CaMSN4 locus in the homozygous mnl1/mnl1 null mutant (Table 1). The loss of CaMSN4 and MNL1 in the corresponding mutants was confirmed by Northern analysis (not shown).

An S. cerevisiae msn2 msn4 double mutant displays sensitivity to general stresses (38), but S. cerevisiae yer130c mutants display no obvious phenotype. If CaMSN4 and MNL1 have been functionally reassigned in C. albicans, the corresponding mutants might not be expected to display stress phenotypes. Hence, we examined the abilities of the C. albicans msn4/msn4, mnl1/mnl1, and double mutants under a wide range of stress conditions. These included mild heat shock (25 to 37°C), an osmotic stress (0.3 M NaCl), or an oxidative stress (0.4 mM H2O2). These conditions were chosen because they had been shown previously to generate specific elements of stress responses in the yeast (2, 63). However, we reasoned that subtle roles for these proteins might be revealed by examining the molecular phenotypes of CaMsn4 and Mnl1. Hence, the C. albicans ACT1 promoter was used to drive ectopic expression of CaMSN4 and MNL1. This promoter has been used successfully to drive ectopic expression of other transcription factors in C. albicans. For example, ACT1-CaNRG1 and ACT1-CaGCN4 fusions have been shown to confer morphological and metabolic phenotypes upon C. albicans (2, 63). However, C. albicans strains carrying ACT1-CaMSN4 or ACT1-MNL1 fusions displayed no significant elevation in resistance to heat shock, osmotic stress, heavy-metal stress, or oxidative stress (not shown). This was consistent with the idea that CaMsn4 and Mnl1 play no significant roles in the stress responses examined.

Molecular phenotypes of C. albicans msn4 and mnl1 mutants. It was possible that by examining the cellular phenotypes of msn4 and mnl1 mutants we might have missed subtle influences of CaMsn4 and Mnl1 upon C. albicans stress responses. However, we reasoned that subtle roles for these proteins would be revealed by analyzing the molecular phenotypes of the corresponding null mutants. Hence, transcript profiling was performed to compare the double msn4/msn4 mnl1/mnl1 mutant with its isogenic wild-type parent during exposure to a mild heat shock (23 to 37°C), an osmotic shock (0.3 M NaCl), or an oxidative stress (0.4 mM H2O2). These conditions were chosen because they had been shown previously to generate specific stress responses in the C. albicans transcriptome (15). The strains were compared at 0, 10, 30, and 60 min after exposure to each stress. A high degree of statistical reproducibility was observed for the three independent experiments that were performed for each time point. However, inactivation of
TABLE 3. Effects of inactivating CaMsn4 and Mnl1 upon gene induction in response to osmotic stress

| Gene       | Expression ratio | Function$^d$ |
|------------|------------------|--------------|
|            | WT stress/WT no stress$^{abc}$ at time (min.) | msn4 mnl1 stress/WT stress$^{abc}$ at time (min.) | |
|            | 0 10 30 60       | 0 10 30 60   | |
| of19.7284  | 0.9 20.2 55.3 1.4 | 0.8 1.1 0.8 0.7 | Unknown function |
| of19.5302  | 0.9 14.8 27.8 4.4 | 1.1 2.0 1.3 0.8 | Unknown function |
| MSC1       | 1.0 14.1 23.2 1.7 | 0.8 0.9 0.9 0.7 | Meiotic sister chromatid recombination |
| of19.5070  | 0.9 13.7 1.3 1.1 | 1.2 1.1 0.9 1.1 | Unknown function |
| CTAl       | 1.0 11.8 7.7 1.2 | 1.0 1.0 1.1 0.8 | Peroxisomal catalase A |
| of19.2048  | 0.9 10.8 3.2 0.7 | 1.5 1.1 1.1 1.1 | Unknown function |
| DDR48      | 1.1 9.9 12.2 5.0 | 1.0 1.1 1.3 1.1 | DNA damage-responsive protein |
| of19.2344  | 1.0 9.7 14.2 1.8 | 1.1 0.8 1.2 0.8 | Unknown function |
| of19.7350  | 1.1 9.3 5.1 2.3 | 0.5 0.5 0.8 0.6 | Unknown function |
| CEX16      | 0.8 8.8 1.3 2.4 | 1.4 1.5 1.8 1.5 | Putative interaction with heat shock proteins and chaperones |
| of19.3932  | 1.1 8.3 7.5 1.2 | 1.0 0.9 0.7 0.8 | Unknown function |
| of19.2737  | 0.9 8.3 8.0 0.9 | 1.0 0.9 1.0 1.0 | FGGY family of carbohydrate kinases |
| of19.7296  | 0.9 8.1 0.6 0.7 | 0.8 0.9 1.0 0.6 | Unknown function |
| ADH6       | 1.1 7.5 6.1 1.0 | 0.9 0.9 1.0 1.0 | Alcohol dehydrogenase |
| DCW2       | 1.0 7.1 1.9 1.3 | 1.1 1.1 1.0 1.0 | GP1-anchored protein with cell wall role |
| SGA1       | 0.9 7.0 0.9 1.3 | 1.1 1.2 1.0 1.2 | Glucoamylase |
| of19.692   | 0.9 6.1 0.6 0.9 | 1.1 1.2 1.0 1.0 | Unknown function |
| YBR56      | 1.0 5.9 6.9 1.0 | 1.0 0.8 1.0 0.8 | Putative exo-1,3-beta-glucanase |
| AGP2       | 1.0 5.8 1.7 2.2 | 1.0 1.0 1.0 1.0 | Putative amino acid permease |
| of19.3007.2| 1.0 5.5 2.7 0.8 | 1.2 1.0 1.0 0.9 | Unknown function |
| LRR        | 0.9 5.1 1.1 1.0 | 0.6 0.8 1.3 0.7 | Leucine-rich repeat protein |

$^a$ Data from Enjalbert et al. (15).
$^b$ The osmotic stress was 0.3 M NaCl. Similar observations were obtained for the heat and oxidative stresses (not shown). WT, wild type.
$^c$ Data from this study (Fig. 4).
$^d$ Gene information from http://candida.bri.nrc.ca/candida/index.cfm and http://genolis1.pasteur.fr//CandidaDB/.

CaMsn4 and Mnl1 had no significant effect upon the transcriptional responses of C. albicans to these stresses (Fig. 4). Wild-type C. albicans cells display a well-defined transcriptional response to the osmotic, oxidative, and heat stresses examined in this study (15). When the transcript profiles of msn4/msn4 mnl1/mnl1 and wild-type cells were compared, the expression ratios for most genes approximated to 1 at each time point under each condition (Fig. 4 and Table 3). This indicates that the inactivation of CaMsn4 and Mnl1 had no significant effect upon the expression of almost all C. albicans genes. Hence, all stress-induced transcription was retained in the msn4/msn4 mnl1/mnl1 cells under the stress conditions examined.

The expression of only one stress-induced gene was consistently reduced more than twofold in the msn4/msn4 mnl1/mnl1 mutant: CaYPL088. Northern analysis confirmed that this transcript was induced moderately by osmotic stress in wild-type cells and that this increase was blocked in msn4/msn4 mnl1/mnl1 cells (Fig. 5). This gene encodes a protein with sequence similarity to Agrobacterium tumefaciens MocA, but the biological significance of this change, if any, is not clear.

Signals for two probes on the microarray were constitutively elevated in msn4/msn4 mnl1/mnl1 cells. However, both probes corresponded to the same transcript (CaYIR035). The elevation in CaYIR035 mRNA levels in the unstressed double mutant was confirmed by Northern analysis (Fig. 5). Northern analysis of the single msn4/msn4 and mnl1/mnl1 mutants suggested that CaMsn4 plays a greater role than Mnl1 in the regulation of CaYIR035, which is predicted to encode a short-chain dehydrogenase. Again, the biological significance of this change is not clear.

The CaMSN4 transcript was constitutively reduced in msn4/msn4 mnl1/mnl1 cells, confirming our earlier Northern analyses, which indicated that msn4/msn4 cells lacked CaMSN4 mRNA (not shown). The transcript profiling data of Enjalbert and coworkers (15) indicate that CaMSN4 and MNL1 are expressed under the heat, osmotic, and oxidative stress condi-
tions examined and that their transcript levels do not change significantly under these conditions.

Although unlikely, it was possible that the effects of the msn4/msn4 mnl1/mnl1 mutations upon the transcriptome might have been masked by functional redundancy with some unknown factor. Therefore, we examined the effects of the ACT1-CaMSN4 and ACT1-MNL1 fusions upon the C. albicans transcriptome. Again, the three independent transcript-profiling experiments performed for each experimental condition were highly reproducible. However, the ectopic expression of CaMsn4 or Mnl1 had no significant effect upon the transcript profile relative to the pACT1 control, even following exposure of the C. albicans cells to heat, osmotic, or oxidative stress (not shown). Therefore, transcript profiling revealed no obvious roles for CaMsn4 or Mnl1 during the C. albicans stress responses tested or during exponential growth on glucose. This was consistent with our working hypothesis.

The STRE in C. albicans. S. cerevisiae Msn2 and Msn4 activate transcription via the STRE (38), and the role of the STRE in mediating transcriptional responses to stress is conserved in other fungi (47). Many C. albicans genes that respond to stress contain STRE-like sequences in their promoters. Furthermore, we have shown that other S. cerevisiae regulatory elements, such as GCRE and YRE, are conserved in C. albicans (34, 63). However, if CaMsn4 and Mnl1 have been functionally reassigned, we predicted that STRE-like sequences might not

FIG. 6. The STRE does not mediate stress-activated transcription in C. albicans. (A) To test the working model that some stresses activate transcription via the STRE in C. albicans, the expression of basal RLUC (B), STRE-RLUC (S), and YRE-RLUC (Y) fusions were monitored in CAI8 (WT) following exposure to a range of stresses: no stress (YPD; 30°C), mild heat shock (25 to 37°C), 0.3 M NaCl, 7% ethanol (EtOH), and 2.5 mM H2O2. The effect of Cap1 inactivation upon the response to 2.5 mM H2O2 was measured as a control using strain MMY301 (Table 1). Luciferase levels were measured in triplicate for three independent transformants (105 relative light units). (B) To test the hypothesis that CaNrg1 might repress STRE activation, the same experiment was performed using strain MMC4 (∆nrg1) (Table 1).

FIG. 7. CaNrg1, CaMsn4, and Mnl1 do not display synthetic stress phenotypes. C. albicans cells were exposed to a wide range of stresses, including no stress (YPD; 30°C), mild heat shock (25 to 37°C), 1.0 M NaCl, 0.4 mM H2O2, and 2.5 mM H2O2. Strains: CAI8 (WT), MMC4 (∆nrg1), MSC12 (∆msn4, ∆mnl1), and SNC10 (∆nrg1, ∆msn4, ∆mnl1) (Table 1). The strains were transformed with CIp10 (URA3) and/or pCRW3 (ADE2) to make them prototrophic.
mediate transcriptional responses to general stresses in *C. albicans*.

To test this, we introduced four tandem STRE upstream of the basal *C. albicans* reporter, *ADH1b-RLUC* (63), to create STRE-RLUC. As a positive control, we constructed an analogous YRE-RLUC reporter containing four tandem YRE. As expected, the YRE-RLUC reporter did not respond to a mild heat shock, 0.3 M NaCl, or 7% ethanol but was activated in response to 2.5 mM H2O2 in a Cap1-dependent fashion (Fig. 6A). In contrast, the STRE reporter displayed no significant activation above basal levels following exposure to these heat, osmotic, ethanol, or oxidative stresses.

It was possible that, although the YRE-RLUC reporter had responded appropriately to an oxidative stress, something about the design of the STRE-RLUC reporter had inhibited STRE-mediated transcriptional activation. Therefore, we generated a second reporter in which the STRE were provided with alternative flanking nucleotides, an alternative basal promoter region (*ScCYC1*), and an alternative reporter (*StlacZ*) (65). However, no STRE-mediated transcriptional activation was observed using this alternative reporter (not shown). Therefore, no obvious role was observed for the STRE with respect to stress-mediated transcription in *C. albicans* and the lack of involvement of CaMsn4 and Mnl1 in general stress responses.

**Potential overlap between CaMsn4, Mnl1, and CaNrg1 regulons in *C. albicans***. Previously, CaNrg1 was identified as a transcriptional repressor that mediates its effects in *C. albicans* via the Nrg1 response element (NRE) (41, 42). The consensus sequence for the NRE [(A/C)(A/C/G)C] (41) is closely related to the STRE (CCCCT), so that STREs appear to be a subset of potential NREs. Therefore, in principle, CaNrg1 might repress STRE-mediated transcriptional activation in *C. albicans*. It follows, therefore, that there might be some overlap between CaMsn4, Mnl1, and CaNrg1 regulons in *C. albicans* and that this overlap might have masked the activities of CaMsn4, Mnl1, and STRE in the above-mentioned experiments. A prediction of this working hypothesis was that these activities would be unmasked by inactivating CaNrg1.

To test this, we generated a *C. albicans msn4/msn4 mnl1/mnl1 nrg1/nrg1* triple mutant and compared its phenotype to those of wild-type, *nrg1/nrg1*, and *msn4/msn4 mnl1/mnl1* cells (Fig. 7). Inactivation of CaNrg1 derepresses filamentous growth in *C. albicans* (2, 41), and therefore, cells carrying the *nrg1/nrg1* mutation formed wrinkly colonies. However, the triple mutant displayed no significant difference from *nrg1/nrg1* cells with respect to stress sensitivity (Fig. 7). Again, this reinforced the idea that CaMsn4 and Mnl1 do not have significant roles in the *C. albicans* stress responses examined, even in the absence of CaNrg1.

Does CaNrg1 repress STRE-mediated transcriptional activation in response to stresses? This was tested by assaying the activity of the STRE-RLUC reporter in *nrg1/nrg1* cells (Fig. 6B). The inactivation of CaNrg1 did not release any significant activation of the reporter following exposure to mild heat, osmotic, ethanol, or oxidative stress. Therefore, no significant overlap between the CaMsn4, Mnl1, and CaNrg1 regulons was observed in this study.
Functionality of CaMsn4 in C. albicans. Northern analysis and transcript profiling had indicated that the CaMSN4 gene is expressed, and the complementation experiment suggested that CaMsn4 might have some transcriptional activity at least in S. cerevisiae (Fig. 2). However, our cellular and molecular analyses had revealed no obvious function for this protein in C. albicans. Therefore, we assayed the activities of the putative transcriptional activation and DNA binding domains of CaMsn4 in C. albicans.

LexA fusions have been used to examine the activities of specific transcription factors in S. cerevisiae (3, 30). Hence, we expressed CaMsn4 as a LexA fusion protein in C. albicans and asked whether it could activate the expression of a reporter gene carrying the corresponding lexA operator in its promoter (Fig. 8A). Staphylococcus aureus lexA was used because it lacks CUG codons (53), which are decoded as serine, not leucine, in C. albicans (55). Hence, a SalexA-CaMSN4 fusion was introduced into a C. albicans strain containing a Streptococcus thermophilus lacZ reporter gene under the control of a SalexA operator sequence (53). Control strains contained a StlacZ reporter lacking the SalexA operator, and these generated basal levels of β-galactosidase in C. albicans (Fig. 8B). These levels were not affected significantly by the introduction of the S. aureus lexA operator. As expected (53), a control LexA-Gcn4 fusion showed significant StlacZ activation. However, the LexA-CaMsn4 fusion displayed no significant activation, even following exposure to osmotic (1.0 M NaCl) or ethanol (7%) stress. Therefore, we were unable to detect any significant transcriptional activation by CaMsn4.

To examine the functionality of the zinc finger domain of CaMsn4, codons 554 to 759 of CaMSN4 were fused to a synthetic codon-optimized VP16 transcriptional activation domain (see Materials and Methods). This VP16 domain has been shown to activate transcription in S. cerevisiae (12, 50). The VP16-CaMsn4DBD fusion was expressed in C. albicans using the methionine-conditional MET3 promoter (7) and tar-

FIG. 9. Expression of a VP16-CaMsn4DBD fusion in C. albicans causes no obvious stress phenotype. (A) Cartoon illustrating the experimental rationale to identify gene targets of CaMsn4. The CaMET3 promoter is repressed by methionine and cysteine (7). (B) Western blot with anti-VP16 antibody showing regulated expression of MET3-VP16-MSN4DBD in C. albicans. Wild type, CAI4 (WT; Table 1); SNC11 (VP16); SNC12 (VP16-Mnl1); SNC13 (VP16-Msn4); no methionine or cysteine (–); 10 mM methionine and 10 mM cysteine (+); open arrow, nonspecific band; closed arrow, VP16 fusion protein. (C) Phenotype of C. albicans CAI4 cells expressing VP16-CaMsn4DBD or the VP16 control on plates lacking methionine and cysteine. No stress (SC; 30°C), mild heat shock (25 to 37°C), 1.0 M NaCl, and 2.5 mM H2O2.
geted to the nucleus using a synthetic SV40 T-antigen nuclear localization signal (Fig. 9A). Control cells contained the empty expression plasmid pMET3-VP16 or pMET3-VP16-MNL1. Methionineconditional expression of the VP16-CaMsn4DBD fusion and the control VP16-Mnl1DBD protein in C. albicans was confirmed by Western blotting (Fig. 9B). However, no obvious growth or stress phenotype was observed for cells expressing the VP16-CaMsn4DBD fusion compared with control cells (Fig. 9C).

We reasoned that C. albicans genes containing the CaMsn4 DNA binding site would be activated artificially following expression of this VP16-CaMsn4DBD fusion. The intention was to identify these CaMsn4 target genes, and hence the CaMsn4 DNA binding site. Therefore, transcript profiling was performed to compare the molecular responses of C. albicans cells to the VP16-CaMsn4DBD fusion and the VP16 control. Highly reproducible signals were obtained for four independent hybridizations, but no significant differences were observed between the transcript profiles of pMET3-VP16-CaMsn4DBD and pMET3-VP16 cells. This reinforced the idea that CaMsn4 no longer retains activity as a transcriptional activator in C. albicans.

**DISCUSSION**

In general, there appears to be a high degree of conservation between S. cerevisiae and C. albicans with respect to their signal transduction modules. These include the MAP kinase module involved in mating responses, the adenyl cyclase-protein kinase A module that regulates cellular morphogenesis, the Rim101 module involved in pH signaling, the Gcn4 module that activates general amino acid control, the Yap1 module that mediates oxidative stress response, and the Hog1 module involved in osmotic stress responses (1, 4, 13, 14, 16, 18, 31, 32, 35, 48, 51, 54, 59, 63, 66, 68). However, transcript profiling has highlighted significant differences between the general stress responses of C. albicans and those of S. cerevisiae (15). Hence, we anticipated significant differences between the pathogenic fungus and the relatively benign fungus with respect to their Snf2- and Msn4-like signaling modules.

C. albicans has two Msn2- and Msn4-like proteins. IPF9939 (orf19.4752) was called CaMSN4 on the basis that its product is most similar to S. cerevisiae Msn4 (Fig. 1), and it was able to complement an S. cerevisiae msn2 msn4 double mutation, albeit weakly (Fig. 2). IPF9113 (orf19.6121), which was most similar to S. cerevisiae YER130c, was called MNL1 on the basis of its similarity to Msn2- and Msn4-like proteins (Fig. 1). Furthermore, MNL1, like S. cerevisiae YER130c, was unable to complement an S. cerevisiae msn2 msn4 double mutation (Fig. 2). The existence of a single C. albicans orthologue (CaMSN4) of the functionally redundant Msn2-MSN4 gene pair in S. cerevisiae is consistent with the idea that, during fungal evolution, genome duplication occurred after the divergence of C. albicans and S. cerevisiae (67).

To test our working model that CaMsn2- and Msn4-like proteins in C. albicans have been functionally reassigned, we examined their roles in detail. As expected, neither CaMsn4 nor Mnl1 appears to play an obvious role in stress responses. This conclusion was based on numerous complementary observations. (i) Inactivation of CaMSN4 and MNL1 did not increase the sensitivity of C. albicans to any of the numerous stresses tested (Fig. 3). This differs from the situation in S. cerevisiae, where an msn2 msn4 mutant is more sensitive to general stresses (38). It also contrasts with CAP1, the inactivation of which renders C. albicans more sensitive to oxidative stresses (Fig. 3) (1). (ii) Inactivation of CaMSN4 and MNL1 did not affect the C. albicans transcriptome during responses to mild heat shock or osmotic or oxidative stress (Fig. 4 and Table 3). Again, this contrasts with S. cerevisiae, in which the inactivation of Msn2 and Msn4 inhibits transcriptional responses to many stresses (8, 21). Subtle effects of CaMsn4 or Mnl1 upon stress responses might have been missed in our analyses of cellular stress responses. However, such effects are unlikely to have been missed by transcript profiling, which is exquisitely sensitive to environmental change (8, 9, 15, 21, 43). (iii) Ecotropic expression of CaMSN4 or MNL1 did not increase the tolerance of C. albicans to stresses and did not affect the C. albicans transcriptome significantly during responses to stress (not shown). The ACT1 promoter has been used successfully to generate overexpression phenotypes for at least two other transcription factors in C. albicans (CaNrg1 and CaGcn4) (2, 63). Nevertheless, we are unable to exclude the possibility that the absence of cellular and molecular phenotypes was due to a lack of overexpression, improper folding, or mislocalization of CaMsn4 and Mnl1. (iv) The STRE did not mediate transcriptional activation in response to stresses in C. albicans (Fig. 6), although CaMsn4 was capable of activating the transcription of a STRE reporter in S. cerevisiae in a stress-dependent fashion, albeit weakly (Fig. 2). Therefore, CaMsn4 and Mnl1 do not appear to play significant roles in responses to cellular stresses in C. albicans. (v) Expression of a protein fusion containing the Msn4DBD domain linked to the VP16 transcriptional activation domain (Fig. 9) did not lead to the activation of any stress-related functions in C. albicans (not shown). Indeed, no significant CaMsn4 targets were observed using this approach. Also, a SaLexA-CaMsn4 fusion showed no transcriptional activation in C. albicans. We are unable to exclude the possibility that the SaLexA-CaMsn4 fusion showed no transcriptional activation in C. albicans. Hence, if CaMsn4 does retain functionality as a transcription factor in C. albicans, this functionality presumably depends on other factors not examined in this study.

Two potential targets of CaMsn4 were identified by transcript profiling and confirmed by Northern blotting (Fig. 5). The induction of CaYPL088 in response to stress appeared to be dependent upon CaMsn4 (and, to a lesser extent, upon CaMnl1). In contrast, CaYIR035 mRNA levels were constitutively elevated in cells lacking CaMsn4. CaMsn4 might act indirectly upon these genes. Nevertheless, these represent the first identified gene targets for CaMsn4.

On the basis of the above observations, we conclude that CaMsn4 and Mnl1 do not play significant roles in the stress responses examined. This conclusion is consistent with the view that there has been significant evolutionary divergence between S. cerevisiae and C. albicans with respect to their stress responses. Such divergence has probably been driven by the evolution of niche-specific environmental responses, because the environmental challenges posed to a fungal pathogen of...
humans are likely to be quite distinct from those posed to a saprophytic fungus. It is not surprising, therefore, that recent transcript-profiling experiments have revealed significant differences in the molecular responses of different fungi to stress. *Schizosaccharomyces pombe* and *S. cerevisiae* exhibit core transcriptional responses to a variety of different stresses, including heat, acid and alkali shifts, and osmotic and oxidative stresses (8, 9, 21). This core transcriptional response is reflected at the cellular level by the phenomenon of “cross-protection,” in which exposure to a mild dose of one form of stress protects the fungus against more severe doses of a quite different type of stress. Interestingly, these core transcriptional responses are regulated in different ways. *S. pombe* appears to exploit a common SAPK signaling pathway in which Sty1 activates its common set of stress genes (9), whereas *S. cerevisiae* uses different signaling pathways to activate its common set of stress genes (8, 9, 21). *C. albicans* differs from these benign fungi in that it did not display a common core transcriptional response to sublethal heat, osmotic, and oxidative stresses that induce such responses in *S. pombe* and *S. cerevisiae* (15). Instead, specific molecular responses to each stress were observed, and this was consistent with the lack of cross-protection provided by mild heat, osmotic, or oxidative stress (15). Hence, these three fungi clearly display specialized stress responses that presumably reflect their contrasting niches.

The situation is complicated by the potential involvement of dose-dependent stress-signaling networks in each fungus. For example, in *S. pombe*, the Sty1 pathway is activated by H$_2$O$_2$ in a dose-dependent fashion via two distinct sensing mechanisms (49). Hence, it could be argued that CaMsn4 (and possibly Mnl1) might be required for responses to only certain doses of a particular stress. However, we observed no phenotypic effects of the *msn4-msn4* and *mnl1-mnl1* mutations following exposure to a wide range of salt, nutrient, or oxidative stresses. Therefore, stress signaling in *C. albicans* appears to have diverged to the extent that Msn2- and Msn4-like proteins no longer play significant roles.

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REFERENCES

1. Alarco, A. M., and M. Raymond. 1999. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. J. Bacteriol. 181:701–708.

2. Braun, B. R., D. Kadosh and A. D. Johnson. 2001. NRG1, a repressor of filamentous growth in *Candida albicans*, is down-regulated during filament induction. EMBO J. 20:4753–4761.

3. Brent, R., and M. Ptashne. 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell 43:729–736.

4. Brown, A. J. P. 2002. Expression of growth form-specific factors during morphogenesis in *Candida albicans*, p. 87–93. In R. Calderone (ed.), *Candida* and Candidiasis. ASM Press, Washington, D.C.

5. Brown, A. J. P., G. Bertram, P. J. Feldmann, M. W. Peggie, and R. K. Sowboda. 1991. Codon utilisation in the pathogenic yeast, *Candida albicans*. Nucleic Acids Res. 19:4298.

6. Calderone, R. A. 2002. Candida and candidiasis. ASM Press, Washington, D.C.

7. Care, R. S., J. Treveithick, K. M. Binley, and P. E. Sudbery. 1999. The TET3 promoter: a new tool for *Candida albicans* molecular genetics. Mol. Microbiol. 34:792–798.

8. Causton, H. C., B. Ren, S. S. Koh, C. T. Harbison, E. Kanin, E. G. Jennings, T. I. Lee, H. L. True, E. S.lander, and R. A. Young. 2001. Remodeling of yeast genome expression in response to environmental changes. Mol. Biol. Cell 12:3233–3272.

9. Chen, D., W. M. Toone, J. Mata, R. Lyne, G. Burns, K. Kivinen, A. Brahma, N. Jones, and J. Baehler. 2003. Global transcriptional responses of fission yeast to environmental stress. Mol. Biol. Cell 14:214–229.

10. Cohen, B. A., Y. Pilpel, R. Mitra, and G. M. Church. 2002. Discrimination between paralogs using microarray analysis: application to the Yap1p and Yap2p transcription networks. Mol. Biol. Cell 13:1608–1614.

11. Cormack, B., G. Bertram, M. Egerton, N. A. R. Gow, S. Fallow, and A. J. P. Brown. 1997. Yeast enhanced green fluorescent protein EGFP: a reporter of gene expression in *Candida albicans*. Microbiology 143:303–311.

12. Cress, W. D., and S. J. Triezenberg. 1991. Critical structural elements of the VP16 transcriptional activation domain. Science 251:87–90.

13. Csank, C., K. Schroppel, E. Leberer, D. Harcus, O. Mohamed, S. Mlecho, D. Y. Thomas, and M. Whiteway. 1998. Roles of the *Candida albicans* mitogen-activated protein kinase homolog, Cek1p, in hyphal development and systemic candidiasis. Infect. Immun. 66:2713–2721.

14. Davis, D., R. B. Wilson, and A. P. Mitchell. 2000. RIM101-dependent and -independent pathways govern pH responses in *Candida albicans*. Mol. Cell. Biol. 20:971–978.

15. Enjalbert, B., A. Nantel, and M. Whiteley. 2003. Stress induced gene expression in *Candida albicans*: absence of a general stress response. Mol. Biol. Cell 14:1460–1467.

16. Estruch, F., and M. Carlson. 1995. Two homologous zinc finger genes identified by multistep suppression in a SNF1 kinase mutant of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 13:3872–3881.

17. Feng, Q., E. Summers, B. Guo, and G. R. Fink. 1999. Ras signaling is required for serum-induced hyphal differentiation in *Candida albicans*. J. Bacteriol. 181:6339–6346.

18. Fonzi, W. A., and M. Y. Irwin. 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 134:717–728.

19. Gorner, W., E. Durchschlag, J. Wolf, E. L. Brown, G. Ammerer, H. Ruis, and C. Schuller. 2002. Nuclear localization of the C$_2$H$_2$ zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes Dev. 12:586–597.

20. Gorner, W., E. Durchschlag, J. Wolf, E. L. Brown, G. Ammerer, H. Ruis, and C. Schuller. 2002. Acute glucose starvation activates the nuclear localization of the C$_2$H$_2$ zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Mol. Biol. Cell 11:4241–4257.

21. Gillum, A. M., E. Y. Tsay, and D. R. Kirsch. 1984. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of the *S. cerevisiae* ura3 and *E. coli* pyrF mutations. Mol. Gen. Genet. 198:179–182.

22. Gorner, W., E. Durchschlag, J. Martinez-Pastor, F. Estruch, G. Ammerer, B. Hamilton, H. Ruis, and C. Schuller. 1998. Nuclear localization of the C$_2$H$_2$ zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes Dev. 12:586–597.

23. Gorner, W., E. Durchschlag, J. Wolf, E. L. Brown, G. Ammerer, B. Hamilton, H. Ruiz, and C. Schuller. 2002. Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. EMBO J. 21:135–144.

24. Hirata, Y., T. Andoh, T. Asahara, and A. Kikuchi. 2003. Yeast glycerol synthase kinase-3 activates Msn2p-dependent transcription of stress responsive genes. Mol. Biol. Cell 14:302–312.

25. Jacquet, M., G. Renaul, S. Lallet, J. De Mey, and A. Goldbeter. 2003. Oscillatory autoregulatopalusmatic chasing of the general stress response transcriptional activator Msn2 and Msn4 in *Saccharomyces cerevisiae*. J. Cell Sci. 161:497–505.

26. Jamieson, D. J., D. W. S. Stephen, and E. C. Terriere. 1996. Analysis of the adaptive oxidative stress response of *Candida albicans*. FEMS Microbiol. Lett. 138:83–88.

27. Kadosh, D., and A. D. Johnson. 2001. Rfg1, a protein related to the *S. cerevisiae* hypoxic regulator Rox1p, controls filamentous growth and virulence in *C. albicans*–*. Mol. Cell. Biol. 21:2496–2505.

28. Kaiser, C., M. Michaelis, and S. Mitchell. 1994. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
48. Keleher, C. A., M. J. Redi, J. Schultz, M. Carlson, and A. D. Johnson. 1992. Snf6-Tup1 is a general repressor of transcription in yeast. Cell 68:709–719.

49. Quinn, J., V. J. Findlay, K. Dawson, J. B. A. Millar, N. Jones, B. A. Morgan, and W. M. Toone. 2002. Distinct regulatory proteins control the graded transcriptional response to increasing H2O2 levels in fission yeast Schizosaccharomyces pombe. Mol. Biol. Cell 13:805–816.

50. Regier, J. L., F. Shen, and S. J. Triezenberg. 1993. Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. Proc. Natl. Acad. Sci. USA 90:883–887.

51. Rocha, C. R. C., K. Schroppel, D. Harscus, A. Marcell, D. Dignard, B. N. Taylor, D. Y. Thomas, M. Whiteway, and E. Leberer. 2001. Signaling through adenyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol. Biol. Cell 12:3631–3643.

52. Rupp, S. 2002. lacZ assays in yeast. Methods Enzymol. 350:112–131.

53. Russell, C. L., and A. J. P. Brown. Expression of Staphylococcus aureus LexA fusions in Candida albicans confirm that CaNrg1 is a transcriptional repressor and that CaGcn4 is a transcriptional activator. Submitted for publication.

54. Santos, M. A. S., G. Keight, and M. F. Tuine. 1993. Non-standard translational events in Candida albicans mediated by an unusual seryl-tRNA with a 5′-CAG-3′ leucine anticodon. EMBO J. 12:607–616.

55. Schnell, N., B. Krens, and K.-D. Entian. 1992. The PAR1 YAP1/SNQ3 gene of Saccharomyces cerevisiae, a cjun homolog, is involved in oxygen metabolism. Curr. Genet. 21:269–273.

56. Sherman, F. 1991. Getting started with yeast. Methods Enzymol. 194:3–21.

57. Sikorski, R. S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics. 122:19–27.

58. Sommeijn, A., D. P. Bockmuhl, M. Gerads, K. Kurpanek, D. Sanglard, and J. F. Ernst. 2000. Protein kinase A encoded by TPK2 regulates dimorphism of Candida albicans. Mol. Microbiol. 38:386–396.

59. Srikantha, T., A. Klagach, W. W. Lorenz, L. K. Tsai, L. A. Laughlin, J. A. Gorman, and D. R. Soll. 1996. The sea pansy Renilla reniformis luciferase serves as a sensitive bioluminescent reporter for differential gene expression in Candida albicans. J. Bacteriol. 178:121–129.

60. Swoboda, R. K., G. Bertram, S. Delbruck, J. F. Ernst, N. A. R. Gow, G. W. Goward, and A. J. P. Brown. 1994. Fluctuations in glycolytic mRNA levels during the yeast-to-hyphal transition in Candida albicans reflect underlying changes in growth rather than a response to cellular dimorphism. Mol. Microbiol. 13:663–672.

61. Thomas, R. J., and R. Rothstein. 1989. The genetic control of direct-repeat recombination in Saccharomyces: the effect of rad52 and rad1 on mitotic recombination at GAL10, a transcriptional regulation gene. Genetics 123:725–738.

62. Tripathi, G., C. Wiltshire, S. Macaskill, H. Tournu, S. Budge, and A. J. P. Brown. 2002. CaGcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in Candida albicans. EMBO J. 21:5448–5456.

63. Tuscher, V. G., R. Tsuchihara, and G. Chu. 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA 98:5116–5121.

64. Uhl, M. A., and A. D. Johnson. 2001. Development of a Streptococcus thermophilus lacZ as a reporter gene for Candida albicans. Microbiology 147:1189–1195.

65. Whiteway, M. 2000. Transcriptional control of cell type and morphogenesis in Candida albicans. Curr. Opin. Microbiol. 3:582–588.

66. Wolfe, K. H., and D. C. Shields. 1989. Mutational analysis of a yeast transcriptional activator. Proc. Natl. Acad. Sci. USA 86:4097–4101.

67. Ziegelbauer, A., S. Rupp, S. C. Pringle, and M. Whiteway. 2001. Development of a Streptococcus thermophilus lacZ as a reporter gene for Candida albicans. Microbiology 147:1189–1195.

68. Wolfe, K. H., and D. C. Shields. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387:708–713.

69. Zhang, X., M. de Micheli, S. Coleman, D. Sanglard, and W. S. Moye-Rowley. 2000. Analysis of the oxidative stress regulation of the Candida albicans transcription factor, Cap1p. Mol. Microbiol. 36:618–629.