We report here that in addition to a cytoplasmic copper-zinc-containing superoxide dismutase (SOD) and a mitochondrial manganese-containing SOD, *Candida albicans* expresses a third SOD gene (SOD3). The deduced amino acid sequence contains all of the motifs found in previously characterized manganese-containing SODs, except the presence of a mitochondrial transit peptide. Recombinant Sod3p expressed and purified from *Escherichia coli* is a homotetramer with a subunit mass of 25.4 kDa. Mass absorption spectrometry detected the presence of both iron and manganese in purified Sod3p but, as determined by metal replacement experiments, the enzyme displays activity only when bound to manganese. Overexpression of *SOD3* was shown to rescue the hypersensitivity to redox cycling agents of a *Saccharomyces cerevisiae* mutant lacking the cytoplasmic copper-zinc-containing SOD. Northern blot analyses showed that the transcription of *SOD3* is induced neither by the transition from the yeast to the mycelial form of *C. albicans* nor by drug-induced oxidative stress. In continuous cultures, the expression of *SOD3* was strongly stimulated upon the entry and during the stationary phase, concomitantly with the repression of *SOD1*. We conclude that Sod3p is an atypical cytosolic manganese-containing superoxide dismutase that is involved in the protection of *C. albicans* against reactive oxygen species during the stationary phase.

*Candida albicans* is a lifelong commensal of the human gastrointestinal tract and vaginal mucosa, and among commensals, *Candida* is an alert opportunist. No ill effect normally results from colonization, but subtle defects in host defenses lead to infection. Its ability to be maintained as commensal involves (i) resistance to the yeast to the mycelial form of *C. albicans* nor by drug-induced oxidative stress. In continuous cultures, the expression of *SOD3* was strongly stimulated upon the entry and during the stationary phase, concomitantly with the repression of *SOD1*. We conclude that Sod3p is an atypical cytosolic manganese-containing superoxide dismutase that is involved in the protection of *C. albicans* against reactive oxygen species during the stationary phase.

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sorption of the SOD1 and SOD3 genes. Because the SOD3 gene is strongly induced upon and during the stationary phase, we propose that this gene may be part of a well orchestrated and distinct defense mechanism for survival in nutrient-limiting conditions.

**Experimental Procedures**

**Yeast and Bacterial Strains**—C. albicans ATCC 32354 was used for the construction of the genomic library (10) and plasmid constructions. The C. albicans strain SC5314 was used for the SOD1 and SOD3 transcriptional studies. The C. cerevisiae strains used, all purchased from ResGen (Invitrogen Corporation), were: BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0), BY4741 sod1Δ (same as BY4741 except sod1Δ::KAN), and BY4742 sod2Δ (MATa his3Δ1 leu2Δ0 by2Δ0 ura3Δ0 sod2Δ::KAN). The Escherichia coli strains MC1061 and M15(RepE) were used for standard recombinant DNA work and the heterologous expression of SOD3, respectively.

**Media and Growth Conditions**—The C. albicans blastospores were routinely cultured at 28 °C in Iscove's modified Dulbecco's medium (IMDM) supplemented with glucose as described previously (11). For the Northern analyses performed with the yeast and mycelial forms of C. albicans, blastospores were first grown overnight at 28 °C in the following media: 1% yeast extract, 2% peptone, and 2% glucose (YPD); YPD supplemented with 2% galactose (YPGal), or 3% glycerol (YPG) were diluted to an A600 of 0.1 and incubated for 60 h at 30 °C in the same media. For the expression of the C. albicans sod genes in response to induced oxidative stresses, a preculture of C. albicans blastospores grown in YPD was diluted to an A600 of 0.1 and incubated for 24 h at 30 °C in the same media. Aliquots were removed every 2 h and further incubated for 1 h at the same temperature in YPD or in YPD supplemented with either hydrogen peroxide (0.4 mM), menadione (0.5 mM), or paraquat (0.5 mM).

The C. albicans strains were grown at 30 °C in either YPD or, when transformed, in synthetic complete medium lacking uracil (SC-ura) as described (12). The E. coli strains were cultured in 2YT medium containing 0.2% glucose supplemented with antibiotics as required (13).

**DNA Manipulations and Transformations**—Screening of the C. albicans genomic library was done as described by Hanahan and Meselson (14) using a radioactive 397-hp EcoRV DNA fragment derived from plasmid p4E1 that was labeled as described previously (10).

For the 5'-rapid amplification of cDNA ends analysis, C. albicans blastospores grown in IMDM at mid-log phase were harvested by centrifugation (4000 × g). Purification of total RNA was performed according to the procedure described by Koller and Domdey (15). The first strand cDNA synthesis was obtained by reverse transcription of the total RNA with the SuperScript II RNase H- reverse transcriptase (Life Technologies, Inc.) and the oligonucleotide RISO3D following the manufacturer's instructions. All of the oligonucleotides used in this study were purchased from Life Technologies, Inc., and their sequences are presented in Table 1. The single-stranded cDNA was then tailed at the 3'-end by the terminal deoxynucleotidyl transferase (New England Biolabs Inc.) and 0.05 mM EDTA and 0.05 M NaCl, and the rate of O2 uptake was measured at 37 °C calibrated with the following gel filtration calibration molecular mass standards (Sigma): bovine gamma globulin (158 kDa), chicken ovalbumin (44 kDa), equine myoglobin (17.5 kDa), and vitamin B12 (1.4 kDa). The resulting tailed cDNA was submitted to a first polymerase chain reaction (PCR) with the Tag DNA polymerase (Life Technologies, Inc.) and the oligonucleotides RISO3D and AAP as primers. A second round of PCR was performed with a 100-fold dilution of the amplicons generated during the first PCR, but this time using the oligonucleotides R2SOD3 and AUAP as primers. The thermal cycling conditions for both PCR were the following: 94 °C for 2 min; then 94 °C for 1 min and 72 °C for 3 min for 40 cycles (72 °C for 10 min at the last cycle); and 4 °C until use. Direct automatic DNA sequencing of the resulting amplicon was done as described above, except that the Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) and the AP primer were used. The single-stranded cDNA obtained was next amplified by PCR using the oligonucleotides AUAP and AAP as primers, and following a 150-fold dilution of the reaction mixture, a second round of PCR was performed using the oligonucleotides FSOD3 and RSO3D. In each case the thermal cycling conditions were: 94 °C for 2 min; then 94 °C for 1 min and 60 °C for 1 min; 72 °C for 2 min for 35 cycles (72 °C/10 min at the last cycle); and 4 °C until use. The resulting amplicon was then cut with BamHI and ligated to the BamHI-digested plasmids pQE30 (Qiagen) and pTV102U (16) to generate pSOD1r and pVTSOD3, respectively. In pSOD1r, a His-tag coding sequence provided by pQE30 flanks the 5'-end of the SOD3 coding sequence. The LacI repressor regulates expression of the chimeric gene in pSOD1r, whereas pVTSOD3 provides constitutive expression through the strong ADH promoter.

Northern analyses and radiolabeling of the probes were performed as described previously (10). For standard recombinant DNA work and the expression of SOD3, respectively, along with cDNA clone as template (this study).

**Expression and Purification of the Sod3 Recombinant Protein**—E. coli M15(RepE) cells transformed with pSOD1r were grown in 2YT medium supplemented with 100 μg ml−1 ampicillin and 25 μg ml−1 kanamycin to early log phase (A600 of 0.6) at 37 °C with vigorous shaking. The expression of SOD3 was then triggered either by the addition 1 mM isopropanol-thiogalactopyranoside (IPTG) followed by a further 5-h incubation (long induction) or by the addition of 0.1 mM EDTA, 1 mM MgCl2, and 0.1 mM IPTG and a 15-min incubation (short induction). All of the purification steps were then performed at 4 °C. The cells were harvested at 4,000 × g for 20 min and resuspended in 10 ml of 50 mM Tris-HCl buffer, pH 7.8, 300 mM NaCl, and 20 mMimidazole. They were lysed by three passages through a French pressure cell (Amicon) at 8000 p.s.i. The cell lysate was centrifuged at 10,000 × g for 30 min, and the recombinant protein was purified with 5 ml of an 50% Ni-NTA suspension of nickel-nitrilotriacetic acid resin has been described by the manufacturer (The QiAexpression; Qiagen). The unbound material was removed by 10 washes with 10 ml of 50 mM Tris-HCl buffer, pH 7.8, 300 mM NaCl, and 20 mM imidazole. The bound recombinant Sod3 fusion protein was eluted with 2 × 1 ml of 50 mM Tris-HCl buffer, pH 7.8, 300 mM NaCl, and 250 mM imidazole. The protein preparation was then dialyzed against 4 liters of 50 mM potassium phosphate, pH 7.8 for 16 h at 4 °C and stored for up to 3 months at −20 °C at 4 °C.

In Vitro and in Vivo Superoxide Dismutase Assays—Superoxide dismutase activity of the purified Sod3pr was assayed by the nitroblue tetrazolium method of Beauchamp and Fridovich (17). For the in vivo assay, 100 μl of the indicated S. cerevisiae strains grown overnight in SC-ura medium were mixed with 8 ml of molten top agar (0.7% w/v agar in SC-ura medium) maintained at 42 °C. The top agar was rapidly overlaid onto SC-ura plates and allowed to solidify. Sterile filter discs were deposited on the plates, and 10 μl of the indicated concentrations of either menadione or paraquat were spotted onto the filter discs. The plates were then incubated at 30 °C for 48 h.

**Metal Content and Reconstitution of SODs with Other Metals**—The metal content of the purified Sod3pr enzyme was determined by atomic absorption spectrophotometry. The purified Sod3pr enzyme and commercial preparations of the E. coli FeSOD and MnSOD (Sigma Chemical Co.) used as controls were treated essentially as described by Gregory and Dapper (18) for metal removal and replacement.

**Miscellaneous Methods**—The molecular mass of the native enzyme was determined by gel filtration on a Superdex 75 HR 10/30 (Amersham Pharmacia Biotech) and eluted with 50 mM Tris-HCl, pH 7.5, 150 mM NaCl (same as BY4741 except sod1Δ::KAN) at a flow rate of 0.9 ml/min. The column was calibrated with the following gel filtration calibration molecular mass markers (Sigma): bovine gamma globulin (158 kDa), chicken ovalbumin (44 kDa), equine myoglobin (17.5 kDa), and vitamin B12 (1.4 kDa). Subunit molecular mass was estimated by 12% SDS-PAGE in denaturing conditions as described previously (10). The protein concentration was measured by the method of Bradford (19).
RESULTS

Identification and Sequence Analysis of a Third SOD Gene from C. albicans—The CSA1 gene, which encodes an abundant mycelial surface antigen in C. albicans (11), was recently sequenced from a 7.4-kilobase genomic DNA fragment cloned into plasmid YEp24 (10). Further sequencing reactions of this plasmid revealed the presence of an incomplete open reading frame in the opposite direction relative to CSA1. This open reading frame encodes a putative protein with sequence similarity to members of the manganese-containing SOD enzyme family. Because the recently characterized C. albicans cytosolic Cu/ZnSOD (7) and mitochondrial MnSOD (8) were named Sod1p and Sod2p, respectively, we designated this novel gene and the corresponding enzyme as SOD3 and Sod3p.

The full-length sequence of this gene was completed with plasmid pSOD3, obtained through colony hybridization of a C. albicans genomic library with a radiolabeled 397-bp EcoRV fragment derived from p4E1 (Fig. 1). The complete 678-bp open reading frame is composed of two exons encoding, respectively, 35- and 171-amino acids segments separated by a 60-bp intron positioned between nucleotides 106 and 165. Typical fungal consensus sequences (20) described for the 5' and 3' intron/exon boundaries, along with the splice signal for lariat formation (WRCRTAC) were identified in the intervening sequence (Fig. 1). Splicing of the intron at these proposed sites was verified by 5'-rapid amplification of cDNA ends analysis. Direct sequencing of the resulting amplicon gave the sequence 5'-CAAACACC-3', hence confirming that nucleotides 105 and 166 were joined together in the cDNA.

This analysis also identified the transcriptional start site that is located 9 bp upstream from the initiator ATG codon.

The deduced amino acid sequence of SOD3 is a 206-residue protein with a molecular mass of 22.7 kDa (Fig. 1). The consensus pattern DXXWEHXXY (i.e. Asp170 to Tyr177 in Sod3p) and the four characteristic residues involved in metal binding (i.e. His32, His81, Asp170, and His174) typical of the Mn/FeSOD family are present within Sod3p (21–23). Parker and Blake (21) identified critically positioned amino acids that allow iron- and manganese-containing SODs to be distinguished based on their primary structure. The amino acids Gly75, Gly76, Gln155, and Asp156 found in Sod3p conform to the signature sequence for the MnSOD subfamily, suggesting that this third C. albicans SOD requires manganese at its catalytic site for activity.

At the amino acid level, Sod3p displays significant sequence identity with several members of the eukaryotic MnSODs and bacterial MnSOD and FeSOD but is most closely related to the C. albicans Sod2p (63% identity) and S. cerevisiae Sod2p (60% identity) (Fig. 2). In sharp contrast to the C. albicans Sod2p and S. cerevisiae Sod2p enzymes, however, as well as to most eukaryotic MnSOD enzymes characterized so far, Sod3p does not possess an amino-terminal extension of 30 residues (transit peptide) that normally targets the enzyme to the mitochondria. It therefore predicts that the enzyme will be sequestrated within the cytosol.

Purified Sod3p Is a Tetrameric Enzyme That Requires Manganese for Activity—To biochemically characterize the C. albicans SOD3 gene product, its cDNA was cloned into the bacterial expression vector pQE30 (see “Experimental Procedures”).
The resulting plasmid (pSOD3r) expresses a histidine-tagged Sod3 enzyme, and a large amount of it (10–100 mg/liter) was purified in its native form by a single affinity chromatography step on nickel-nitrioltriacetic acid resin. When analyzed by SDS-PAGE under denaturing conditions, the highly purified preparation migrated as a single band of 25.4 kDa, in agreement with the predicted molecular mass of 24.1 kDa for the His-tagged modified Sod3p subunit (Fig. 3, inset). The molecular mass of the native enzyme was also evaluated by gel filtration (Fig. 3). The elution profile showed a prominent, nearly symmetrical, peak of ~85 kDa and a minor peak of very low molecular mass. This is therefore consistent with a homotetrameric structure for native Sod3p, a result that further extends the similarity between the C. albicans Sod2 and Sod3 enzymes to their quaternary structure (8).

A low level of dismutase activity was measured in initial experiments where Sod3p was purified from E. coli recombinants cultured in 2YT medium and induced for 5 h with IPTG (Table II). Consistent with its requirement for Mn$^2+$ at the catalytic site, a nearly 7-fold increase in specific activity was observed (~2100 units/mg versus ~350 units/mg; Table II) when Sod3p was purified from E. coli recombinants cultured in a manganese-supplemented medium and induced for a shorter period with IPTG (15 min). Atomic absorption spectrometry performed on these two enzyme preparations, while indicating the presence of both Fe$^2+$ and Mn$^2+$ within Sod3p, also revealed a positive correlation between the manganese/iron ratio and the activity (the greatest manganese/iron ratio was found in the most active enzyme preparation). Finally, and in further support that Sod3p belongs to the MnSOD family, the activity of Sod3p was not affected by the presence of known inhibitors of either bacterial FeSODs (hydrogen peroxide) or Cu/ZnSODs (potassium cyanide). However, because neither enzyme preparation was filtered exclusively with Fe$^{3+}$ ions, these results could not rule out the possibility that Sod3p is a member of the so-called cambialistic SODs. These enzymes represent a special group of Mn/FeSODs that are active, albeit at variable levels, with either metal ion present at their active site (24). We directly tested this possibility by first denaturing Sod3p in the presence of a chelator to generate apoSod3p and subsequently allowed the enzyme to refold in the presence of either metal cofactor (Table III). As expected, the apo form of Sod3p, as well as bacterial apoMnSOD and apoFeSOD used as controls, was totally inactive. When renatured in the presence of Fe$^{2+}$, Sod3p displayed a very low background activity (~130 units/mg). This activity was similar to, although somewhat lower than, that measured for the E. coli apoMnSOD reconstituted with Fe$^{2+}$ (~310 units/mg). In contrast, when apoSod3p was refolded in the presence of Mn$^{2+}$, the activity of the reconstituted enzyme was significantly higher (~3900 units/mg) than the native enzymes purified from E. coli (~350 and ~2000 units/mg; Table II). Similarly, the E. coli apoMnSOD and apoFeSOD reconstituted with their corresponding metal cofactor were more active than the commercial preparations. Collectively, we concluded from these experiments that the C. albicans Sod3p is a bona fide manganese-containing SOD, active as a tetramer, that is predicted to reside rather unusually into the cytosolic compartment.

![Fig. 2. Alignment of the amino-terminal amino acid sequences of the C. albicans and S. cerevisiae MnSODs.](http://www.jbc.org/figure/2)

![Fig. 3. Gel filtration chromatography and SDS-PAGE analysis of the Sod3r enzyme purified from E. coli transformants.](http://www.jbc.org/figure/3)
sion of the \textit{C. albicans} SOD3 cDNA did not alleviate the known paraquat hypersensitivity of \textit{sod2Δ} yeast (26). These data are thus consistent with the predicted localization of Sod3p. They also indicate that the presence of either a MnSOD or a Cu/ZnSOD within the cytosol similarly protects the cell upon induced oxidative stresses.

\textit{C. albicans} SOD1 and SOD3 Genes Are Divergently Expressed in Nutrient-limiting Conditions and upon Induced Oxidative Stress—The existence in \textit{C. albicans} of two cytoplasmic SOD enzymes that, based on functional assays in \textit{S. cerevisiae} mutants, appear to have redundant roles in the detoxification of ROS prompted us to investigate the expression of the corresponding genes under various growth conditions. An important aspect of \textit{C. albicans} physiology is its dimorphic nature, being able to grow as and to switch to a yeast or a mycelial form. Numerous factors, including the temperature, the pH level, the growth phase, and the presence of serum are known to favor or trigger the transition from the yeast to the hyphal form. To test whether the expression of \textit{SOD1} or \textit{SOD3} is associated with a particular morphological form, we performed a series of Northern hybridizations with total RNA prepared from \textit{C. albicans} growing in various culture media at 28 and 37 °C. As a control for the temperature shift, \textit{C. albicans} were also grown in YPD medium where the yeast form was either the exclusive (28 °C) or the predominant (37 °C) species observed (Fig. 5B). In this medium \textit{SOD3} was expressed at both temperatures, whereas the transcription of \textit{SOD1} was largely repressed (Fig. 5A). When cultured in YPD supplemented with serum (YPDS), the opposite was observed. The \textit{C. albicans} \textit{SOD1} gene was strongly expressed at both 28 and 37 °C, whereas the \textit{SOD3} mRNA was not detected at 28 °C and present albeit at reduced level at 37 °C when compared with that observed in YPD. Because hyphal structures were present at 37 °C exclusively (Fig. 5B), this provided a first indication that the growth conditions (i.e., nature of the culture medium) rather than the transition from the yeast to the mycelial phase per se greatly influenced the expression of these two \textit{C. albicans} \textit{SOD} genes. This was further supported by the expression profile observed in Lee’s medium. In this medium, \textit{C. albicans} rapidly switched from the yeast to the mycelial form upon raising the temperature from 28 to 37 °C under both acidic (pH 4.5) and nearly neutral (pH 6.5) conditions (Fig. 5B). Yet, only the \textit{SOD1} gene was expressed under all conditions (Fig. 5A). For \textit{C. albicans} grown in IMDM pH 6.5 medium, only the \textit{SOD1} mRNA was detected at 28 °C (predominantly the yeast form). The transition from the yeast to the mycelial form induced by the temperature shift (37 °C) was accompanied by a slight induction in the transcription of \textit{SOD3}, a sharp decrease in the \textit{SOD1} mRNA level, and the accumulation of a novel mRNA species hybridizing with the \textit{SOD1} probe. The nature of this mRNA, larger in size than the \textit{SOD1} mRNA, is at present not clear but could be derived from the transcriptional induction of a \textit{SOD1} homologous gene whose existence was revealed by the completed genome sequence of \textit{C. albicans}. More importantly, our data indicated that neither of the \textit{SOD} genes is specifically regulated by the dimorphism. Because the \textit{SOD1} and \textit{SOD3} genes were rarely expressed or repressed together, it also suggested that their transcriptional regulation may be oppositely coordinated. To further substantiate this hypothesis, we studied their expression profile in different metabolic conditions as well as following various oxidative stresses.

Northern hybridizations performed with total RNA extracted at different time points in glucose-grown (YPD) \textit{C. albicans} did not alleviate the known paraquat hypersensitivity of \textit{sod2Δ} yeast (26). These data are thus consistent with the predicted localization of Sod3p. They also indicate that the presence of either a MnSOD or a Cu/ZnSOD within the cytosol similarly protects the cell upon induced oxidative stresses.

### Table II

| Source | Enzyme | Activity (units/mg protein) | Inhibition | Metal content (mol metal/mol protein) |
|--------|--------|----------------------------|------------|----------------------------------------|
|        |        |                            | 5 mm H₂O₂ | 2 mm KCN |
|        |        |                            | %          |          |
| \textit{C. albicans} | Sod3r (LI) | 370 | ND<sup>a</sup> | 0.4 |
|        | Sod3r (SI) | 2240 | 7 | 0.9 |
|        | FeSOD | 4840 | 10 | 0.9 |
|        | MnSOD | 4570 | ND | ND |
| \textit{E. coli} | ApoFeSOD | 80 | ND | ND |
|        | FeSOD | 405 | ND | ND |

<sup>a</sup> LI, long induction with IPTG (5 h); SI, short induction with IPTG (0.25 h).

<sup>b</sup> ND, not determined.

### Table III

| Source | Enzyme | Specific activity (units/mg of protein) |
|--------|--------|----------------------------------------|
| \textit{C. albicans} | Sod3r | 355 |
|        | ApoSod3r | 135 |
|        | ApoSod3r + Fe<sup>2+</sup> | 3925 |
|        | ApoSod3r + Mn<sup>2+</sup> | 4155 |
| \textit{E. coli} | MnSOD | 315 |
|        | FeSOD | 1235 |
|        | FeSOD + Fe<sup>2+</sup> | 4495 |
|        | FeSOD + Mn<sup>2+</sup> | 5760 |

<sup>a</sup> The native \textit{C. albicans} Sod3r enzyme expressed in \textit{E. coli} and the commercial \textit{E. coli} MnSOD and FeSOD were denatured to generate the Apo forms (e.g., ApoSod3r) and reconstituted with either Fe<sup>2+</sup> or Mn<sup>2+</sup>.

<sup>b</sup> The specific activity shown is the average of triplicate samples except for the ApoMnSOD + Mn<sup>2+</sup> sample (duplicate). In all cases the standard deviation did not exceed 10% of the value.

<sup>c</sup> NDA, no detectable activity.

Fig. 4. Heterologous expression of the \textit{C. albicans} SOD3 gene in \textit{S. cerevisiae}. \textit{S. cerevisiae} wild type cells (WT) and mutants lacking either the copper-zinc superoxide dismutase (sod1Δ) or the manganese superoxide dismutase (sod2Δ) expressing (pVTsod3Δ) or not (pVT) the \textit{C. albicans} SOD3 cDNA were assayed for hypersensitivity to the indicated concentrations of menadione and paraquat (see “Experimental Procedures”). Yeast killing was assessed by the presence of a clear zone surrounding the disc.

<sup>2</sup> C. Lamarre, J.-D. LeMay, N. Deslauriers, and Y. Bourbonnais, unpublished data.
albicans cultures detected the presence of SOD1 mRNA at the earliest time point considered (2 h), but its abundance was maximal after 8 h of growth (Fig. 6B). As the cells entered the slow growth phase (by ~12 h of growth; Fig. 6A), the expression of SOD1 was gradually repressed, and no SOD1 mRNA could be detected beyond 16 h of growth. Concomitant to this repression of SOD1, the transcription of SOD3 was induced at 12 h and maintained as the cells entered the stationary phase. This profile of expression was not specific to glucose-grown cultures because nearly superimposable patterns of expression were observed when C. albicans was grown in an alternative fermenting (galactose) or a nonfermenting (glycerol) carbon source (Fig. 6). Hence nutrient limiting conditions rather than the respiratory chain seem to regulate both the transcriptional repression of SOD1 and the induction of SOD3.

In many organisms, including the yeast S. cerevisiae (27, 28), the gene encoding the cytoplasmic SOD is transcriptionally stimulated by treatment with various oxidative agents such as hydrogen peroxide, menadione, and paraquat. The expression of the SOD1 and SOD3 genes in response to oxidative stresses was studied with C. albicans cells taken at different time points in the growth phase (Fig. 7). Control samples taken at 4, 8, 18, and 22 h of growth in glucose medium (YPD) were representative of that observed previously (Fig. 6B); SOD1 mRNA was the only species detected at 4 h, both SOD1 and SOD3 mRNAs were present after 8 h, and only the SOD3 mRNA was observed at later time points (18 and 22 h). In all treated samples (i.e., hydrogen peroxide, menadione, and paraquat), and irrespective of the age of the culture, the oxidative stress stimulated the transcription of SOD1 (Fig. 7). At later time points (18 and 22 h), this was accompanied by the transcriptional repression of SOD3. Remarkably, the decrease in SOD3 mRNA paralleled in magnitude the relative increase in SOD1 mRNA. These results, along with the expression profiles

FIG. 5. Expression of the SOD1 and SOD3 genes in the yeast and hyphal forms of C. albicans. A, Northern blot analysis of the SOD1 and SOD3 transcripts in C. albicans strain SC5314 grown for 16 h in the indicated media at 28 °C (28) and 37 °C (37). The ethidium bromide-stained 25 and 18 S ribosomal RNAs are also shown as loading control. B, C. albicans cells grown for 16 h in the indicated media, and temperatures were visualized with Nomarski optics (400×).

FIG. 6. Expression of the SOD1 and SOD3 genes in continuous cultures at 28 °C. A, growth curve, expressed in absorbance units at 600 nm (A600) of the C. albicans strain SC5314 in YPD (glucose), YPGal (galactose), and YPG (glycerol) media. B, Northern blot analysis of the SOD1 and SOD3 transcripts in the continuous cultures of C. albicans shown in A. The ethidium bromide-stained 25 and 18 S ribosomal RNAs are also shown as loading control.
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The expression of the SOD1 and SOD3 genes under various
growth conditions rather suggested that C. albicans, by coor-

dinating inversely the transcription of these genes, ensures a
constant presence of superoxide dismutase activity within the
cytosol. In this hypothesis the differential expression of
SOD1 and SOD3 would be dictated by the availability of the metal
ions (Cu2+, Zn2+, and Mn2+) and/or the need for the presence of a
specific metal scavenger. The S. cerevisiae SOD1 gene was
shown to be essential for the stationary phase survival (4). In
C. albicans, SOD1 is repressed concomitantly to the induction
of SOD3 upon the entry and during the stationary phase (Fig.
6), indicating that in C. albicans, it is the expression of an usual
cytoplasmic MnSOD that confers antioxidant protection during
these growth phases. Based on this and previous studies, sev-

eral lines of evidence suggest that this switch from SOD1 to
SOD3 expression reflects: (i) the requirement for increased
protection against both ROS and copper toxicity during the
stationary phase and (ii) the difference between S. cerevisiae
and C. albicans in the mechanisms involved in the homeostatic
control of copper (see below).

Consistent with the requirement for an added protection
against the deleterious effect of copper in stationary phase
cells, a recent genome-wide expression profiling study con-
ducted in S. cerevisiae revealed that the entry into the station-
ary phase was accompanied by a strong induction of the genes
involved in copper resistance (e.g. ACE1 and CUP1) and by the
down-regulation of the genes implicated in copper uptake (e.g.
CTR1 and CTR3) (28). Recent works performed with the fila-
mentous yeast Podospora anserina provided direct evidence
that copper is implicated in the aging process (32, 33). A mu-
tant (grisea) defective in copper uptake survived nearly twice
as long as wild type P. anserina in continuous cultures.

Furth-

ermore, a similar extension in life span was observed when
wild type P. anserina was cultured in copper-depleted media.
In C. albicans, an added protection against copper toxicity
during the stationary phase provides an explanation for the
repression of the SOD1 gene and the induction of an alternate
cytoplasmic SOD activity.

In S. cerevisiae the main defense mechanism against copper
toxicity is the expression of copper scavengers such as the
metallothionein encoded by CUP1 and the Sod1 enzyme, which
was shown to suppress the copper toxicity phenotype of a cup1Δ
mutant (34). Consistent with this role of Sod1p in copper buff-
ering, its gene is induced by the Ace1p copper sensor, a DNA
transactivator (25). In contrast, despite possessing an ortho-
lolog of CUP1 (CaCUP1), the main defense mechanism of C. albicans
against copper appears to rely predominantly on the copper-
transporting P-type ATPase encoded by the CaCRP1/CaCRD1
gene (35, 36). This plasma membrane transporter, not present
in S. cerevisiae, was shown to extrude the cytosolic copper ions
into the medium, hence leading to depletion of this ion from
the cytosolic compartment. Under these conditions, the expres-
sion of the Cu/ZnSOD encoded by SOD1 would therefore be inap-
propriate. The expression of both CaCUP1 and CaCRP1/
CaCRD1 was found to be stimulated by copper and proposed to
involve an Ace1p-like DNA transactivator. Unlike CaCUP1
and CaCRP1/CaCRD1 and in support of this hypothesis, no
Ace1-responsive elements are present in the promoter region of
C. albicans SOD1 (not shown).

Although most eukaryotic MnSODs are targeted to the mi-
tochondria, cytoplasmic manganese-containing SODs have
been found in few organisms, namely in unicellular green algae
(37) and in various species of filamentous fungi (38). Interest-
ingly, unicellular green algae (39) and filamentous fungi (40,
41), including C. albicans (42), are also known to possess an
alternative respiratory pathway in which the mitochondrial
cytochrome c oxidase, requiring copper as cofactor, is replaced
by an alternate oxidase (Aox) using iron as cofactor. In the P.
A Third SOD in C. albicans

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Candida albicans Expresses an Unusual Cytoplasmic Manganese-containing Superoxide Dismutase (SOD3 Gene Product) upon the Entry and during the Stationary Phase

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