High Resistance to Oxidative Stress in the Fungal Pathogen *Candida glabrata* Is Mediated by a Single Catalase, Cta1p, and Is Controlled by the Transcription Factors Yap1p, Skn7p, Msn2p, and Msn4p

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We characterized the oxidative stress response of *Candida glabrata* to better understand the virulence of this fungal pathogen. *C. glabrata* could withstand higher concentrations of H$_2$O$_2$ than *Saccharomyces cerevisiae* and even *Candida albicans*. Stationary-phase cells were extremely resistant to oxidative stress, and this resistance was dependent on the concerted roles of stress-related transcription factors Yap1p, Skn7p, and Msn4p. We showed that growing cells of *C. glabrata* were able to adapt to high levels of H$_2$O$_2$ and that this adaptive response was dependent on Yap1p and Skn7p and partially on the general stress transcription factors Msn2p and Msn4p. *C. glabrata* has a single catalase gene, CTA1, which was absolutely required for resistance to H$_2$O$_2$ in vitro. However, in a mouse model of systemic infection, a strain lacking CTA1 showed no effect on virulence.

Recent surveys show that *Candida* species are responsible for about 8% of all hospital-acquired bloodstream infections, and among these species, the two most frequently isolated are *Candida albicans* and *Candida glabrata* (60, 73). *C. glabrata* is an opportunistic fungal pathogen that is a commensal in human gastrointestinal and genitourinary tracts but that also causes severe invasive infections. Phylogenetically, *C. glabrata* is quite distinct from *C. albicans* but is closely related to *Saccharomyces cerevisiae*. The virulence attributes that allow *C. glabrata* to colonize human tissues and cause disseminated infections have recently started to be identified (reviewed in reference 40).

Phagocytic cells are the first line of defense against fungal infections (49). These cells generate reactive oxygen species (ROS), including superoxide, hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals, that can damage all biomolecules and destroy phagocytosed pathogens (27, 70). ROS are also by-products of normal aerobic metabolism, and all aerobic organisms possess mechanisms to maintain very low levels of these species. In particular, a variety of small antioxidant molecules, such as glutathione and thioredoxin, are synthesized to scavenges ROS, and even tyrosine has been proposed to have a protective role against oxidative stress (48). In addition, several well-characterized enzymes, such as the superoxide dismutases, catalases, peroxidases, and glutathione peroxidases, are produced to eliminate ROS. Pathogens have coopted these well-conserved antioxidation mechanisms to evade phagocyte defenses (5, 27, 69, 70); thus, the production of these enzymes is directly related to virulence (35, 76).

When cells are under oxidative stress, transcriptional remodeling occurs to ensure the proper response. The enzymes and the regulation of the oxidative stress response (OSR) are well conserved among fungal species. Catalases are well-conserved detoxifying enzymes catalyzing the conversion of H$_2$O$_2$ to H$_2$O and molecular oxygen (reviewed in references 1 and 2). *S. cerevisiae* has two catalase genes, both of which are required for detoxifying H$_2$O$_2$ (13, 30, 65, 66, 72). Both *C. albicans* and *C. glabrata* carry only one catalase gene, and *C. albicans* catalase has been shown to play an important role in the virulence of *C. albicans* (56, 74, 76). The OSR in *S. cerevisiae* is in part under the control of the well-studied transcription factors Yap1p, Skn7p, Msn2p, and Msn4p (21, 43, 44, 46, 53, 64). *S. cerevisiae* Yap1p (ScYap1p) belongs to the family of basic leucine zipper domain transcription factors and controls the expression of at least 32 proteins of the H$_2$O$_2$ stimulus (46). Strains lacking Yap1p are hypersensitive to H$_2$O$_2$. The Yap1p orthologs in *C. albicans* (Cap1p) (3, 78), *Schizosaccharomyces pombe* (Pap1p) (71), and *Ustilago maydis* (Yap1p) (52) have been characterized previously, and they are involved in the OSR. The *C. glabrata* Yap1p ortholog is functionally involved not only in the OSR, but also in resistance to different drugs (11). ScSkn7p contains a receiver domain found in the family of two-component signal transduction systems of prokaryotes and a DNA-binding domain similar to that of heat shock factor Hsf1p (6, 54). The target genes of ScSkn7p overlap with those of ScYap1p, and a skn7Δ strain is hypersensitive to H$_2$O$_2$ (46, 53). The *C. albicans* Skn7p ortholog has been characterized previously, and cells lacking Skn7p are modestly attenuated in virulence (68). ScMsn2p and ScMsn4p are functionally nonre-

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TABLE 1. Strains used in this study

| Strain          | Parent          | Genotype and/or description                                                                 | Reference or source |
|-----------------|-----------------|---------------------------------------------------------------------------------------------|---------------------|
| **E. coli**      |                 |                                              |                     |
| DH10B           |                 | F− mcrA Δ(mrr-hsdRMS-mcrBC) ΔΩ800lacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara,leu)7697 galU galK ΔrpsL ΔnapG | 7                   |
| JM109           |                 | recA1 endA1 glyA96 thi hsdR17(tK− mK+) relA1 supE44 Δ(lac-proAB) [F' traD36 proAB lacPZΔM15] | 77                  |
| **S. cerevisiae**|                 |                                              |                     |
| W303            |                 | MATα ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100 ade2-1 ade3-2 hisG                           | 51                  |
| YJM128          |                 | Clinical isolate                                                                           | 12                  |
| YJM356          |                 | Clinical isolate                                                                           | 12                  |
| **C. albicans**  |                 |                                              |                     |
| CA14            |                 | ura3Δ::imm434/ura3Δ::imm434                                                                  | 25                  |
| CA5             |                 | Clinical isolate                                                                           | Lab collection      |
| CA7             |                 | Clinical isolate                                                                           | Lab collection      |
| **C. glabrata**  |                 |                                              |                     |
| MC7             |                 | Clinical isolate                                                                           | Lab collection      |
| MC22            |                 | Clinical isolate                                                                           | Lab collection      |
| BG2             |                 | Clinical isolate (strain B)                                                                  | 22                  |
| BG14            | BG2             | ura3Δ::Tn903 G418′; wt Ura− strain used in this study                                       | 15                  |
| BG462           | BG14            | URA3                                                                                       | 18                  |
| BG1739 (msn2Δ mutant) | BG14       | ura3Δ::Tn903 G418′ msn2Δ Hyg′ Ura− pRD96/BcgI                                               | 15                  |
| BG1740 (msn4Δ mutant) | BG14       | ura3Δ::Tn903 G418′ msn4Δ Hyg′ Ura− pRD97/BcgI                                               | R. Domergue and B. Cormack |
| BG1742 (msn2Δ msn4Δ mutant) | BG1739 | ura3Δ::Tn903 G418′ msn2Δ msn4Δ Δ::hyg′                                                     | R. Domergue and B. Cormack |
| CGM295 (cta1Δ mutant) | BG14       | ura3Δ::Tn903 G418′ cta1Δ::hyg′ Hyg′ pCV15/BcgI                                             | This work           |
| CGM297 (yap1Δ mutant) | BG14       | ura3Δ::Tn903 G418′ yap1Δ::hyg′ Hyg′ pCV17/BcgI                                             | This work           |
| CGM306 (skn7Δ mutant) | BG14       | ura3Δ::Tn903 G418′ skn7Δ::hyg′ Hyg′ pCV21/BcgI                                             | This work           |
| CGM307 (yap2Δ mutant) | CGM297     | ura3Δ::Tn903 G418′ yap2Δ::hyg′ Ura−                                                       | This work           |
| CGM310 (yap1Δ skn7Δ mutant) | CGM307 | ura3Δ::Tn903 G418′ yap1Δ skn7Δ::hyg′                                                      | This work           |
| CGM351 (cta1Δ mutant) | CGM295     | URA3 cta1Δ::hyg′                                                                            | This work           |
| CGM385 (yap1Δ skn7Δ mutant) | CGM310 | ura3Δ::Tn903 G418′ yap1Δ skn7Δ::hyg′                                                      | This work           |
| CGM386 (yap1Δ skn7Δ msn2Δ mutant) | CGM385 | ura3Δ::Tn903 G418′ yap1Δ skn7Δ msn2Δ::hyg′                                                  | This work           |
| CGM388 (yap1Δ skn7Δ msn4Δ mutant) | CGM386 | ura3Δ::Tn903 G418′ yap1Δ skn7Δ msn4Δ::hyg′                                                  | This work           |
| CGM480 (yap1Δ skn7Δ msn4Δ mutant) | CGM388 | ura3Δ::Tn903 G418′ yap1Δ skn7Δ msn4Δ::hyg′                                                  | This work           |

...dundant Zn2+ finger transcription factors involved in the general stress response, including the response to oxidative stress (reviewed in references 20 and 55). They control the expression of about 27 gene products regulated in response to H2O2 (31). Msn2p and Msn4p play an important role in stationary-phase (SP) survival under oxidative stress. S. cerevisiae cells lacking Msn2p and Msn4p are sensitive to H2O2 (21, 31, 50, 55, 63). Interestingly, C. albicans Msn2p (CaMsn2p) and CaMsn4p play no obvious role in the stress response, including the response to oxidative stress (58).

The OSR of C. glabrata has not been analyzed previously. In this study, we showed that the growth of C. glabrata could withstand higher concentrations of H2O2 than that of S. cerevisiae and even that of C. albicans (see below) (5, 9). This phenotype could be seen in both log-phase (LP) and SP cells, but the phenotype was more profound in the latter case. SP resistance was dependent on the concerted roles of Yap1p, Skn7p, and Msn4p. We also showed that C. glabrata was able to adapt to high levels of H2O2 and that this adaptive response was dependent on the stress-related transcription factors Yap1p and Skn7p and partially on the general stress transcription factors Msn2p and Msn4p. Lastly, we showed that the C. glabrata catalase gene CTA1 was absolutely required for resistance to H2O2 in vitro. However, a strain lacking CTA1 had no obvious phenotype in vivo in a mouse model of systemic infection.

**MATERIALS AND METHODS**

**Strains.** All strains used in this study are summarized in Table 1.

**Plasmids.** All plasmids used in this study are summarized in Table 2.

**Primers.** All primers used for cloning are summarized in Table 3.

**Media.** Yeast media were prepared as described previously (67), and 2% agar was added for plates. Yeast extract-peptone-dextrose (YPD) medium contained yeast extract at 10 g/liter and peptone at 20 g/liter and was supplemented with 2%...
TABLE 2. Plasmids used in this study

| Plasmid | Description and/or relevant genotype | Reference or source |
|---------|-------------------------------------|---------------------|
| pGEM-T | Cloning vector; Km<sup>+</sup> Ap<sup>+</sup> | Promega 19 |
| pAP599 | Cloning vector with 2 FRT direct repeats flanking the hygromycin marker [FRT-P<sub>PGK</sub>-hph:(3′ UTR of HIS3)-FRT] for construction of multiple mutants; URA4 His<sup>+</sup> Amp<sup>+</sup> | |
| pMZ17 | Replicative vector expressing ScFLP1 (recombinase gene) for removing the hygromycin marker; P<sub>PGK</sub>:FLP1:(3′ UTR of HIS3) CgCEN ARS Amp<sup>+</sup> | Cormack lab collection 15 |
| pBC34.1 | pUC19::CgUrA43; 2.2-kb PstI fragment; Ap<sup>+</sup> | |

Plasmids for deletion

| pCV15 cta1Δ | A 0.906-kb SpeI/BamHI PCR fragment (corresponding to primers 57 and 58) carrying the promoter region of CTA1 and a 0.682-kb HindIII/KpnI PCR fragment (corresponding to primers 60 and 61) carrying the 3′ UTR of CTA1 were cloned into pAP599 Ap<sup>+</sup> | This work |
| pCV17 yap1Δ | A 0.846-kb Xhol/HindIII PCR fragment (corresponding to primers 7 and 8) carrying the promoter region of YAPI and a 0.652-kb BamHI/Sacl PCR fragment (corresponding to primers 11 and 12) carrying the 3′ UTR of YAPI were cloned into pAP599 Ap<sup>+</sup> | This work |
| CV21 skn7Δ | A 0.875-kb KpnI/Xhol PCR fragment (corresponding to primers 4 and 5) carrying the promoter region of SKN7 and a 0.929-kb BamHI/Sacl PCR fragment (corresponding to primers 1 and 2) carrying the 3′ UTR of SKN7 were cloned into pAP599 Ap<sup>+</sup> | This work |
| pRD96 msn2Δ | A 0.691-kb KpnI/Xhol PCR fragment (corresponding to primers 2984 and 2985) carrying the promoter region of MSN2 and a 0.520-kb BamHI/Sacl PCR fragment (corresponding to primers 2986 and 2987) carrying the 3′ UTR of Msn2 were cloned into pAP599 Ap<sup>+</sup> | R. Domergue and B. Cormack |
| pRD97 msn4Δ | A 0.564-kb KpnI/Xhol PCR fragment (corresponding to primers 2990 and 2991) carrying the promoter region of Msn4 and a 0.535-kb BamHI/Sacl PCR fragment (corresponding to primers 2992 and 2993) carrying the 3′ UTR of Msn4 were cloned into pAP599 Ap<sup>+</sup> | R. Domergue and B. Cormack |

Plasmids for cloning, construction, and marker removal

| TABLE 2. Oligonucleotides used in this study

| Primer | Sequence<sup>a</sup> | Description or restriction site(s) |
|--------|----------------------|-----------------------------------|
| 13 | CTGTTAAGAACAGGCGGAGTG | pUC forward |
| 17 | GAAAGACGCTTACGACAGTT | pUC reverse |
| 1 | CCGAAGCTTCGATTAATCGGCAATCAG | BamHI |
| 2 | CAAGGACGCTTCTGTCGCGTGCGTGAATGATCCAG | SacI/BglI |
| 4 | TCGAATTCCTATGGGTACATGACCCAAA | Xhol |
| 5 | CGGGAGCTCGGACAAGCTATGACGATCATC | KpnI/BglI |
| 7 | AACCTCCGATGCGTCGATGCGGATACAGG | Xhol/BglI |
| 8 | CCGAAGCTTATATCATATCGGTCGAT | HindIII |
| 11 | CGGGAGCTTATATCATATCGGTCGAT | BamHI |
| 12 | CAGAAGCTTATATCATATCGGTCGAT | SacI/BglI |
| 57 | CGCGGATCTTATATCATATCGGTCGAT | BamHI |
| 58 | CAGCTCTAGATGAACTTTTCGATG | SpeI |
| 60 | CCGAAGCTTGCAATGAACTTTTCGATG | HindIII |
| 61 | CCGAAGCTTGCAATGAACTTTTCGATG | KpnI/BglI |
| 2984 | GTGATCCGAGATAGTTATGGCAGTTATG | KpnI/BglI |
| 2985 | CTGATCCGAGATAGTTATGGCAGTTATG | Xhol |
| 2986 | GAGCTCTACGAGATAGTTATGGCAGTTATG | BamHI |
| 2987 | GAGCTCTACGAGATAGTTATGGCAGTTATG | SacI/BglI |
| 2990 | GTGATCCGAGATAGTTATGGCAGTTATG | KpnI/BglI |
| 2991 | CGCGGATCTTATATCATATCGGTCGAT | Xhol |
| 2992 | CGCGGATCTTATATCATATCGGTCGAT | BamHI |
| 2993 | CGCGGATCTTATATCATATCGGTCGAT | SacI/BglI |

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<sup>a</sup> Restriction sites are indicated in boldface.
RESULTS

*C. glabrata* is resistant to high levels of hydrogen peroxide. In order to characterize the OSR in *C. glabrata*, we investigated the resistance of *C. glabrata* LP cells to H₂O₂. *C. glabrata* strains (BG14 [lab reference strain] and clinical isolates [CI] MC7 and MC22) were treated with H₂O₂ as described in the legend to Fig. 1 and Materials and Methods. As shown in Fig. 1A, *C. glabrata* BG14 was able to survive exposure to H₂O₂ at concentrations up to 40 mM. There was a severe loss of viability at 100 mM and a complete loss at 200 mM (data not shown), while CI MC7 and MC22 showed reduced resistance at 40 mM. We also performed an adaptation experiment by pretreating the LP cells with 10 mM H₂O₂ for 1 h and then treating them with 100 mM H₂O₂ for 2 additional hours. As shown in Fig. 1A (lane 10+100), the survival rate of BG14 cells pretreated with a low dose of H₂O₂ was much higher than that of cells without this treatment, and CI MC7 and MC22 showed reduced adaptation compared to that of BG14. Experiments similar to those described above were also performed with *C. albicans* strains (CAI4 [lab reference strain] and CI CA5 and CA7) and *S. cerevisiae* strains (W303 [lab reference strain] and CI YJM128 and YJM336). *C. albicans* CAI4 was resistant to 40 mM H₂O₂ and showed complete sensitivity to 100 mM H₂O₂, while *C. albicans* CI showed reduced resistance compared to that of CAI4 (Fig. 1B). As shown in Fig. 1C, *S. cerevisiae* was resistant only to 6 mM H₂O₂ and completely sensitive to 8 mM H₂O₂. Interestingly, CI YJM128 and YJM336 were more resistant than the W303 lab reference strain. These experiments indicate that LP *C. glabrata* cells are modestly more resistant to H₂O₂ than LP *C. albicans* cells and significantly more resistant than LP *S. cerevisiae* cells. In addition, *C. glabrata* and *C. albicans* cells were able to adapt, though to different levels, suggesting that adaptation is a common trait for both pathogens (Fig. 1A and B). Similarly, *S. cerevisiae* has been shown previously to be able to adapt to higher concentrations of H₂O₂ when pretreated with a lower dose (14, 17, 37, 38).

It has been reported previously that SP cells of various bacterial and fungal pathogens are more resistant to oxidants than LP cells (16, 29). To determine if this is true also for *C. glabrata*, we treated *C. glabrata* strain BG14 and CI MC7 and MC22 as described in the legend to Fig. 2 and Materials and Methods. As shown in Fig. 2A, *C. glabrata* SP cells exhibited resistance to H₂O₂ at concentrations up to 1,000 mM, significantly higher than those to which LP cells showed resistance. The same experiments were performed with *C. albicans* strain CAI4 and CI CA5 and CA7 and *S. cerevisiae* strain W303 and CI YJM128 and YJM336. As shown in Fig. 2B, *C. albicans* CAI4 showed resistance to up to 300 mM H₂O₂ while CI showed resistance to up to 100 mM. *S. cerevisiae* W303 SP cells showed an increase in resistance compared to that of LP cells, exhibiting resistance to up to 100 mM H₂O₂ and complete sensitivity to 200 mM. Interestingly, *S. cerevisiae* CI were more resistant than the lab reference strain W303 (Fig. 2C). Overall, the SP levels of resistance were much higher than those in LP. These data are in agreement with previous findings that SP cells are more resistant to oxidative stress than LP cells. Moreover, our data confirm that *C. glabrata* strain CAI4 is intrinsically more resistant to H₂O₂ than *S. cerevisiae* and *C. albicans* in SP.

Role of the transcription factors Snk7p, Yap1p, Msn2p, and Msn4p in the OSR. The key regulators of the OSR in *S. cerevisiae*, such as Skn7p, Yap1p, Msn2p, and Msn4p, are well characterized. *C. glabrata* orthologs of *S. cerevisiae* genes encoding these transcription factors were identified in the *C. glabrata* genome database (http://dbielewicz.org/Genolevures/elt/CAGL), and an analysis of the amino acid sequence homology of *C. glabrata* and *S. cerevisiae* transcription factors was done by ClusalW alignment, with the following results: *C. glabrata* Skn7p (CGSkn7p; CAGL0F09097g), 48% identical and 15% similar to ScSkn7p; CgMsn2p (CAGL0F09599g), 25% identical and 16% similar to ScMsn2p; CgMsn4p (CAGL0M13189g), 22% identical and 13% similar to ScMsn4p; and CgYap1p (CAGL0H04631g), 36% identical and 12% similar to ScYap1p (11). We simply asked whether these transcription factors participate in the OSR in *C. glabrata*. We constructed *C. glabrata* mutant strains containing single (yap1Δ or skn7Δ), double (yap1Δ skn7Δ), triple (yap1Δ skn7Δ msn2Δ or yap1Δ skn7Δ msn4Δ), and quadruple (yap1Δ skn7Δ msn2Δ msn4Δ) deletions. Additional null strains including msn2Δ, msn4Δ, and msn2Δ msn4Δ strains were provided by R. Domergue and B. Cormack. We first characterized the sensitivities to H₂O₂ treatment of the LP cells of the various *C. glabrata* deletion strains.
described above. As shown in Fig. 3A, the yap1Δ and skn7Δ strains showed reduced resistance to 10 mM H2O2 compared to that of the parental wt strain. When treated with 100 mM H2O2, both the yap1Δ and skn7Δ strains lost viability, in contrast with the parental strain. The same phenotype was seen in the adaptation experiment. As shown in the bottom panel of Fig. 3A, when pretreated with a low dose of H2O2 of 10 mM, the yap1Δ and skn7Δ strains could not adapt to the stress, in contrast with the parental strain. A yap1Δ skn7Δ strain showed the same phenotype as the strains carrying a single deletion of either gene, indicating that the corresponding transcription factors are needed for adaptation and resistance in LP and that they do not compensate for each other. Furthermore (Fig. 3A), since (i) the yap1Δ skn7Δ msn2Δ triple mutant had the same reduced resistance phenotype at 10 mM as the single and double mutants (yap1Δ, skn7Δ, and yap1Δ skn7Δ strains), (ii) the yap1Δ skn7Δ msn4Δ triple mutant was more sensitive than the single and double mutants to 10 mM, and (iii) the quadruple mutant (yap1Δ skn7Δ msn2Δ msn4Δ) behaved the same as the yap1Δ skn7Δ msn4Δ triple mutant (see Fig. 6A), Skn7p, Yap1p, and Msn4p together, but not Msn2p, coordinate the overall resistance in LP cells. Msn2p and Msn4p may play a role in adaptation since the msn2Δ msn4Δ double mutant but not the corresponding single mutants showed a subtle but reproducible defect in adaptation (Fig. 3B). These results indicate, first, not only that C. glabrata has conserved the adaptive response to oxidative stress in LP cells but also that this adaptation is dependent mainly on Skn7p and Yap1p and partially on both Msn2p and Msn4p and, second, that Skn7p, Yap1p, and Msn4p coordinate the control of the OSR since

FIG. 1. C. glabrata, C. albicans, and S. cerevisiae LP resistance to H2O2. Saturated cultures of C. glabrata (C.g.) strain BG14 and CI MC7 and MC22 (A), C. albicans (C.a.) strain CAI4 and CI CA5 and CA7 (B), and S. cerevisiae (S.c.) strain W303 and CI YJM128 and YJM336 (C) were diluted with fresh medium (YPD) so that all strains reached an OD600 of 0.5 after seven doublings at 30°C. C. glabrata and C. albicans strains were divided and exposed to 0, 10, 20, 30, 40, 50, and 100 mM H2O2 and S. cerevisiae strains were exposed to 2, 4, 6, and 8 mM H2O2 for 3 h. For adaptation experiments, C. glabrata and C. albicans cells were pretreated for 1 h with 10 mM H2O2 and then with 100 mM H2O2 for 2 additional hours. After the treatment, H2O2 was removed by centrifugation. The cultures were resuspended in distilled water, and the OD600 was adjusted when needed to 0.5. Cultures were serially diluted, and each dilution was spotted onto YPD plates, ensuring that the same amounts of cells were plated. Plates were incubated at 30°C. See Materials and Methods.
the triple and quadruple mutants were almost as sensitive as the cta1Δ mutant in LP (see below and Fig. 6A).

We then tested the role of these transcription factors for resistance in SP. Saturated cultures (OD_{600}, 30.0) of the parental strain BG14 and single (yap1Δ, skn7Δ, msn2Δ, and msn4Δ), double (yap1Δ skn7Δ and msn2Δ msn4Δ), triple (yap1Δ skn7Δ msn2Δ and yap1Δ skn7Δ msn4Δ), and quadruple (yap1Δ skn7Δ msn2Δ msn4Δ) mutants were treated as described in the legend to Fig. 2. Figure 4A shows that the yap1Δ mutant behaved as the parental strain, that the skn7Δ mutant

**FIG. 2.** *C. glabrata*, *C. albicans*, and *S. cerevisiae* SP resistance to H\textsubscript{2}O\textsubscript{2}. Saturated cultures of *C. glabrata* (C.g.) strain BG14 and CI MC7 and MC22 (A), *C. albicans* (C.a.) strain CA14 and CI CA5 and CA7 (B), and *S. cerevisiae* (S.c.) strain W303 and CI YJM128 and YJM336 (C) were diluted to an OD\textsubscript{600} of 0.5 with spent medium from the same cultures. The cells were divided into aliquots and treated for 3 h with H\textsubscript{2}O\textsubscript{2} at different concentrations: for *C. glabrata*, 500, 800, 1,000, and 1,500 mM; for *C. albicans*, 0, 50, 100, 300, and 500 mM; and for *S. cerevisiae*, 0, 10, 50, 100, and 200 mM. After the treatment, the cultures remained at an OD\textsubscript{600} of 0.5, oxidant was removed by centrifugation, cells were resuspended in distilled water, and suspensions were diluted and spotted onto YPD plates. Plates were incubated at 30°C. See Materials and Methods.

**FIG. 3.** Regulation of the OSR to H\textsubscript{2}O\textsubscript{2} in LP. The wt (BG14) and single, double, and triple mutants with yap1Δ, skn7Δ, msn2Δ, and msn4Δ mutations were grown and treated with H\textsubscript{2}O\textsubscript{2} as described in the legend to Fig. 1. See Materials and Methods.
was more sensitive to H$_2$O$_2$ than the wt, and that the skn7Δ mutation is epistatic to yap1Δ (compare the data for the yap1Δ skn7Δ double mutant and the single yap1Δ and skn7Δ mutants). This finding indicates that, surprisingly, Skn7p but not Yap1p is required in SP. As in LP cells, Msn4p was required for resistance in SP cells: an msnΔ single mutant and a triple mutant with msn4Δ in combination with skn7Δ showed reduced resistance to H$_2$O$_2$ (Fig. 4). Interestingly, Msn2p has a role in SP since the msn2Δ msn4Δ double mutant was more sensitive than the msn2Δ and msn4Δ single mutants (Fig. 4B). These data suggest that Msn2p and Msn4p are both important for resistance in SP cells: an isoyme, the mitochondria, or the cytoplasm.

**CTA1 is absolutely required for resistance to H$_2$O$_2$ in vitro.** Catalase plays a central role in the cell response against oxidative stress. We characterized the function of C. glabrata catalase Cta1p in the resistance to H$_2$O$_2$ treatment. S. cerevisiae has two catalases, cytoplasmic (Ctt1p) and peroxisosomal (Cta1p) (13, 30, 65, 66, 72), and C. albicans has only one catalase (Ctt1p) (76). Interestingly, the single C. glabrata catalase gene, CTA1 (CAGL0K10868g), is the ortholog of S. cerevisiae CTA1, the gene for the peroxiosomal catalase. CgCta1p and ScCta1p are 85% similar (78% identical and 7% similar) over the entire lengths of the proteins, and CgCta1p has two putative internal peroxiosomal targeting signals (SKF) (59) (Fig. 5). It is not known whether CgCta1p is targeted to the peroxosome, the mitochondria, or the cytoplasm.

We tested whether CTA1 is required in C. glabrata for the high-level resistance to H$_2$O$_2$ displayed by both the LP and SP cells. A CTA1 null strain (cta1Δ) was constructed. cta1Δ LP cells (Fig. 6A) and SP cells (Fig. 6B) behaved in the same way: cta1Δ cells completely lost their ability to survive at high concentrations of H$_2$O$_2$ (>4 mM) (Fig. 6). Interestingly, cta1Δ LP cells were still able to adapt but only when exposed to low levels of H$_2$O$_2$ (Fig. 6A, lane 1 + 4). This result may indicate the presence of a catalase-independent pathway to respond to H$_2$O$_2$. In SP, the cta1Δ mutant also became sensitive to H$_2$O$_2$ (Fig. 6B). These results clearly indicate that this single Cta1p plays a central role in the resistance of C. glabrata to H$_2$O$_2$ either in LP or in SP cells. In addition, CTA1 regulation in LP is likely controlled primarily by Skn7p, Yap1p, and Msn4p since the removal of these transcription factors rendered the cells almost as sensitive to H$_2$O$_2$ as those of the cta1Δ strain (Fig. 6A). In SP, by contrast, the regulation was more complex since the triple and quadruple mutants lacking three or four of the transcription factors (skn7Δ yap1Δ msn4Δ and skn7Δ yap1Δ msn2Δ msn4Δ strains), while being less resistant than the wt, were still able to grow at 200 mM H$_2$O$_2$, displaying a level of resistance well above that of the cta1Δ strain (Fig. 6B). This result suggests the possibility of additional regulators of CTA1 or an independent pathway to respond to H$_2$O$_2$.

Cta1p is not necessary for virulence in C. glabrata. Since Cta1p is entirely responsible for the extremely high level of resistance to H$_2$O$_2$ in vitro (Fig. 6), CTA1 is induced in macrophages (41), and CaCTA1 is required for virulence (56, 76), we investigated whether C. glabrata catalase plays a role during disseminated infection. Prior to the in vivo analysis, the parental and the mutant strains were made to express the Ura$^+$ phenotype by restoring URA3 at the URA3 locus to generate BG462 and CGM351 (see Materials and Methods). The two strains grew with identical doubling times in rich medium at 30 or 37°C (Fig. 7). To test for the virulence of the cta1Δ strain, we infected groups of 10 mice by tail vein injection using 2 × 10$^7$ cells of strain BG462 (wt expressing URA3) or CGM351 (cta1Δ URA3). Mice were sacrificed on day 7 after infection, and CFUs in kidneys, spleens, and livers were enumerated (Materials and Methods). The average numbers of CFU of the mutant strain and the wt strain recovered from the three organ types showed no difference (Fig. 8). The results of these experiments indicate that Cta1p is required in vitro but is dispensable in the murine disseminated-infection model.

**DISCUSSION**

Higher eukaryotes use ROS through the oxidative burst to eliminate invading pathogens (42, 57). During the coevolution of pathogens and their hosts, pathogens have coopted the antioxidant enzymes and molecules for normal ROS elimination to evade oxidative killing so that survival and persistence...
are ensured. *C. glabrata* is no exception; it possesses a defined genetic program to respond to oxidative killing by the host. *C. glabrata* adapts by reprogramming its gene expression, providing a clear advantage to this fungal pathogen upon infection. In this paper, we present evidence that the OSR in *C. glabrata* is controlled in part by the transcription factors Yap1p, Skn7p, Msn2p, and Msn4p. *C. glabrata* is resistant to extreme concentrations of H$_2$O$_2$, and this resistance is mediated in vitro by the single catalase Cta1p. This single catalase is, however, dispensable in vivo.

**Resistance and adaptation to H$_2$O$_2$.** Both the enzymes and the regulation of the OSR are well conserved among *S. cerevisiae*, *C. albicans*, and *C. glabrata*. Searching for genes involved in oxidative stress in the genome databases (http://www

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**FIG. 5.** ScCta1p and CgCta1p alignment. ScCta1p and CgCta1p are 85% similar across the entire lengths of the proteins. Identical residues are boxed and shaded. SKF (indicated by boxes and asterisks) is the peroxisomal targeting signal.

**FIG. 6.** Analysis of Cta1p in the OSR. Experiments with both LP and SP wt (BG14), cta1$\Delta$, yap1$\Delta$, skn7$\Delta$, msn2$\Delta$, and yap1$\Delta$ skn7$\Delta$ msn2$\Delta$ msn4$\Delta$ cells were performed as described in the legends to Fig. 1 and 2. See Materials and Methods.
yeastgenome.org, http://www.candidagenome.org, and http://cbi.labri.fr/Genolevures/elt/CAGL), we found that of 96 oxidative stress-related genes in *S. cerevisiae*, 67 are present in *C. glabrata* and 49 are present in *C. albicans*. Additionally, six oxidative stress-related genes are present only in *C. albicans*. In *S. cerevisiae*, the OSR is partly under the control of the well-studied transcription factors Yap1p, Skn7p, Msn2p, and Msn4p. These transcription factors have orthologs in *C. glabrata*, as follows: Skn7p (63% similar), Msn2p (41% similar), Msn4p (35% similar), and Yap1p (48% similar). From the results of our study, the roles of these transcription factors seem to be conserved as well.

*C. glabrata* was naturally resistant to higher levels of H$_2$O$_2$ than *C. albicans* and *S. cerevisiae* (Fig. 1 and 2). LP cells of *C. glabrata* were able to detect low levels of oxidant and induce a set of enzymes that would allow resistance to high levels and adaptation to the new environment. This response was mediated mainly by the transcription factors Skn7p and Yap1p but also by Msn2p and Msn4p (Fig. 3). This adaptation was present among cells of both *C. albicans* (Fig. 1) and *S. cerevisiae*, in which both catalases and the transcription factors Yap1p and Skn7p are required for this response (14, 17, 23, 27, 37, 39). Furthermore, Yap1p, Skn7p, and Msn4p coordinated the response to oxidative stress in LP cells, which required at least the activation and induction of the catalase gene (Fig. 3). The roles of Msn2p and Msn4p are interesting in two ways: first, CaMsn2p and CaMsn4p have no obvious role in oxidative stress (58), and second, ScMsn2p and ScMsn4p perform non-redundant functions depending on the stress (reviewed in references 20 and 55). In *C. glabrata* upon oxidative stress, these two transcription factors appeared to work independently of each other. Both were needed for SP resistance (see below), and Msn4p was required for LP resistance, along with Skn7p and Yap1p (Fig. 3 and 4).

**SP is important for resistance.** It has been shown previously that not only yeast but other microorganisms in SP are more resistant to oxidants than the same organisms in LP (38, 61, 62), and *C. glabrata* is no exception. *C. glabrata* in SP was naturally resistant to high levels of H$_2$O$_2$, up to 1,000 mM, compared to about 100 mM H$_2$O$_2$ for *S. cerevisiae* and 300 mM H$_2$O$_2$ for *C. albicans* (Fig. 2) (5, 9). This resistance was controlled by Msn2p, Msn4p, and Skn7p (Fig. 4), whereas Yap1p did not appear to have a central role. This high-level resistance suggests that *C. glabrata* has an extremely efficient Cta1p and it is probably able to repair efficiently the damage generated by the oxidant. It is possible that *C. glabrata* catalase activity may increase post-exponential phase. For example, *S. cerevisiae* catalase activity increases in SP (37). *C. albicans* shows growth phase-dependent resistance to H$_2$O$_2$ (38, 75), and the *C. albicans* Mn-SOD3 superoxide dismutase is induced in SP and is needed for the OSR (45). This high-level natural resistance to H$_2$O$_2$ may, in part, explain why *C. glabrata* is able to evade elimination by macrophages (41). Another clear advantage would be that *C. glabrata* can compete with H$_2$O$_2$-generating pathogens for specific niches inside the host. It has been shown previously that H$_2$O$_2$-producing bacteria inhibit the proliferation of *C. albicans* (75). Interestingly, CI of the nonpathogenic *S. cerevisiae* showed increased resistance to H$_2$O$_2$ relative to that of the reference strain (Fig. 1C and 2C). This result is consistent with the idea that pathogens, in order to survive, require a proper response to oxidative stress.
Catalase and virulence. The *C. glabrata* single catalase (Cta1p) was absolutely required to confer resistance on LP and SP cells in vitro. *cta1Δ* cells were extremely sensitive (Fig. 6) to H₂O₂. CgCta1p is the ortholog of the *S. cerevisiae* peroxisomal catalase (ScCta1p; 85% similar) (Fig. 5), which converts H₂O₂ formed by acyl coenzyme A oxidase (Pox1p) during fatty acid beta-oxidation (34) to H₂O and O₂ in the peroxisomal matrix. CgCta1p is a multifunctional 57-kDa protein classified as a group III catalase (42). It is interesting that *S. cerevisiae* has two catalases, cytoplasmic (Ctt1p) and peroxisomal (Cta1p), and that *S. cerevisiae* was about 10 times less resistant than *C. glabrata* (Fig. 2). Surprisingly, it has been shown previously that both catalases in *S. cerevisiae* (Ctt1p and Cta1p) are dispensable in growing cells and that glutathione compensates for the lack of the catalases (37). The fungal pathogens *C. albicans* and *C. glabrata*, though distantly related phylogenetically, show increased resistance to oxidative stress relative to that of *S. cerevisiae*.

The results presented in Fig. 6 suggest that in LP cells CTA1 may be controlled by the concerted actions of Yap1p, Skn7p, and Msn4p. The triple (yap1Δ skn7Δ msn4Δ) and quadruple (yap1Δ skn7Δ msn2Δ msn4Δ) mutants behaved exactly the same, rendering LP cells almost as sensitive as the *cta1Δ* strain (Fig. 6A). In fact, bioinformatic analyses of the CgCta1 promoter have previously revealed putative conserved cis-acting elements for each of these transcriptional regulators (reviewed in references 24, 32, and 36). In SP, however, both triple (yap1Δ skn7Δ msn4Δ) and quadruple (yap1Δ skn7Δ msn2Δ msn4Δ) mutants showed increased resistance to H₂O₂ relative to that of the reference strain W303. (Fig. 6B). These mutants could still grow at elevated levels of H₂O₂ (up to 200 mM). This finding would indicate that in SP but not in LP, other transcriptional regulators of the CTA1 gene besides Yap1p, Skn7p, Msn2p, and Msn4p are in play.

Is the catalase a virulence factor? (i) *cta1Δ* strains are extremely sensitive to H₂O₂ in vitro, (ii) CaCta1p is important for virulence (56, 76; reviewed in reference 10), and (iii) CTA1 is induced after phagocytosis (26, 41, 47). We therefore assayed *cta1Δ* cells in a mouse model of systemic infection. The experiment showed no difference in the colonization of the kidney, spleen, and liver by the *cta1Δ* strain (Fig. 8). This finding is in strong contrast with the results of the in vitro experiments, in which *cta1Δ* cells were extremely sensitive to H₂O₂. Our results suggest either that the catalase is not important in the OSR in vivo or that there are additional factors that may compensate for the lack of Cta1p in vivo. One possibility is that glutathione may mediate H₂O₂ resistance in vivo, since it has been shown previously that both catalases and glutathione provide an overlapping antioxidant defense system in *S. cerevisiae* (28). The results also suggest that these additional factors are silent in vitro. In fact, the *cta1Δ* strain was still able to adapt to oxidative stress in vitro, though at low levels of H₂O₂ (Fig. 6A). This finding indicates that there may be an additional catalase-independent pathway to respond to H₂O₂. Currently, we are working to identify these additional regulators/effectors of the OSR.

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REFERENCES

1. Aguirre, J. W., Hansberg, and R. Navarro. 2006. Fungal responses to reactive oxygen species. Med. Mycol. 44(Suppl.):101–107.
2. Aguirre, J. M. Rios-Momborg, B. Hewitt, and W. Hansberg. 2005. Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol. 13:1118.
3. Alarcoc, 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. 181:700–708.
4. Ainsworth, R. B., R. E. Kingston, D. D. Moore, J. A. Smith, and K. Struhl. 2001. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, NY.
5. Avery, A. M., and S. V. Avery. 2001. Saccharomyces cerevisiae expresses three phospholipid hydperoxide glutathione peroxidases. J. Biol. Chem. 276:33730–33735.
6. Brown, J. L., H. Bussey, and R. C. Stewart. 1994. Yeast Skn7p functions in a eukaryotic two-component regulatory pathway. EMBO J. 13:5186–5194.
7. Cabot, N. M., and P. C. Hanshaw. 1985. High-efficiency transformation of bacterial cells by electroporation. J. Bacteriol. 170:2796–2801.
8. Castano, L., R. Kaur, S. Pan, R. Cregg, A. De Las Penas, N. Guo, M. C. Bier, N. L. Craig, and B. P. Corman. 2003. Tn7-based genome-wide random insertional mutagenesis of Candida glabrata. Genome Res. 13:905–915.
9. Chauhan, N., D. Inglis, E. Roman, J. Pla, D. Li, J. A. Calera, and R. Calderone. 2003. Candida albicans response regulator gene SKN1 regulates a subset of genes whose functions are associated with cell wall biosynthesis and adaptation to oxidative stress. Eukaryot. Cell 2:1018–1024.
10. Chauhan, N., J. P. Latge, and R. Calderone. 2006. Signalling and oxidative adaptation in Candida albicans and Aspergillus fumigatus. Nat. Rev. Microbiol. 4:335–444.
11. Chen, H., T. Miyazaki, H. F. Tsai, and J. E. Bennett. 2007. The bZIP transcription factor Cpg1p is involved in multidrug resistance and required for activation of multidrug transporter gene CgFRL1 in Candida glabrata. Gene 386:63–72.
12. Clemons, K. V., J. H. McCusker, R. W. Davis, and D. A. Stevens. 1994. Comparative pathogenesis of clinical and nonclinical isolates of Saccharomyces cerevisiae. J. Infect. Dis. 169:859–867.
13. Cohen, G., W. Rapata, and H. Ruis. 1988. Sequence of the Saccharomyces cerevisiae CTA1 gene and amino acid sequence of catalase A derived from it. Eur. J. Biochem. 176:159–163.
14. Collinson, L. P., and I. W. Dawes. 1992. Inducibility of the response of yeast cells to peroxide stress. J. Gen. Microbiol. 138:329–335.
15. Cormack, B. P., and S. F. Fink. 1998. Efficient homologous transformation and illegitimate recombination in the opportunistic yeast pathogen Candida glabrata. Genetics 151:979–987.
16. Cyrne, L., L. Martinis, L. Fernandes, and H. S. Marinho. 2003. Regulation of antioxidant enzymes gene expression in the yeast Saccharomyces cerevisiae during stationary phase. Free Radic. Biol. Med. 34:385–393.
17. Davies, J. M., C. V. Lowry, and K. J. Davies. 1995. Transient adaptation to oxidative stress in yeast. Arch. Biochem. Biophys. 317:1–6.
18. De Las Penas, A., S. J. Pan, J. Castano, J. Alder, R. Cregg, and B. P. Corman. 2003. Virulence-related surface glycoproteins in the yeast pathogen Candida glabrata are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. Genes Dev. 17:2245–2250.
19. Domerque, R., I. Castano, A. De Las Penas, M. Zupanic, V. Lockatelli, J. R. Hebel, D. Johnson, and B. P. Corman. 2005. Nicotinic acid limitation regulates silencing of Candida adhesins during UTI. Science 308:866–870.
20. Estruch, F. 2000. Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol. Rev. 24:469–486.
21. Estruch, F., and M. Carlson. 1993. Two homologous zinc finger genes identified by multicopy suppression in a S. cerevisiae protein kinase mutant of Saccharomyces cerevisiae. Mol. Cell. Biol. 13:3872–3881.
22. Flattery-O’Brien, J., L. P. Collinson, and I. W. Dawes. 1993. Saccharomyces cerevisiae has an inducible response to menadione which differs from that to hydrogen peroxide. J. Gen. Microbiol. 139:501–507.
24. Foch-Mallol, J. L., A. Garay-Arroyo, F. Ledias, and A. A. Covarrubias-Robles. 2004. The stress response in the yeast Saccharomyces cerevisiae. Rev. Latinoam. Microbiol. 46:24–46. (In Spanish.)

25. Fonzi, W. A., and M. Y. Irwin. 1993. Inositol strain segregation and gene conversion in Candida albicans. Genetics 134:175–177.

26. Fradin, C., P. De Groot, D. MacCallum, M. Schaller, F. Klis, F. C. Odds, and B. Hube. 2005. Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. Mol. Microbiol. 56:397–415.

27. Gonzalez-Parraga, P., J. A. Hernandez, and J. C. Arguelles. 2003. Role of antioxidant enzymatic defences against oxidative stress (H2O2) and the acquisition of oxidative tolerance in Candida albicans. Yeast 20:1161–1169.

28. Grilli, C. M., S. G. Cummins, and L. D. W. Moreira. 2006. Glutathione and catalase provide overlapping defenses for protection against hydrogen peroxide in the yeast Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun. 235:893–898.

29. Gray, J. V., G. A. Petsko, G. C. Johnston, D. Ringe, R. A. Singer, and M. Werner-Washburne. 2004. “Sleeping beauty”: quiescence in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 68:187–206.

30. Hartig, A., and H. Ruis. 1986. Nucleotide sequence of the Saccharomyces cerevisiae CTT1 gene and deduced amino-acid sequence of yeast catalase T. Eur. J. Biochem. 160:487–490.

31. Hasan, R., C. Leroy, A. D. Isnard, J. Labarre, E. Boy-Marcotte, and M. B. Kuge, S., and N. Jones. 2002. Interactions of fungi with phagocytes: a bacterial view of the tuber–fungus relationship. Mol. Biol. Evol. 19:233–241.

32. Higgins, D. G., J. D. Thompson, and T. J. Gibson. 1996. CLUSTAL W: multiple sequence alignment with inter-active graphics and sequenceediting facilities. Nucleic Acids Res. 24:3383–3388.

33. Hiliounen, T., A. M. Mursula, H. Rottensteiner, R. K. Wierenga, A. J. Kastaniotis, and A. Gurvitz. 2003. The biochemistry of peroxisomal beta-oxidation in the yeast Saccharomyces cerevisiae. FEBS Microbiol. Rev. 27:35–64.

34. Hwang, C. S., G. E. Rhie, J. H. Oh, W. K. Huh, H. S. Yim, and S. O. Kang. 2002. Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of Candida albicans against oxidative stresses and the expression of its full virulence. Microbiology 148:3705–3713.

35. Ilner, A., and K. Shiozaki. 2005. Yeast signaling pathways in the oxidative stress response. Mutat. Res. 59:13–27.

36. Izawa, S., Y. Inoue, and A. Kimura. 1996. Importance of catalase in the adaptive response to hydrogen peroxide: analysis of acatalasemic Saccharomyces cerevisiae. Biochem. J. 320:61–67.

37. Jamieson, D. J. 1992. Saccharomyces cerevisiae has distinct adaptive responses to both hydrogen peroxide and menadione. J. Bacteriol. 174:6678–6681.

38. Jamieson, D. J., S. L. Rivers, and D. W. Stephen. 1994. Analysis of Saccharomyces cerevisiae proteins induced by peroxide and superoxide stress. Microbiology 140:3277–3283.

39. Kasur, R., R. Dominguez, M. L. Zapancic, and P. B. Cormack. 2005. A yeast by any other name: Candida glabrata and its interaction with the host. Microbiol. Mol. Biol. Rev. 69:1089–1112.

40. Krems, B., C. Charizanis, and K. D. Entian. 1996. The response regulator-like protein Poa/Skn7 of Saccharomyces cerevisiae is involved in oxidative stress resistance. Curr. Genet. 29:327–334.

41. Kuge, S., and N. Jones. 1997. YAP1 dependent activation of TRX2 is essential for the response of Saccharomyces cerevisiae to oxidative stress by hydrogenperoxides. EMBO J. 16:1035–1044.

42. Morgan, B. A., G. R. Banks, W. M. Toone, D. Raitt, S. Kuge, and L. H. Johnston. 1997. The Skn7 response regulator controls gene expression in the oxidative-stress response of the budding yeast Saccharomyces cerevisiae. EMBO J. 16:1035–1044.

43. Morgan, B. A., N. Bouquin, G. F. Merrill, and L. H. Johnston. 1995. A yeast transcription factor bypassing the requirement for SBF and DSC1/MFB in budding yeast has homology to bacterial signal transduction proteins. EMBO J. 14:5679–5689.

44. Moyer-Rowley, W. S. 2002. Transcription factors regulating the response to oxidative stress in yeast. Antioxid. Redox Signal. 4:125–140.

45. Nakagawa, Y., T. Kanbe, and I. Mizuguchi. 2003. Disruption of the human pathogenic yeast Candida albicans catalase gene decreases survival in mouse models of infection and toxicants. J. Bacteriol. 185:3356–3361.

46. Lee, J., C. Godon, G. Lagniel, D. Spector, J. Garin, J. Labarre, and M. B. Babcook, A. J. Brown. 2004. Dual targeting of yeast catalase A to peroxisomes and mitochondria. Biochem. J. 380:393–400.

47. Petrou, V. Y., D. Drescher, A. V. Kujundzicvea, and M. J. Schmitt. 2004. “Sleeping beauty”: quiescence in Candida albicans: a persistent public health problem. Clin. Microbiol. Rev. 20:133–163.

48. Schenberger-Frascino, A. 1972. Lethal and mutagenic effects of elevated temperature on haploid yeast. II. Recovery from thermolosions. Mol. Gen. Genet. 117:239–253.

49. Schenberger-Frascino, A., and E. Moustacchi. 1972. Lethal and mutagenic effects of elevated temperature on haploid yeast. I. Variations in sensitivity during the cell cycle. Mol. Gen. Genet. 115:243–257.

50. Schmitt, A. P., and K. McEntee. 1996. MnS2p, a zinc finger DNA-binding protein, is the transcriptional activator of the multistress response in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 93:5775–5782.

51. Schnell, N., B. Krems, and K. D. Entian. 1992. The PAR1 (YAP1/SNQ3) gene of Saccharomyces cerevisiae, a c-jun homologue, is involved in oxygen metabolism. Curr. Genet. 21:269–273.

52. Seah, T. C., and J. G. Kaplan. 1973. Purification and properties of the catalase of bakers' yeast. J. Biol. Chem. 248:2889–2893.

53. Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

54. Singh, P., N. Chauhan, A. Ghosh, F. Dixon, and R. Calderone. 2004. SKN7 of Candida albicans: mutant construction and phenotype analysis. Infect. Immun. 72:2390–2394.

55. Temple, M. D., G. G. Perrone, and I. W. Dawes. 2005. Complex cellular responses to reactive oxygen species. Trends Cell Biol. 15:319–326.

56. Thorpe, G. W., C. S. Fong, N. Alic, V. J. Higgins, and I. W. Dawes. 2004. Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. Proc. Natl. Acad. Sci. USA 101:6556–6569.

57. Toda, T., M. Shimannuki, and M. Yanagida. 1991. Fission yeast genes that confer resistance to staurosporine encode an AP1-like transcription factor and a protein kinase related to the mammalian ERK1/ERK2 and budding yeast FUS3 and KSS1 kinases. Genes Dev. 5:560–73.

58. Tracey, A., T. Bilinski, J. Litwinska, M. Skoneczny, and J. Rytd. 1985. Catalase T deficient mutants of Saccharomyces cerevisiae. Acta Microbiol. Pol. 34:231–245.

59. Traczyk, A., T. Bilinski, J. Litwinska, M. Skoneczny, and J. Rytka. 1997. Catalase A from Candida albicans: a persistent public health problem. Acta Med. Ent. 103:1569–1576.

60. Traczyk, A., T. Bilinski, J. Litwinska, M. Skoneczny, and J. Rytka. 1997. Catalase A from Candida albicans: a persistent public health problem. Acta Med. Ent. 103:1569–1576.

61. Traczyk, A., T. Bilinski, J. Litwinska, M. Skoneczny, and J. Rytka. 1997. Catalase A from Candida albicans: a persistent public health problem. Acta Med. Ent. 103:1569–1576.
ditioned medium protects yeast cells from oxidative stress: a possible link between quorum sensing and oxidative stress resistance. Eukaryot. Cell 4:1654–1661.
76. Wysong, D. R., L. Christin, A. M. Sugar, P. W. Robbins, and R. D. Diamond. 1998. Cloning and sequencing of a Candida albicans catalase gene and effects of disruption of this gene. Infect. Immun. 66:1953–1961.

77. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119.
78. Zhang, X., M. De Micheli, S. T. 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.