The *Candida albicans* CTR1 gene encodes a functional copper transporter

Marcus E. Marvin, Peter H. Williams and Annette M. Cashmore

Department of Genetics and Department of Microbiology and Immunology, University of Leicester, Leicester LE1 7RH, UK

Copper and iron uptake in *Saccharomyces cerevisiae* are linked through a high-affinity ferric/cupric-reductive uptake system. Evidence suggests that a similar system operates in *Candida albicans*. The authors have identified a *C. albicans* gene that is able to rescue a *S. cerevisiae* ctr1/ctr3-null mutant defective in high-affinity copper uptake. The 756 bp ORF, designated *CaCTR1*, encodes a 251 amino acid protein with a molecular mass of 27.8 kDa. Comparisons between the deduced amino acid sequence of the *C. albicans* Ctr1p and *S. cerevisiae* Ctr1p indicated that they share 39.6% similarity and 33.0% identity over their entire length. Within the predicted protein product of *CaCTR1* there are putative transmembrane regions and sequences that resemble copper-binding motifs. The promoter region of *CaCTR1* contains four sequences with significant identity to *S. cerevisiae* copper response elements. *CaCTR1* is transcriptionally regulated in *S. cerevisiae* in response to copper availability by the copper-sensing transactivator Mac1p. Transcription of *CaCTR1* in *C. albicans* is also regulated in a copper-responsive manner. This raises the possibility that *CaCTR1* may be regulated in *C. albicans* by a Mac1p-like transactivator. *A. albicans* ctr1-null mutant displays phenotypes consistent with the lack of copper uptake including growth defects in low-copper and low-iron conditions, a respiratory deficiency and sensitivity to oxidative stress. Furthermore, changes in morphology were observed in the *C. albicans* ctr1-null mutant. It is proposed that *CaCTR1* facilitates transport of copper into the cell.

**INTRODUCTION**

Copper is an important cofactor for a wide variety of cellular enzymes that carry out essential biological processes such as respiration, iron acquisition and protection against oxidative stress. The problem, however, is that copper exists in the environment as insoluble complexes and is toxic in the presence of oxygen because of the formation of destructive hydroxyl free radicals. Therefore, organisms have evolved mechanisms for the transport of copper into the cell and for maintaining intracellular concentrations at non-toxic levels.

In the baker’s yeast *Saccharomyces cerevisiae*, a well-documented system has been described where the acquisition of copper is linked to the acquisition of iron. Like copper, iron presents a similar problem to the cell as it is essential for numerous biological processes, is found in the environment as insoluble complexes and is toxic in the presence of molecular oxygen. In *S. cerevisiae*, copper and iron acquisition is achieved through a specific high-affinity reductive uptake system that is negatively regulated by both metals. Both copper and iron are rendered soluble by a cell-surface ferric/cupric-reductase complex encoded by the genes *FRE1* and *FRE2* (Dancis *et al.*, 1990; Georgatsou & Alexandraki, 1994). Uptake of iron is facilitated by a specific iron transporter complex comprising an iron permease, Ftr1p, and a multicopper ferroxidase, Fet3p (Askwith *et al.*, 1994; Stearman *et al.*, 1996). Copper uptake requires two functionally redundant high-affinity copper transporters, Ctr1p and Ctr3p, localized in the plasma membrane (Dancis *et al.*, 1994a, b; Knight *et al.*, 1996). Cells that express either Ctr1p or Ctr3p are competent for high-affinity copper transport. However, *ctr1Δctr3Δ* mutants exhibit copper starvation phenotypes that include the inability to grow on non-fermentable carbon sources, lack of measurable high-affinity iron uptake, and defective copper/zinc-superoxide dismutase activity (Dancis *et al.*, 1994a, b; Knight *et al.*, 1996). Three copper chaperones that deliver copper to specific intracellular targets have also been identified, Lys7p to superoxide dismutase, Cox17p to the mitochondria and Atx1p to the gene product of *CCC2* (Culotta *et al.*, 1997; Glerum *et al.*, 1996; Lin *et al.*, 1997; Pufahl *et al.*, 1997). Ccc2p is an intracellular copper transporter that in turn delivers copper to the lumen of the Golgi for incorporation into Fet3p (Yuan *et al.*, 1995; Lin *et al.*, 1997). Since Fet3p
relied on copper for biological activity, inhibition of copper uptake has the secondary effect of significantly reducing iron uptake (Askwith et al., 1994; Yuan et al., 1995; De Silva et al., 1995).

A further five additional FRE-like genes (FRE3–7) have been identified by their sequence similarity to FRE1 and FRE2. It has been demonstrated that the proteins encoded by FRE1, FRE2, FRE3 and FRE4 can reduce iron bound to low-molecular-mass iron-binding compounds called siderophores (Yun et al., 2001). As S. cerevisiae cannot produce its own siderophores, these gene products may facilitate the scavenging of iron bound to siderophores produced by other species. The specific function of the additional three reductases (encoded by FRE5–7) remains unclear.

The interdependence of high-affinity copper and iron uptake in S. cerevisiae extends to the transcriptional activators Mac1p and Aft1p that operate in conditions of copper or iron depletion and recognize specific consensus sequences in the promoters of various genes (Radisky & Kaplan 1999; Yamaguchi-Iwai et al., 1993; Jungmann et al., 1993). The copper-responsive activator Mac1p regulates FRE1, CTR1, CTR3 and FRE7, the uncharacterized homologue of FRE1, while the iron-responsive activator Aft1p regulates FRE1, FRE2, FRE3-6, FTR1, FET3, ATX1, CCC2, and a family of intracellular siderophore transporter genes ARN1–4 (Dancis et al., 1992; Lin et al., 1997; Jungmann et al., 1993; Yamaguchi-Iwai et al., 1996; Labbe et al., 1997; Martins et al., 1998; Lesuisse et al., 1998; Heymann et al., 2000; Yun et al., 2000a, b). Thus FRE1 is regulated by both copper and iron through the activity of Mac1p and Aft1p, whereas the copper-trafficking genes ATX1 and CCC2 are regulated by iron through the activity of Aft1p. Two low-affinity transporters have also been described, Ctr2p for copper and Fet4p for iron and copper (Dix et al., 1994; Kampfenkel et al., 1995; Hassett et al., 2000).

Candida albicans is a dimorphic opportunistic pathogen that is a commensal of the human mouth and gastrointestinal tract in about 30–50% of the population. However, in immunocompromised patients, C. albicans can cause both superficial and life-threatening diseases (Scherer & Magee 1990). Two genes have been identified in C. albicans (CaCUP1 and CaCRP1) that confer an unusually high resistance to copper (Weissman et al., 2000). The CaCUP1 gene encodes a metallothionein that sequesters intracellularized copper and CaCRP1 encodes a plasma-membrane P-type ATPase pump that transports copper out of the cell. Physiological concentrations of copper have been shown to be toxic to C. albicans under the acidic anaerobic conditions found in the gastrointestinal tract (Weissman et al., 2000). Therefore, by reducing intracellular free copper concentrations the products of these genes are believed to facilitate the survival of C. albicans in this environmental niche.

Iron has been implicated as an important factor for the growth, survival and virulence of C. albicans (Valenti et al., 1986; Chaffin et al., 1998; Fratti et al., 1998). The organism can acquire iron from haem and can bind to and lyse erythrocytes (Manns et al., 1994; Moors et al., 1992). Although C. albicans can produce siderophores (Ismail et al., 1985; Sweet & Douglas, 1991) it is still not clear how it obtains ferrous iron from these complexes or from other sources. Growing evidence suggests that a similar reductive system to that described for S. cerevisiae operates in C. albicans and several homologous genes have been identified. Our laboratory has previously reported that C. albicans has a cell-surface-associated ferric/cupric-reductase that is regulated similarly to the S. cerevisiae enzyme (Morrissey et al., 1996). We have also reported a C. albicans gene (CFL1) that is able to rescue mutant phenotypes associated with a S. cerevisiae fre1-null mutant and, like the S. cerevisiae FRE1 gene, is regulated in response to both copper and iron availability (Hammacott et al., 2000). Eck et al. (1999) described a C. albicans multicopper oxidase gene (CaFET3) that shares 55% identity with the S. cerevisiae FET3 gene. A C. albicans fet3Δ/fet3A mutant strain displayed slow growth in low-iron medium, suggesting a loss of high-affinity iron uptake. Ramanan & Wang (2000) described two C. albicans high-affinity iron permeases, encoded by CaFTR1 and CaFTR2. C. albicans mutants lacking CaFTR1 displayed a severe growth defect in low-iron conditions and, importantly, were unable to establish a systemic infection in mice. These observations suggest that, as in S. cerevisiae, copper and iron uptake in C. albicans are intimately linked such that reduced copper uptake may also affect the uptake of iron.

To further investigate copper uptake in this organism, we have isolated and characterized a C. albicans gene, CaCTR1. This gene is able to rescue the mutant phenotypes associated with a S. cerevisiae ctr1/ctr3-null mutant and we describe the sequence, transcriptional regulation and functional analyses of this gene. Our findings support the role of CaCTR1 as a copper transporter gene. This is believed to be the first report of a transporter facilitating the uptake of copper by C. albicans.

**METHODS**

**Strains.** The S. cerevisiae strain W303 (MATa; SUC2; ade2–1; can1–100; his3–11,115; leu2–3,112 trp1–1; ura3–1; R. J. Rothstein, Columbia University, New York, USA) is a derivative of strain S288C (Yeast Genetic Stock Center), which is known to harbour a ctr3-null allele due to a Ty2 insertion in the promoter separating the TATA box from the transcriptional start site by 6 kb (Knight et al., 1996). One-step gene replacement (Rothstein, 1983) was used to generate a ctr1-null allele in strain W303 (to generate S. cerevisiae strain W303ctr1Δ::URA3) using a disruption cassette as follows. Primers containing XbaI and KpnI sites (ScCTR1–233 and ScCTR1 +1525, Table 1) were used to amplify a 1758 bp fragment containing the entire CTR1 ORF. The resulting fragment was cloned into the KpnI and XbaI sites of the vector pUC19 to generate recombinant plasmid pCO1. Additional primers incorporating BamHI sites (ScCTR1 +62 and ScCTR1 +1131, Table 1) were then used in an inverse PCR reaction with pCO1 in order to delete 1068 bp from the cloned CTR1 ORF. The amplification product was digested with BamHI,
and allowed to self-ligate, generating plasmid pCD1. A 1141 bp fragment of plasmid YDpU (Berben et al., 1991) carrying the S. cerevisiae URA3 gene was inserted into the BamHI site of pCD1. The resulting construct, designated pCD2, carried a 1798 bp CTR1 disruption cassette comprising the URA3 marker flanked by 280 bp and 377 bp of genomic targeting sequences. The disruption cassette was removed from pCD2 by digestion with KpnI and XbaI, and used to transform S. cerevisiae strain W303 selecting for uracil prototrophy. Homologous recombination leading to disruption of the CTR1 gene was confirmed by Southern blot analysis of genomic DNA digested with EcoRV and probed with a [α-32P]CTP-labelled CTR1 KpnI and XbaI fragment excised from plasmid pCOI RV and probed with a [α-32P]CTP-labelled CTR1 KpnI and XbaI fragment excised from plasmid pCOI RV.

Wild-type C. albicans strain SC5134 was isolated from a systemic infection (Gillum et al., 1984). A C. albicans ctrl-null strain was generated from a derivative of this strain, BW17 (ura3Δ::imm434/ ura3Δ::imm434; arg4::hisG/arg4::hisG; his1::hisG/his1::hisG), using PCR-directed mutagenesis (Wilson et al., 1999). Primers containing 70 bp of homology to C. albicans genomic DNA (CaCTR1−70 and CaCTR1+753, Table 1) were designed to enable the deletion of 683 bp of the genomic CaCTR1 ORF, including the ATG start codon. These primers were used to amplify disruption cassettes containing the selectable markers URA3 and ARG4 from plasmid templates pGEM-URA3 and pRSARG4SpeI (Wilson et al., 1999). Two rounds of successive transformations generated strains BW17 ctrl1Δ::URA3/ CTR1 and BW17ctrl1Δ::URA3/ctrl1Δ::ARG4 selecting for uracil and arginine prototrophy respectively. Homologous recombination was confirmed by Southern blot analysis by digesting genomic DNA with HindIII and probed with a [α-32P]CTP-labelled 1904 bp fragment generated from primers CaCTR1−863 and CaCTR1+1057 (Table 1). In order to construct strain BW17 ctrl1Δ/His1::CTR1 a 1888 bp fragment was amplified by PCR using genomic DNA from strain SC5134 (Gillum et al., 1984) as a template and primers CaCTR1−854 and CaCTR1+1057 (Table 1). The resulting fragment was digested with Sall and introduced into the equivalent restriction site in pGEM-HIS1 (Davis et al., 2000; Wilson et al., 1999). The resulting construct (pGEM-HIS1/CTR1) contained the entire CaCTR1 ORF flanked by 844 bp of upstream and 288 bp of downstream sequences. BW17 ctrl1Δ::URA3/ctrl1Δ::ARG4 was then transformed with pGEM-HIS1/CTR1 which had been linearized by digestion with NruI and the colonies were selected for histidine prototrophy. Strain BW17 ctrl1Δ/HIS1 was constructed by transforming strain BW17 ctrl1Δ::URA3/ctrl1Δ::ARG4 with pGEM-HIS1 that had been linearized by digestion with NruI. Targeted integration at the his1::hisG locus in strains BW17 ctrl1Δ/HIS1::CTR1 and BW17 ctrl1Δ/HIS1 was confirmed by PCR analysis using primers CaHis1−287, CaHis1+1153, CaCTR1+1057 and 5DR (Table 1; data not shown).

Escherichia coli strain DH5α [rps8lacZAM15 recA1 endA1 gyrA96 thi-1 hsdR17(rK− m8') supE44 relA1 deo1 Δ(lacZΔM15-argF1)U169] was used for propagation of library clones and plasmid constructs. For transposon mutagenesis the donor strain was streptomycin-sensitive E. coli DH1 [recA1 endA1 gyrA96 thi-1 hsdR17(rK− m8') supE44 relA1] carrying plasmid R388 transposed with modified Tn1000 containing the S. cerevisiae HIS3 gene as a selectable marker. The recipient strain was MH1578 [recA1 endA1 gyrA96 thi-1 hsdR17(rK− m8') supE44 relA1] carrying plasmid pCRC1 was used to transform E. coli strain DH1 [R388::Tn1000(HIS3)], which was then used as a donor strain for conjugal mating with recipient strain MH1578 with selection for resistance to ampicillin and streptomycin. Plasmid DNA was isolated from transconjugants and used to transform strain W303/ctrl1Δ::URA3 with selection for leucine and histidine prototrophy.

**Table 1. Sequences of primers used in this study**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′→3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ScCTR1−233</td>
<td>GTCATCTAGAGGAGGTCTTG</td>
</tr>
<tr>
<td>ScCTR1 +1525</td>
<td>GGGAGAACAAGGTACCTCG</td>
</tr>
<tr>
<td>ScCTR1 +62</td>
<td>GTCTTGGATCCACTGACATGG</td>
</tr>
<tr>
<td>ScCTR1 +1131</td>
<td>GGGCGGGATCTTGGAGAATTTC</td>
</tr>
<tr>
<td>CaCTR1−70</td>
<td>ATATATTGCAACCTAATTGATATTTCTCTGATTATCATG</td>
</tr>
<tr>
<td>CaCTR1 +753</td>
<td>GCAACCATTTTCTAGGAGGACCTTTTTATTTCTGATGTTTCCAT</td>
</tr>
<tr>
<td>CaCTR1−854</td>
<td>GTGAAGCATGTCGACCTCGATGATGTCG</td>
</tr>
<tr>
<td>CaCTR1−863</td>
<td>TGGGATCTTGAGACATGGTGTCG</td>
</tr>
<tr>
<td>CaCTR1+1057</td>
<td>GCAAAAGCTGCGTACACTAAGAGCGC</td>
</tr>
<tr>
<td>CaCTR1 +60</td>
<td>GGAATGTCCGGCAATTTCAGC</td>
</tr>
<tr>
<td>CaCTR1 +568</td>
<td>CTCTAGAATAATTGATGTCG</td>
</tr>
<tr>
<td>5DR</td>
<td>TGTGGAATTTGTAGCGGATA</td>
</tr>
<tr>
<td>CaHis1−287</td>
<td>GAGGATACGTGACATCGAGG</td>
</tr>
<tr>
<td>CaHis1 +1153</td>
<td>CTTATGGGCTCATGGCTACGC</td>
</tr>
</tbody>
</table>

**Growth conditions.** S. cerevisiae and C. albicans cultures were grown at 30°C unless otherwise stated. For growth in non-selective
conditions yeast-extract peptone medium was used, with glucose added at a final concentration of 2 % w/v (YPD). To test the strains for the ability to grow on non-fermentable carbon sources, glucose was replaced with either 3 % (v/v) glycerol (YPG) or 3 % (v/v) ethanol (YPE). To test for sensitivity to oxytetracycline, overnight cultures were transferred into fresh yeast-extract peptone medium and grown to a cell density of $1 \times 10^7$ cells ml$^{-1}$. The cells were then diluted back to $1 \times 10^6$ cells ml$^{-1}$ and 100 μl was transferred to solid YPD medium containing 100 μg ml$^{-1}$ and 1 mg ml$^{-1}$ oxytetracycline, respectively. For yeast grown in selective conditions SD medium [0.67 % (w/v) yeast nitrogen base with ammonium sulphate (B101)] was used. Minimal defined medium (MD) was used to verify the ability of strains to grow in low-copper or low-iron conditions; the medium was based on the yeast nitrogen base recipe of Wickerham (1951) with the addition of 20 mM sodium citrate.

Restriction analysis, DNA manipulation and sequence analysis. A C. albicans Sau3AI partial digest genomic library cloned into the BamHI site of vector YEp213 was obtained from P. Meacock, Department of Genetics, University of Leicester, UK. Transformation of S. cerevisiae and C. albicans was achieved by the lithium acetate method described by Geitz et al. (1992) and Braun & Johnson (1997), respectively. Isolation of plasmid DNA from S. cerevisiae was achieved using the procedure described by Holm et al. (1986). Plasmid DNA was isolated and purified from E. coli by alkaline lysis (Isa-Horowitz & Burke, 1981) and transformation of E. coli was performed using the calcium chloride method (Mandel & Higa, 1970).

**Functional rescue of S. cerevisiae mutant strain W303ctr1Δ::URA3**

High-affinity copper uptake in S. cerevisiae is facilitated by two genes, CTR1 and CTR3 (Dancis et al., 1994a, b; Knight et al., 1996). However, the parent of the S. cerevisiae strain used in this study (W303) is S288C, which is known to carry a defective allele of CTR3 due to a Ty2 insertion (Knight et al., 1996). Therefore, it was only necessary to disrupt CTR1 in strain W303 in order to construct a mutant that was defective in high-affinity copper uptake. A S. cerevisiae strain carrying a null allele of CTR1 was generated using one-step gene disruption (Rothstein, 1983). The resulting strain, W303ctr1Δ::URA3, displayed phenotypes typical of a strain defective in high-affinity copper uptake, specifically a respiratory deficiency resulting in the inability to grow on yeast-extract peptone medium containing non-fermentable carbon sources such as glycerol and ethanol (YPG and YPE), high ferric-reductase activity on iron-rich medium (Dancis et al., 1994a) and the inability to grow on MD medium depleted of copper or iron (data not shown).

The respiratory-deficient phenotype of strain W303ctr1Δ::URA3 was exploited to isolate a C. albicans gene functionally analogous with S. cerevisiae CTR1 or CTR3. Strain W303ctr1Δ::URA3 was transformed with a YEp213 C. albicans Sau3AI partial digest genomic library (Peter A. Meacock, personal communication) and screened on YPE medium. Selected colonies were tested for ferric-reductase activity using a qualitative plate assay (Dancis et al., 1990). A single colony that exhibited wild-type low ferric-reductase activity on high-iron medium was picked for further study. Transformation of W303ctr1Δ::URA3 with plasmid DNA (designated pCRC1) from this colony resulted in complementation of all the ctrl1/ctr3-null-associated phenotypes (data not shown). Since these phenotypes reflect the lack of high-affinity copper transport, we propose that pCRC1 contains a C. albicans gene encoding a protein that facilitates copper transport into the cell.
Localization and sequence analysis of the rescuing gene in pCRC1

Restriction digest analysis indicated that plasmid pCRC1 carried a 9·3 kb genomic insert. To identify the rescuing ORF within this insert, a library of pCRC1 derivatives that carried random transposon insertions was assembled using transposon mutagenesis (Sedgewick & Morgan, 1994). Transformants were screened on YPE; three colonies unable to grow on this medium were picked and plasmid DNA used to retransform strain W303ctr1Δ::URA3 in order to confirm the mutant phenotype. Restriction analysis showed that transposon insertion sites in these three plasmids were located within a 1·1 kb EcoRI fragment of insert DNA (Fig. 1).

Primers 5′GGGGGACTGAGGCTCTAC3′ and 5′TCAATAAGTTATACCAT3′ were used in big dye terminator sequencing reactions to obtain DNA sequence flanking the transposon in the three mutant plasmids. Alignment of these sequences revealed a 756 bp ORF with 97% identity over 395 bp to a genomic fragment (accession number 265216D11.y1.seq) designated as CTR1-like on the C. albicans information page (http://alces.med.umn.edu/Candida.html). Further analysis at Stanford University’s C. albicans sequencing project website (http://www-sequence.stanford.edu/group/candida/index.html) revealed that this fragment is part of a 28731 bp contiguous sequence (accession number contig4-3041) that contains four putative ORFs designated CTR1, FAT1, PGK1 and SEC8. Additional primers were used to generate a total of 1650 bp of genomic sequence, including the entire rescuing gene, which we have named CaCTR1, and flanking sequences (Fig. 1). These sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession number AJ277398. A BLASTN search using the CaCTR1 ORF as a query against contig4-041 revealed seven ORFs within this insert, a library of pCRC1 derivatives that contained the entire CaCTR1 ORF and included 863 bp of genomic sequence (accession number contig4-3041) that corresponds to the transcriptional ATG start site with A of the start codon as +1. The putative TATA box is underlined with a double score. Transposon insertions mapped within a 1·1 kb EcoRI fragment are denoted as double-headed horizontal black arrows at nucleotides 62 (C for T), 65 (T for C), 81 (A for T), 115 (G for A), 219 (A for G), 468 (A for G) and 743 (A for G). To isolate the gene responsible for mutant rescue of strain W303ctr1Δ::URA3, we generated 1920 bp of genomic sequence using primers CaCTR1–863 and CaCTR1+1057, which included BamHI and SalI restriction sites (Table 1). The resulting fragment contained the entire CaCTR1 ORF and included 863 bp of upstream and 301 bp of downstream sequences. We then digested the PCR-generated fragment using BamHI and SalI and cloned the resulting 1907 bp fragment into the corresponding sites in YEp213 to generate plasmid pCaCO1. This construct was used to transform strain W303ctr1Δ::URA3 and the mutant phenotypes were subsequently tested. The plasmid (pCaCO1) was able to rescue all the mutant phenotypes associated with strain W303ctr1Δ::URA3 (data not shown), confirming that the PCR-generated fragment that included CaCTR1 was sufficient for mutant rescue.

Analysis of the promoter region of CaCTR1 using the S. cerevisiae promoter regulatory sequence analysis website (http://rsat.ulb.ac.be/rsat/; Van Helden et al., 2000), revealed four motifs with the consensus sequence 5′GGTTTATTCAT3′ (Fig. 1). Three of the four motifs were immediately preceded by 5′TTT3′, making them identical to cis-acting copper response elements (CuREs) found in the promoters of CTR1, CTR3, FRE1 and FRE7 of S. cerevisiae (Labbe et al., 1997; Yamauchi-Iwai et al., 1997; Martins et al., 1998). The core sequence of these transcription

http://mic.sgmjournals.org
regulatory elements, \(5'-\text{TTCG(TG)C(A/G)}^3\), is essential for binding of the copper-sensing transactivator Mac1p \(\text{(Jungmann et al., 1993; Georgatsou et al., 1997; Graden & Winge, 1997; Joshi et al., 1999)}\). The fourth \(C. \text{ albicans}\) motif differed from the \(S. \text{ cerevisiae}\) consensus by the lack of two of the three T residues at the 5'-end. Three of the potential \(C. \text{ albicans}\) CuREs are preceded immediately by A, which has been shown to facilitate more efficient binding of Mac1p to the \(S. \text{ cerevisiae}\) high-affinity copper transporter \(\text{CTR1}\) \(\text{(Joshi et al., 1999)}\).

The predicted protein product of \(C. \text{ albicans CTR1}\)

A BLASTp search (Altschul et al., 1997) using the predicted \(C. \text{ albicans}\) Ctr1p sequence as a query against the SWISS-PROT database revealed significant similarity with the \(S. \text{ cerevisiae}\) high-affinity copper transporter Ctr1p, as well as the \(H. \text{ sapiens}\) hCtr1p (Zhou & Gitschier, 1997) and the high-affinity copper transporter of \(S. \text{ pombe}\), Ctr4p (Zhou & Thiele, 2001). Comparison with the \(S. \text{ cerevisiae}\) genome database (http://genome-www.stanford.edu/Saccharomyces) using the FastA program (Pearson & Lipman, 1988) revealed significant similarity to all three of the \(S. \text{ cerevisiae}\) copper transporters Ctr1–3p. Direct comparison using the Needleman & Wunsch algorithm alignment (GAP) from the GCG package (Wisconsin package version 9.1, Genetics Computer Group, Madison, WI, USA) showed that the \(C. \text{ albicans}\) Ctr1p had 39-6% similarity and 33-0% identity to the \(S. \text{ cerevisiae}\) Ctr1p.

Analysis of the \(C. \text{ albicans CTR1}\) protein sequence using the SAPS analysis program (http://www.isrec.isb-sib.ch/software/SAPS_form.html; Brendel et al., 1992) revealed a predicted protein of 251 amino acids with a molecular mass of 27.8 kDa, lacking an amino-terminal leader sequence. Dancis et al. (1994a) previously reported that \(S. \text{ cerevisiae}\) Ctr1p has a methionine- and serine-rich amino-terminal region that contains a putative copper-binding domain. This region includes three 19 amino acid repeats and 11 examples of the motif Met-XX-Met, which is also found in bacterial copper-binding proteins (Cha & Cooksey, 1991; Odermatt et al., 1993). Compositional analysis against all proteins in the SWISS-PROT database indicated that both methionine and serine are highly represented in the \(C. \text{ albicans}\) predicted protein, predominantly at the amino end (residues 1–71). A search for repetitive sequences within this 71 amino acid region revealed four repeats of the motif M(A/X)M(S/A)(S/A/X)(S/A/T)(S/A/T/X)(S/X) in and around two repeated core blocks of SAT(M/_)(SMAM(S/_)(A/S)TS [where _ = no residue and X = other uncharged or non-polar amino acids D/M/V with one exception, H], shown in Fig. 1. Only one example of a MetXX-Met motif was found at residues 52 to 55 (MEGM), but there were eight examples of the motif Met-X-Met (residues 11–13, 20–22, 29–31, 36–38, 38–40, 50–52, 62–64 and 64–66). Distribution of all other amino acid types revealed two high-scoring transmembrane segments at residues 90–104 and 202–229, while pSORT type II analysis (http://psort.nibb.ac.jp/) indicated three transmembrane domains at residues 91–107, 194–210 and 216–232 (Fig. 1).

\(C. \text{ albicans CTR1}\) expression responds to copper availability in the growth medium

Northern blot analysis was carried out to examine expression of \(C. \text{ albicans CTR1}\) in low- and high-copper medium (Fig. 2). Total RNA was isolated from mid-exponential-phase cultures \(1 \times 10^7\) cells ml\(^{-1}\) of \(C. \text{ albicans}\) strain SC5314 grown in unsupplemented MD-BCS medium or in MD-BCS medium supplemented with 50 \(\mu\)M, 100 \(\mu\)M or 250 \(\mu\)M cupric chloride and probed with a 508 bp internal fragment of the \(CaCTR1\) gene. After an exposure of 1 h the autoradiograph revealed a 1.1 kb band.

**Fig. 2.** Northern blot analysis of the \(C. \text{ albicans CTR1}\) transcripts. Total RNA was isolated from cells growing exponentially in MD-BCS medium containing 0 \(\mu\)M (lane 1), 50 \(\mu\)M (lane 2), 100 \(\mu\)M (lane 3) and 250 \(\mu\)M (lane 4) cupric chloride. Following electrophoresis and transfer to a nylon membrane, duplicate sets of the four RNA samples were probed with either an [\(\alpha\)-\(\text{32P}\)]CTP-labelled 508 bp fragment of the \(C. \text{ albicans}\) \(\text{CTR1}\) gene or a 1.3 kb EcoRI fragment of the \(C. \text{ albicans}\) \(\text{URA3}\) gene as a loading control. Autoradiographs were then exposed to the labelled membrane for 1 h (a) or 48 h (b, c). After 48 h exposure further bands appeared, one fractionally greater than 1.1 kb observed in high-copper conditions and another much larger band (3.1 kb) in low-copper conditions.
representing a highly transcribed gene in low-copper conditions.

The expression of CaCTR1 in S. cerevisiae is high in low-copper conditions and is regulated by copper availability through the activity of Mac1p

Transcription of CaCTR1 in S. cerevisiae was investigated using Northern blot analysis. Wild-type S. cerevisiae strain (BY4741) and mac1-null mutant strain (BY4741mac1Δ::KanMX4) were transformed with the plasmids YEp213, pCRC1 and pCaCO1 and grown in unsupplemented MD-BCS medium or in MD-BCS medium supplemented with 100 μM cupric chloride. Cells were harvested at mid-exponential phase (1 × 10⁷ cells ml⁻¹) and the total RNA was extracted. This was probed with the 508 bp internal fragment of CaCTR1 and the results are shown in Fig. 3. High expression of CaCTR1 was observed in strain BY4741 only when it was transformed with pCRC1 and pCaCO1 and grown in unsupplemented MD-BCS medium (Fig. 3, lanes 3 and 5). No expression was observed in strain BY4741 transformed with pCRC1 and pCaCO1 when it was grown in MD-BCS medium supplemented with 100 μM cupric chloride (Fig. 3, lanes 4 and 6) or in the mac1-null strain when it was transformed with pCRC1 or pCaCO1 (Fig. 3, lanes 9–12). These results indicate that high expression of CaCTR1 in S. cerevisiae is in response to low-copper conditions and is under the control of Mac1p.

A C. albicans ctr1-null strain is unable to grow on solid low-copper and low-iron medium and displays altered morphology in response to copper-depleted conditions

To determine the functional role of CaCTR1 in C. albicans we constructed single and double deletions of the gene in strain BWP17 using PCR-directed mutagenesis (Wilson et al., 1999), generating BWP17ctr1Δ::URA3/CTR1 and BWP17ctr1Δ::URA3/ctr1Δ::ARG4, respectively. In each mutant strain, 683 bp of the genomic CaCTR1 ORF (including the ATG start codon) were deleted and replaced by either the CaURA3 or the CaARG4 gene as a prototrophic marker. Homologous recombination in the resulting transformants was confirmed by digestion of genomic DNA with the HinIII and Southern blot analysis (data not shown).

When grown on YPD medium, strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 was slow-growing and formed small white colonies resembling S. cerevisiae petite mutants. It was also observed that with prolonged incubation (30°C for ≥7 days), colonies of both strain BWP17ctr1Δ::URA3/CTR1 and strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 produced invasive filaments that penetrated the growth medium (Fig. 4). In addition, strain BWP17ctr1Δ::URA3/CTR1 always produced wrinkly colonies on YPD medium (Fig. 4b, e). To investigate whether copper available in the growth medium had an effect on morphology, overnight liquid cultures of BWP17ctr1Δ::URA3/ctr1Δ::ARG4, and wild-type BWP17 were set up in unsupplemented MD-BCS medium. The cells were then transferred at a concentration of 1 × 10⁶ cells ml⁻¹ into fresh pre-warmed unsupplemented MD-BCS or MD-BCS supplemented with 100 μM cupric chloride. Growth was continued with agitation and the cells were observed at 15 min intervals.

The results from triplicate experiments showed strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 to have an increased doubling time of 96·9 ± 8·9 min (mean ± SD) in unsupplemented MD-BCS medium compared with 77·5 ± 5·4 min for the wild-type strain BWP17. In MD-BCS medium supplemented with 100 μM cupric chloride the doubling time of strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 was restored to wild-type levels (74·4 ± 3·1 min compared to 77·8 ± 5·7 min for strain BWP17). Changes in morphology

Fig. 3. Northern blot analysis of the CaCTR1 transcripts in S. cerevisiae. Wild-type strain BY4741 (MAC1) and mutant strain BY4741mac1Δ::KanMX4 (mac1Δ) were transformed with plasmid YEp213 (lanes 1–2 and 7–8), pCRC1 (lanes 3–4 and 9–10) or pCaCO1 (lanes 5–6 and 11–12). The resulting transformed strains were grown in either unsupplemented MD-BCS medium (lanes 1, 3, 5, 7, 9 and 11) or MD-BCS medium supplemented with 100 μM cupric chloride (lanes 2, 4, 6, 8, 10 and 12). Total RNA was isolated from the transformed S. cerevisiae cells growing exponentially. Following electrophoresis and transfer to a nylon membrane the RNA samples were probed with an [α-32P]CTP-labelled 508 bp fragment of the C. albicans CTR1 gene or a 342 bp fragment of the S. cerevisiae ACT1 gene as a loading control. Autoradiographs were then exposed to the labelled membrane for 30 min (CaCTR1) or 4 h (ACT1) to visualize hybridized bands.
were also observed in strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 when grown in unsupplemented MD-BCS medium, with many of the cells present in the culture displaying untypical profiles (Fig. 5a). Cells that were in yeast form were much-reduced in size and many were elongated when compared to the wild-type strain BWP17 (Fig. 5b). In addition to this, a significant number (~20–60% depending on the field of view) of the BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 cells produced pseudo-hyphae during mid-exponential phase (Fig. 5a). In MD-BCS medium supplemented with 100 μM cupric chloride no morphology switch was observed and strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 grew like the wild-type strain BWP17 in yeast form (Fig. 5c). The addition of 100 μM cupric chloride to an exponential-phase culture of strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 growing in unsupplemented MD-BCS medium was also sufficient to revert the cells to grow like the wild-type. In stationary phase no filamentous forms of strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 were observed in unsupplemented MD-BCS medium but the cells appeared smaller than the wild-type and remained elongated (data not shown).

We also tested the ability of the C. albicans ctr1-null strain to grow on solid MD-BCS and MD-BPS media. Slow growth of strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 was observed on unsupplemented MD-BCS medium containing 50 μM of the copper chelator (Fig. 6a) and the mutant strain was unable to grow at concentrations of 2 mM BCS or higher (data not shown). Addition of 100 μM cupric chloride to the medium was sufficient to restore growth to the mutant strain. In depleted-iron conditions we found that strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 was unable to grow on unsupplemented MD-BPS medium containing 50 μM of the iron chelator (Fig. 6b). The addition of 100 μM cupric chloride to MD-BPS medium not supplemented with ferric chloride was sufficient to restore growth of the mutant strain (Fig. 6b). We also tested the ability of strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 to produce true hyphae in liquid YPD medium. However no difference was observed between the wild-type (BWP17) or the mutant strain (BWP17 ctr1Δ::URA3/ctr1Δ::ARG4) after induction with bovine calf serum (data not shown).

A C. albicans ctr1-null strain displays a respiratory deficiency when grown on non-fermentable carbon sources and is sensitive to oxidative stress

The growth of strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 was tested on medium containing non-fermentable carbon sources and hydrogen peroxide. The results in Fig. 6(c) show that the Cactr1-null strain is unable to grow on yeast-extract peptone medium containing either glycerol (YPG) or ethanol (YPE) added as a sole carbon source. The results in Fig. 6(d) indicate that strain BWP17 ctr1Δ::URA3/ctr1Δ::ARG4 has increased sensitivity to hydrogen peroxide when grown on unsupplemented MD-BCS medium; the addition of 100 μM cupric chloride to the growth medium.
restored growth on YPG and YPE and decreased the sensitivity of the mutant strain to wild-type levels (data not shown).

It has previously been reported that S. cerevisiae strains carrying null mutations of the SOD1 gene, which encodes Cu/Zn-superoxide dismutase, are sensitive to the antibiotic oxytetracycline (Avery et al., 2000). Levels of oxytetracycline as low as 20 µg ml⁻¹ in yeast-extract peptone medium have been shown to arrest growth of sod1Δ strains, whereas wild-type strains are unaffected at levels as high as 1 mg ml⁻¹. S. cerevisiae ctr1Δ and mac1Δ strains show similar sensitivities to oxytetracycline (Angrave et al., 2001) as a result of deficient levels of intracellular copper. This may be due to defective Cu/Zn superoxide dismutase activity, or through the direct antioxidant capability of copper (Liu & Culotta, 1994). Therefore we tested the growth of strains BWP17, BWP17 BWP17ctr1Δ/CTR1 and BWP17ctr1Δ::URA3/ctr1Δ::ARG4 on yeast-extract peptone medium (YPD) containing oxytetracycline at concentrations of 100 µg ml⁻¹ and 1 mg ml⁻¹ (data not shown). Colony formation by the wild-type strain BWP17 and strain BWP17ctr1Δ::URA3/CTR1 was unaffected by both concentrations of oxytetracycline, with colonies being formed on all the media after 2 days incubation. However, only very small colonies of BWP17ctr1Δ::URA3/ctr1Δ::ARG4 were apparent on YPD containing 1 mg oxytetracycline ml⁻¹ even after 7 days incubation. Colonies of BWP17ctr1Δ::URA3/ctr1Δ::ARG4 on YPD containing 100 µg oxytetracycline ml⁻¹ were observed after 3 days incubation although they were reduced in size in comparison to the other two strains.

Finally, we reconstituted CaHIS1 alone, and CaHIS1 along with CaCTR1, in strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 in order to construct the control strains BWP17ctr1ΔΔ/HIS1 and BWP17ctr1ΔΔ/HIS1::CTR1 (see Methods). We subsequently tested these strains along with the wild-type control strain, DAY185 (Davis et al., 2000), for the mutant phenotypes associated with the lack of copper uptake (Fig. 6). Strain BWP17ctr1ΔΔ/HIS1 displayed phenotypes that were identical to the Cactr1-null strain, BWP17ctr1Δ::URA3/ctr1Δ::ARG4. Strain BWP17ctr1ΔΔ/HIS1::CTR1 displayed phenotypes that were similar to strain BWP17ctr1Δ::URA3/CTR1, showing that reintegration of the wild-type CaCTR1 allele to the his1::hisG locus was sufficient for mutant rescue. Interestingly, strain BWP17ctr1ΔΔ/HIS1::CTR1 also grew as wrinkly colonies on YPD medium.

**DISCUSSION**

We have isolated and characterized a C. albicans genomic clone that is able to complement phenotypes displayed by a S. cerevisiae ctr1/ctr3-null mutant defective in high-affinity copper transport. In the S. cerevisiae mutant, the lack of high-affinity copper transport results in defective high-affinity iron transport as a result of insufficient delivery of copper to Fet3p (Askwith et al., 1994; Yuan et al., 1995; De Silva et al., 1995). The S. cerevisiae ctr1/ctr3 mutant was therefore unable to grow on copper- or iron-restrictive medium and the respiratory-deficient phenotype can be attributed to defective incorporation of both copper and
iron into mitochondrial enzymes that require the transition metals as prosthetic groups. Low copper and iron levels have also been shown to affect cell-surface reductase activity (Lesuisse & Labbe, 1989; Dancis et al., 1990, 1994a; Georgatsou & Alexandraki, 1994; Georgatsou et al., 1997). This resulted in high ferric-reductase activity in the ctrl1/ctrl3 mutant when grown on high-iron medium. Therefore we propose that the rescuing clone facilitates restoration of high-affinity copper transport in the C. albicans ctrl1/ctrl3-null strain. Due to the ability of the C. albicans genomic clone to rescue a S. cerevisiae ctrl1/ctrl3-null mutant and the significant similarity of the predicted protein and promoter elements to the S. cerevisiae Ctr1p we have named the C. albicans copper transporter gene CaCTR1.

The ORF that complements the S. cerevisiae ctrl1/ctrl3-null mutant shares 99% identity with an ORF designated as CTR1-like on the Stanford C. albicans sequencing project website, with seven nucleotide mismatches between the two sequences along the whole ORF. Four of these mismatches result in amino acid substitutions: alanine for valine, methionine for threonine, alanine for threonine, and lysine for arginine at residues 21, 22, 39 and 248 respectively. The predicted amino acid sequence of the CTR1-like ORF is 251 residues long with a molecular mass of 27-8 kDa. A BLAST search using the C. albicans predicted protein sequence against the SWISS-PROT database revealed significant similarity to three high-affinity copper transporters from different species, with the S. cerevisiae Ctr1p scoring highest. Direct comparison to the S. cerevisiae Ctr1p sequence gave 39-6% similarity and 33-0% identity, respectively.

Sequence analysis of the predicted C. albicans Ctrl1 protein revealed that methionine and serine are significantly overrepresented within 71 residues at the amino terminus. Within this highly structured region are repeated motifs that may signify a copper-binding domain. Similar methionine- and serine-rich domains have previously been described in prokaryotic copper-binding proteins (Cha & Cooksey, 1991; Odermatt et al., 1993) and the S. cerevisiae high-affinity copper transporter Ctrl1p (Dancis et al., 1994a, b; Knight et al., 1996) that include repeats of the motif Met-XX-Met. Only one example of the Met-XX-Met motif was found in the C. albicans predicted protein but there were eight examples where the two methionines were separated by one amino acid residue (Met-X-Met). Distribution of all other amino acid types revealed a possibility of two or three transmembrane domains situated in the middle and at the carboxy terminus of the protein. As the predicted protein lacks an amino-terminal leader sequence it may utilize one of these domains for membrane insertion. The S. cerevisiae Ctr1p also lacks a leader sequence, has two or three transmembrane domains and exists as a multimer in the plasma membrane (Dancis et al., 1994b). The lack of a significant number of transmembrane domains in the C. albicans predicted protein probably means that it also exists as a multimer in the plasma membrane.

Similarities between the C. albicans CTR1 and the S. cerevisiae CTR1 extend to the promoter of each respective gene. Analysis of the CaCTR1 promoter sequence revealed four sequences resembling the S. cerevisiae copper response elements (CuREs). These motifs facilitate binding of the copper-sensing transacti- vator Mac1p (Dancis et al., 1992; Jungmann et al., 1993; Yamaguchi-Iwai et al., 1997; Labbe et al., 1997; Martins et al., 1998). The presence of CuRE-like elements in the promoter of CaCTR1 may explain how expression of the gene is controlled in response to copper availability in S. cerevisiae by the copper-sensing transacti- vator Mac1p. However, it also raises the possibility of the existence of a similar copper-sensing transactivator in C. albicans. A putative metal-binding transcriptional regulator is present on the Institut Pasteur C. albicans genomic database (http://genolist.pasteur.fr/CandidaDB/ genome.cgi; accession number CA5628) that displays significant sequence homology to Mac1p of S. cerevisiae. Future studies on this ORF may reveal a similar copper-responsive transactivator in C. albicans. Three of the identified C. albicans Mac1p-like binding motifs found in the promoter of CaCTR1 are identical to the S. cerevisiae CuREs and the fourth is similar due to two mismatches. Electrophoretic mobility shift assays have previously shown that Mac1p binding is stronger to 5' TATTGCTC3' than to 5' TTTTGCTC3' and the transactivator makes specific and favourable contact to an adenine residue immediately 5' to the core sequence compared to a thymine (Joshi et al., 1999). Three of the C. albicans putative core sequences have adenine rather than thymine at this position and one has an identical sequence of 5' TATTGCTC3' that may facilitate

**Fig. 6.** Strain BWP17ctr1::URA3/ctr1::ARG4 displays a growth defect on low-copper and low-iron media, and is respiratory-deficient and sensitive to hydrogen peroxide. (a, b) Cells grown in unsupplemented MD-BCS medium were harvested at mid-exponential phase, washed three times and suspended in distilled water at a concentration of 1 x 10⁷ cells ml⁻¹. Fivefold serial dilutions were made (1 x 10⁻⁷ to 5 x 10⁻⁸ cells ml⁻¹, left to right) and 2 ml of each suspension was spotted in duplicate onto (a) MD medium containing 50 µM of the copper chelator BCS and supplemented with 100 µM cupric chloride or with no added copper, or (b) MD medium containing 50 µM of the iron chelator BPS and supplemented with 100 µM cupric chloride or with no added copper. (c) Single colonies were streaked onto unsupplemented YP medium containing either 2% (w/v) glucose (YPD), or 3% (w/v) glycerol (YPG) or ethanol (YPE) as a carbon source, or onto YPD, YPG and YPE medium containing 100 µM cupric chloride. (d) C. albicans strains were grown in unsupplemented MD-BCS medium to a cell density of 1 x 10⁷ cells ml⁻¹. The cells were then washed and 1 ml was suspended in 12 ml 1 M molten soft agarose equilibrated to 37°C. This was used as a top layer to either unsupplemented MD-BCS medium or MD-BCS medium supplemented with 100 µM cupric chloride, and 10 µl hydrogen peroxide (8·8 M) was dispensed onto a nylon disc placed at the centre of the plate. The lack of growth forming a 'halo' around the disc was observed after 5 days incubation.

http://mic.sgmjournals.org
more efficient binding of a putative transactivator to these sequences. In *S. cerevisiae* the distance between the motifs has also been shown to have a limited effect on transcription, with greater spacing attenuating expression (Martins et al., 1998). Therefore the two *C. albicans* putative CuRE motifs with the least distance between them (−268 to −78 and −239 to −229) are the stronger candidates to facilitate the most effective transcriptional control. In *S. cerevisiae* the CTR1, CTR3 and FRE7 CuREs are inverted repeats whilst the FRE1 CuRE is a direct repeat. The two *C. albicans* putative CuREs with the least distance between them make up an inverted repeat. However, to date an inverted CuRE repeat rather than a direct CuRE repeat has not been shown to convey more effective transcription in *S. cerevisiae*. Northern blot analysis shows that CaCTR1 is negatively regulated by copper in *C. albicans* and is highly expressed in copper depleted conditions. Extra bands present after a 48 h exposure of the autoradiograph may reveal other copper-regulated *C. albicans* genes that contain significant sequence identity to CaCTR1.

A *C. albicans* ctr1-null mutant displays phenotypes consistent with the lack of copper transport, including the inability to grow on low-copper or low-iron medium, the inability to grow on non-fermentable carbon sources and an increased sensitivity to hydrogen peroxide and oxytetracycline. These phenotypes are directly comparable to those of *S. cerevisiae* mutants defective in high-affinity copper transport (Dancis et al., 1994b; Knight et al., 1996; Angrave et al., 2001). The *C. albicans* ctr1-null strain did not grow on low-copper or low-iron medium when the cells had previously been starved of copper. However, a much higher concentration of the copper chelator, BCS (≥ 2 mM), was required to arrest growth completely when compared to a corresponding *S. cerevisiae* ctr1/ctr3-null mutant (50 μM). Addition of copper rescued the Cactr1-null mutant when it was grown on low-copper or low-iron medium. Strain BWP17ctr1Δ::URA3/ctr1Δ::ARG4 also displayed an increased sensitivity to the oxidative-stress-generating agents hydrogen peroxide and oxytetracycline. This may be attributed to deficient intracellular copper conditions that may lead to defective Cu/Zn superoxide dismutase activity, or to lowered copper antioxidant capability (Liu & Culotta, 1994). However, a decrease in Cu/Zn superoxide dismutase activity has previously been described in a *S. cerevisiae* ctr1/ctr3-null mutant (Knight et al., 1996). The inability of the *C. albicans* ctr1-null strain to grow on non-fermentable carbon sources such as glycerol or ethanol can be attributed to defective mitochondria. This is presumably a secondary effect resulting from the deficient delivery of copper and possibly iron to the respiratory enzymes that provide electron transport.

The lack of CaCTR1 activity, and therefore deficient intracellular copper concentrations, may contribute to the change in morphology observed in strains BWP17ctr1Δ::URA3/CTR1 and BWP17ctr1Δ::URA3/ctr1Δ::ARG4 when grown on yeast-extract peptone medium. In support of this is the observation that strain BWP17ctr1A::URA3/ctr1Δ::ARG4 changed morphology in medium depleted of copper and the addition of 100 μM cupric chloride to the growth medium rescued this. The production of pseudohyphae by strain BWP17ctr1A::URA3/ctr1Δ::ARG4 in low-copper medium may result from the cell responding to copper-starved conditions. However, it has previously been observed that low-iron conditions affect hyphal growth in *C. albicans* (Sweet & Douglas, 1991) and so the lack of copper transport affecting iron uptake due to inactive CaFet3p activity may also be a contributing factor.

Copper is believed to play a detrimental role in oxidative stress for *C. albicans* when the organism is in its environmental niche of the gastrointestinal tract. Both CaCUP1 and CaCRP1 may play an important role for the protection of *C. albicans* in this environment (Weissman et al., 2000). We have now isolated and characterized a *C. albicans* gene that may also play a role in this protection. The presence of CuRE-like elements in the promoter of CaCTR1 may facilitate tight control of copper uptake by a Mac1p-like transactivator, leading to copper homeostasis. Iron availability has been shown to affect *C. albicans* growth, hyphal production, adherence to host cells and the ability to set up an infection in mice (Sweet & Douglas, 1991; Valenti et al., 1986; Moors et al., 1992; Fratti et al., 1998; Eck et al., 1999; Ramanan & Wang, 2000). In *S. cerevisiae* the dependence of iron acquisition on the uptake and delivery of copper to Fet3p is well documented and evidence suggests that a similar system operates in *C. albicans* (Morrissey et al., 1996; Eck et al., 1999; Hamacott et al., 2000; Ramanan & Wang, 2000). The CaCTR1 gene product may provide the first step in the chain of events that leads to the incorporation of copper into CaFet3p. We have now cloned the first copper transporter that provides *C. albicans* with this essential metal from the environment. Studies are in progress to give a greater understanding of the relationship between copper and iron uptake in *C. albicans* and the role they may play in survival and proliferation in the mammalian host.

**ACKNOWLEDGEMENTS**

We would like to thank Colin Brooks for technical help with photography, Aaron P. Mitchell for providing guidance, strains and materials for PCR-directed mutagenesis of *C. albicans*. We would also like to thank Simon P. Avery for helpful advice on testing for oxytetracycline sensitivity and Peter A. Meacock for helpful discussions.

**REFERENCES**

Copper uptake in *Candida albicans*


