Candida albicans CFL1 encodes a functional ferric reductase activity that can rescue a Saccharomyces cerevisiae fre1 mutant

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Candida albicans, like other pathogens, has to compete with the host for a limited supply of available iron. Consequently, iron acquisition is likely to be an important factor for the growth, survival and virulence of this organism. It was previously demonstrated that C. albicans has a surface-associated ferric reductase similar to that of Saccharomyces cerevisiae. Therefore, functional rescue of a S. cerevisiae fre1 mutant was used to isolate a C. albicans ferric reductase gene (CFL1). This gene has been previously identified. However, the workers had not observed any functional reductase activity associated with the gene. The discrepancy with the findings in this report appears to be due to the clone previously reported carrying a non-contiguous piece of C. albicans DNA. Results shown in this paper demonstrate that CFL1 transcription is regulated in response to levels of iron and copper. This is the first demonstration of a functional ferric reductase gene from C. albicans.

Keywords: Candida albicans, CFL1, iron uptake, ferric reductase, Saccharomyces cerevisiae

INTRODUCTION

The imperfect fungus Candida albicans is a common commensal of the mouth, gut and vagina in humans, although under certain conditions it is capable of causing both superficial and systemic disease. Systemic infection by C. albicans is usually confined to immunocompromised patients, and is frequently fatal (Komshian et al., 1989). As with other microbial pathogens, virulence of C. albicans is likely to be multifactorial; characteristics such as proteinase secretion, adherence to host cells and hyphal production have all been implicated in virulence (Hube et al., 1997; Gale et al., 1998). Moreover, hyphal production and adherence have both been shown to be altered by growth in low-iron conditions (Sweet & Douglas, 1991a). It is probable, therefore, that iron acquisition plays a key role in the pathogenesis of C. albicans disease in ways similar to those proposed for many bacterial infections (reviewed by Wooldridge & Williams, 1993). Iron is an essential nutrient for most living organisms, playing a crucial role in many redox reactions within the cell. Iron levels must, however, be tightly regulated within the cell as high concentrations are toxic due to the ability of iron to catalyse the production of free radicals from molecular oxygen. For this reason, iron levels in the human host are highly restricted as free iron is sequestered to blood-borne proteins such as transferrin. As a result, invading pathogens require specialized high-affinity mechanisms to acquire iron, and iron acquisition is often found to play an important role in virulence. The entry of the pathogen into the iron-restrictive environment of the host may also trigger the expression of an array of other virulence-determinant genes unrelated to iron acquisition (Wooldridge & Williams, 1993).

It has been shown that C. albicans demonstrates reduced growth in human serum due to the presence of transferrin (Moors et al., 1992), and lactoferrin and ovo-transferrin are also antifungal (Valenti et al., 1986). C. albicans can acquire iron from haem, and can both bind and lyse erythrocytes (Manns et al., 1994; Moors et al., 1992). Moreover, siderophores of both phenolate and hydroxamate types have been reported (Ismail et al., 1985; Sweet & Douglas 1991b). However, it is not yet

Abbreviations: BCS, bathocuproine disulphonic acid; BPS, bathophenanthroline disulphonic acid; FNR, ferredoxin-NADP+ reductase.

The EMBL accession number for the sequence reported in this paper is AJ387722.
clear how iron is released from siderophores or haem for utilization by the cell. Previous work from our laboratory (Morrissy et al., 1996) presented biochemical evidence that C. albicans possesses a cell-surface ferric reductase which is regulated in response to levels of iron and copper. This reductase is similar in its activity and regulation to the surface reductase encoded by the FRE1 and FRE2 genes of Saccharomyces cerevisiae, which play a pivotal role in iron acquisition in this organism.

S. cerevisiae has often been used as a tool and model in the study of C. albicans since genetic analysis of C. albicans is made difficult by its lack of a sexual cycle and perpetual diploid state. S. cerevisiae possesses a well-characterized reductive-iron-uptake system which is capable of obtaining iron from a wide range of sources. This system comprises a cell-surface reductase and a transporter complex. FRE1 and FRE2 encode components of the cell-surface ferric reductase (Dancis et al., 1990; Georgatsou & Alexandraki, 1994) and the main transporter complex is encoded by genes FTR1 (Stearman et al., 1996) and FET3 (Askwith et al., 1994). Fet3p is a multicopper oxidase, which converts Fe²⁺ to Fe³⁺ during the uptake process, and requires copper for activity. Thus iron and copper uptake are inextricably linked and, indeed, the cell-surface ferric reductase is capable of reducing copper as well as iron (Hassett & Kosman, 1995). The genes encoding components of the iron-uptake system are transcriptionally regulated in response to iron concentrations in the growth media (Dancis et al., 1992). In iron-replete conditions genes are expressed at basal levels, whilst in low-iron conditions transcription is up-regulated. In addition, FRE1 transcription is negatively regulated in response to copper (Hassett & Kosman, 1995). In fact there are five other ferric reductase-like genes in S. cerevisiae, all of which are expressed and regulated in response to iron or copper, or both (Martins et al., 1998). Their roles in iron uptake have not yet been established. They may be involved with the surface-associated complex or, alternatively, may have an intracellular role.

A C. albicans ferric reductase-like gene, CFL1, has been identified on the basis of sequence homology with S. cerevisiae FRE1 but no functional ferric reductase activity was observed to be associated with it (Yamada-Okabe et al., 1996). Moreover, a recent project to sequence the C. albicans genome (http://alces.med.umn.edu/Candida.html) has identified 12 different sequences showing some identity with ferric reductase genes, but it is not yet known whether any of these short sequences are indeed part of expressed genes.

In this paper we describe the use of functional complementation to isolate a gene from C. albicans capable of restoring reductase activity to S. cerevisiae mutants defective in FRE1. The gene we identified was, in fact, the same one, CFL1, that had previously been described by Yamada-Okabe et al. (1996). However, our analysis shows that the sequence reported by these workers arose from a clone carrying non-contiguous pieces of C. albicans chromosomal DNA, which explains why no reductase activity was observed. Our work therefore represents the first demonstration of a functional ferric reductase gene from C. albicans.

METHODS

Strains, growth conditions and genetic techniques. S. cerevisiae strain S150-2B (MATa leu2-3 112his3A trp1-289 ure3-52), obtained from J. Hicks, Cold Spring Harbor, NY, USA, was the parental strain used in these experiments. Strain fre1::Δnuc (HS) was derived from S150-2B by single-step gene disruption as described in Results. C. albicans strain S/01, originally isolated from a human systemic infection, was obtained from R. Matthews, Dept. of Microbiology, University of Manchester, UK. Escherichia coli strain DH5x [Δ80lacZAM15 recA1 endA1 gyrA96 thi-1 hsdR17(rk mK) supE44 relA1 deoR A(lacZYA–argF)U169] was used for the propagation of plasmid pCRII DNA and library clones. The donor E. coli strain used in transposon mutagenesis was DH1 [recA1 endA1 gyrA96 thi-1 hsdR17(rk mK) supE44 relA1] carrying a derivative of the plasmid R388 transposed with a modified Tn1000 containing the yeast HIS3 selectable marker. The recipient strain was MH1578 [recA1 endA1 gyrA96 thi-1 hsdR17(rk mK) supE44 relA1 rpsL].

Yeast cultures were grown at 30 °C in either YNB [0.67 % yeast nitrogen base with ammonium sulphate (bio101)] or minimal defined medium (MD) based on Wickerham’s yeast nitrogen base recipe (Wickerham, 1951) with the addition of 20 mM sodium citrate (pH 4.2) (Eide et al., 1992). Amino acids were added to YNB and MD media at the final concentrations used by Sherman et al. (1986) and glucose was added at 2 % to all media. For growth on solid medium, 2 % agar was added to YNB or MD medium. MD medium was rendered iron restricted by the addition of 300 μM z′-dipyridyl (Sigma) (MD-dipyridyl), 50 μM BPS (bathophenanthroline disulphonic acid; Sigma), or 1 mM EDTA (MD-EDTA). Copper-depleted medium was made by the addition of 50 μM BCS (bathocuproine disulphonic acid; Sigma). E. coli cultures were grown at 37 °C in Luria–Bertani medium [1 % Bacto tryptone, 0.5 % Bacto yeast extract, 0.5 % NaCl, pH 7.2].

The C. albicans genomic library was obtained from P. Meacock, Dept. of Genetics, University of Leicester, UK. Plasmids were isolated from S. cerevisiae by the method of Holm et al. (1986). The procedure for S. cerevisiae transformation was the lithium acetate method of Geitz et al. (1992). Plasmid DNA was extracted from E. coli by the alkaline lysis method of Ish-Horowicz & Burke (1981). E. coli transformations were performed using the cold calcium chloride method (Maniatis et al., 1982). Transposon mutagenesis was performed using a derivative of the E. coli transposon Tn1000 containing the yeast HIS3 gene for selection (Sedgwick & Morgan, 1994).

Qualitative solid-phase ferric reductase assay. A modified version of a qualitative ferric reductase assay (Dancis et al., 1990) was used to identify extracellular ferric reductase activity. Cells were grown on YNB agar plates for 3–5 d and placed at 4 °C overnight. Colonies were transferred to nylon filters (Hybond-N) and incubated for 5 h on solid MD-dipyridyl media containing 300 μM FeCl₃. The filters were removed from the plates, incubated for 5 min on Whatman 3MM paper soaked in assay buffer (50 mM sodium citrate, pH 6.5, 5 % glucose), and then transferred to fresh 3MM paper.
soaked in assay buffer containing 1 mM FeCl₃ and 1 mM BPS for a further 5 min. Colonies with ferric reductase activity stained the filter red due to the formation of BPS[Fe²⁺] complexes.

**DNA manipulation and sequence analysis.** Restriction, cloning, agarose gel electrophoresis and Southern blot analysis were performed by standard methods (Maniatis et al., 1982). DNA sequencing was carried out using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) and DNA sequencer (ABI model 377XL). The protocol used was that provided by the manufacturers.

**RNA extraction and Northern analysis.** *C. albicans* cells were grown in MD medium containing either 50 µM BCS or 50 µM BPS to restrict copper and iron availability, respectively. High-copper conditions were made by adding 100 µM CuCl₂ to MD-BCS and high-iron conditions were made by adding 100 µM or 250 µM FeCl₃ to MD-BPS. Total RNA was extracted from these cells using the SDS-hot phenol method of Schmidt et al. (1990). RNA was electrophoresed through a 1.5% denaturing agarose gel, blotted onto nitrocellulose and hybridized overnight in Church–Gilbert buffer (0.5 M sodium phosphate, pH 7.4, 7% SDS, 1 mM EDTA; Church & Gilbert, 1984) at 65 °C using ³²P-labelled DNA as a probe. Stringent washes were carried out at 65 °C for 30 min in 3 × SSC, 0.1% SDS followed by 1 × SSC, 0.1% SDS and 0.5 × SSC, 0.1% SDS. (20 × SSC is 3 M sodium chloride, 0.3 M trisodium citrate). The filter was then exposed to Fuji Medical X-ray film at −80 °C for 2 weeks prior to developing.

**RESULTS**

**Construction of *S. cerevisiae* strain fre1::Tn(HIS3)**

*S. cerevisiae* strain *fre1::Tn(HIS3)* was generated from parental strain S150-2B by transposon disruption of the 5′ end of the *FRE1* gene. A sequence of 1146 bp at the 5′ end of the *FRE1* ORF was PCR amplified using primers 5′-ATGTTAGAACCCTGTATTTCTGC-3′ and 5′-CAGGTTGTCATAATGGTAGCATGGCC-3′, and cloned into the vector plasmid pCRII (Invitrogen). This construct was then used to transform *E. coli* strain DH1 harbouring the conjugative plasmid R388::Tn1000(HIS3). This plasmid was used to introduce the transposon into the cloned sequence by co-integrate formation during conjugal mating with the streptomycin-resistant strain MH1578, with selection for transfer of the ampicillin marker of the cloning vector pCRII and the streptomycin resistance of the recipient strain. Plasmid DNA was re-isolated and linearized using *XbaI* and *EcoNI* and used to transform S150-2B, with selection for histidine prototrophy, to generate strain *fre1::Tn(HIS3)*. Disruption of the wild-type *FRE1* allele was confirmed by Southern blot analysis (data not shown); the site of transposon insertion was upstream of the region encoding conserved motifs likely to play a catalytic role, such as those implicated in FAD and NAD(P)H binding (Fig. 1). Strain *fre1::Tn(HIS3)* was defective in reductase activity as measured by the solid-phase assay described in Methods (Fig. 2a). In addition, the mutant showed significantly reduced ability to grow in low-iron conditions (Fig. 2b), consistent with previous reports (Dancis et al., 1992). Addition of iron back to the medium restored growth to almost wild-type levels, indicating that it was lack of iron that was causing the lack of growth (Fig. 2c).

**Cloning of a *C. albicans* ferric reductase gene**

A *C. albicans* library of genomic fragments from a partial *Sac3A1* digest cloned into the BamHI site of YEp213 (P. Meacock, personal communication) was used to transform *S. cerevisiae fre1::Tn(HIS3)* and screened for clones able to complement the reductase-deficient phenotype using the solid-phase ferric reductase assay. Two positive clones were identified from 36000 colonies screened (Fig. 2a). One of these plasmids designated pJDF1.3, gave consistent rescue of reductase activity upon isolation and retransformation and was therefore chosen for further study. Furthermore, poor growth of the *fre1::Tn(HIS3)* mutant on low iron was restored to approximately wild-type levels by the presence of the plasmid pJDF1.3 (Fig. 2b). Since slow growth on iron-restricted medium must be due to a defect in iron uptake resulting in reduced intracellular iron levels, we propose that the ferric reductase activity associated with pJDF1.3 plays a role in iron accumulation.

**Localization and sequence analysis of the ferric reductase gene pJDF1.3**

Restriction analysis of plasmid pJDF1.3 (Fig. 3) indicated that it carried an insert of 7·6 kb which was likely to contain more than one ORF, and so transposon mutagenesis was used to localize the region responsible for ferric reductase activity. Plasmid pJDF1.3 DNA was used to transform *E. coli* strain DH1/ R388::Tn1000(HIS3), which was then used as the donor for conjugal matings with selection for the ampicillin-resistance marker of the vector. Plasmid DNA isolated from the transconjugants represented pJDF1.3 derivatives containing random transposon insertions; this DNA was used to transform *fre1::Tn(HIS3)*, with
Fig. 2. C. albicans genomic clone pJDF1.3 rescues ferric reductase activity and growth on low iron in a S. cerevisiae fre1 mutant. (a) Reductase activity of rescuing clones was assessed using a solid-phase reductase assay. Cells grown on SD medium were replica plated on to nylon filters placed on the surface of MD-dipyridyl plates containing 300 µM FeCl₃. The plates were incubated at 30 °C for 5 h and the filters removed. Ferric reductase activity was identified as described in Methods. The controls on the bottom row of the filter show that ferric reductase activity of the fre1 mutant is lost relative to the parental strain (S150-2B). Two C. albicans genomic clones, pJDF1.3 and pJDF2.3, restore ferric reductase activity to the fre1 mutant. Other clones shown did not rescue ferric reductase activity. (b) Cells growing in YPD medium were harvested at mid-exponential phase, washed and resuspended in water at 1 × 10⁷ cells ml⁻¹. A series of 10-fold dilutions were made and 5 µl cells at concentrations ranging from 1 × 10⁷ to 1 × 10⁵ cells ml⁻¹ were spotted on to agar plates made up of MD-EDTA media. The plates were incubated at 30 °C for 5 d. (c) The S. cerevisiae fre1 mutant and S150-2B were treated as described above (b) but the serial dilutions plated onto MD-EDTA medium containing 800 µM FeCl₃. The plates were incubated at 30 °C for 5 d.

More extensive sequencing of pJDF1.3, using different primers to walk along the insert, showed 99% identity with the sequence within the CFL1 ORF predicted by Yamada-Okabe et al. (1996) and with 269 bp to the 3' end of it extending into the adjacent gene CGT1. Homology broke down abruptly, however, 122 bp upstream of the original (previously published) predicted ATG translation-start site (Fig. 4). The discrepancy arises at a SacI restriction site, suggesting that the sequence published by Yamada-Okabe et al. (1996) may in fact be derived from the incorrect juxtaposition of two non-contiguous genomic DNA fragments, thus explaining the observed inability of their plasmid to rescue a ferric reductase mutant. Our sequencing data predict that the CFL1 open reading frame extends 276 bp upstream of the published ATG (Fig. 4), giving a predicted product of 761 amino acids, with a putative cleavable N-terminal signal sequence of 18 amino acids, consistent with Cfl1p being a cell-surface integral-membrane protein (PSORT; http://psort.nibb.ac.jp:8800/).

We performed a comparative PCR analysis of pJDF1.3 plasmid and genomic DNA using primers designed to amplify the 5' terminus of CFL1 and the untranslated region upstream of CFL1 (Fig. 5). PCR products of identical sizes were obtained from both templates with each combination of primers used, including some that spanned the SacI site at which homology with the previously published sequence broke down. These data confirm that the DNA cloned in plasmid pJDF1.3

Fig. 3. Restriction map of the pJDF1.3 C. albicans genomic clone showing sites of transposon insertion. The flanking sequence of the plasmid is shown as a thin line and the genomic insert as a thick line. The vertical arrows show the sites of transposon insertion. The plasmid carrying the transposon marked with the asterisk (*) (pJDF1.3a) was used for sequencing. Restriction sites are: B, BglII; C, ClaI; E, EcoRI; H, HindIII; S, SalI; X, XbaI.

selection for leucine prototrophy conferred by the LEU2 gene on the library plasmid, and the resulting colonies were assayed for reductase activity. Plasmid DNA was isolated from four colonies that showed no activity, retransformed into fre1::Tn(HIS3) and reassayed to confirm that the observed phenotype, failure to rescue ferric reductase deficiency, was indeed plasmid-associated. Transposon-insertion sites in these plasmids were located by restriction mapping; all four were in a 1.8 kb EcoRI fragment (Fig. 3), indicating that the gene of interest lay in this region. One of these plasmids (designated pJDF1.3a) was picked for sequence analysis using primers to sequence outwards from the transposon (Fig. 4). Searches of the C. albicans information pages (http://alces.med.umn.edu/Candida.html) revealed that the gene had previously been sequenced and named CFL1 (for Candida ferric reductase-like gene) (Yamada-Okabe et al., 1996). However, these authors were unable to demonstrate rescue by CFL1 either of the ferric reductase deficiency of a S. cerevisiae fre1 mutant or of the characteristic poor growth of the fre1 mutant in low-iron conditions.
A ferric reductase gene from *C. albicans*

The predicted protein product of *CFL1*

Comparison of the predicted Cfl1p amino acid sequence with the *S. cerevisiae* Genome Database (http://genome-www.stanford.edu/Saccharomyces/) using the FASTA program (Pearson & Lipman, 1988) revealed similarities with two *S. cerevisiae* proteins, Fre1p and Fre2p, both of which are structural components of the cell-surface ferric reductase complex. Direct comparison using the program with gap from the GCG package (Wisconsin Package version 9.1, Genetics Computer Group, Madison, WI) showed Cfl1p to have 37.7% similarity and 27.5% identity with Fre2p and 37.4% similarity and 26.8% identity with Fre1p. Significantly, several motifs within the predicted Cfl1p protein sequence are found in all known ferric reductases; three of these are implicated in FAD and NAD(P)H binding and are found in the wider family of ferredoxin-NADP+ reductase (FNR) proteins (Fig. 6a). Transposon insertions that resulted in loss of reductase activity were upstream of at least one of these motifs (Fig. 3). The transposon used for sequencing was mapped to upstream of both NAD(P)H binding sites (motifs 2 and 3) as well as motif 4. Perhaps most interestingly, one transposon was mapped to the 3′ end of the gene approximately 200 bp upstream of motif 3, which is implicated in NAD(P)H binding, suggesting that even this most 3′ motif is required for the proper function of this gene. A hydropathy plot (Kyte & Doolittle, 1982) of Cfl1p shows that it has multiple hydrophobic regions (Fig. 6b) consistent with its being a multi-spanning membrane protein like both Fre1p and Fre2p (Georgatsou & Alexandraki, 1994). Finegold et al. (1996) demonstrated that there are four critical histidine residues in the transmembrane domains of the FRE1 protein, which are important for binding of the haem co-factor. Four histidine residues are also found at comparable locations in the predicted Cfl1p (Fig. 6b).

**CFL1 expression is negatively regulated by copper**

Northern blot analysis was used to investigate the possibility that the expression of *CFL1* may be regulated in response to iron or copper levels, similar to that reported for the *FRE* genes of *S. cerevisiae*. (Fig. 7). RNA was prepared from *C. albicans* S/01 cultures grown in MD medium containing high- and low-iron concentrations. **RNA samples were analysed for** *CFL1* mRNA using the 1.8 kb fragment obtained by *EcoRI* digestion of pJDF1.3 as a probe (Fig. 3). Samples were also probed with the housekeeping gene *CaURA3* to ensure equal loading of samples. The *CFL1* transcript was more abundant in samples from cells grown in conditions of low iron or low copper than in cells grown in media replete for these metals (Fig. 7).
Fig. 6. Sequence comparison of CFL1, FRE1 and FRE2. (a) The predicted amino acid sequences of CFL1, FRE1 and FRE2 were aligned using PILEUP from the GCG package and examined for motifs shown to be conserved throughout the FNR family of proteins. Three conserved motifs implicated in FAD and NAD(P)H binding are shown. Two other motifs of unknown function are also shown. (b) Hydropathy plots of Cfl1p and Fre1p performed using the Kyte-Doolittle algorithm (Kyte & Doolittle, 1982). Putative transmembrane regions are shown as black bars and the four conserved histidine residues thought to play a role in haem binding are shown as white triangles. Other conserved motifs (see above) are shown as black triangles.

Fig. 7. Northern blot analysis of CFL1 transcripts. Total RNA was extracted from exponentially growing cultures of C. albicans grown in MD-BPS media containing 0 µM (lane 1), 100 µM (lane 2) or 250 µM (lane 3) FeCl3 or from cultures grown in MD-BCS media containing 0 µM (lane 4) or 100 µM (lane 5) CuCl2. Following electrophoresis and transfer to a nylon membrane, duplicate sets of the three RNA samples were probed with either a 32P-labelled 1-8 kb EcoRI fragment of CFL1 (see Fig. 3) or with the C. albicans URA3 gene as a loading control.

DISCUSSION

We have used functional homology to identify a C. albicans genomic clone capable of rescuing a S. cerevisiae ferric reductase mutant. This is the first report of the isolation of a functional ferric reductase gene from C. albicans, and provides support for the previous biochemical evidence from our laboratory showing that C. albicans possesses a cell-surface ferric reductase activity (Morrissey et al., 1996). The gene, CFL1, had previously been noted but no ferric reductase had been shown to be associated with it (Yamada-Okabe et al., 1996). In contrast to this previously published report, we have shown that CFL1 can rescue both the ferric reductase deficiency and slow growth on low-iron phenotypes of a S. cerevisiae fre1 mutant. The contradiction between our findings and those of Yamada-Okabe et al. (1996) is due to a divergence of sequence at the 5′ end of the gene which we believe arose by the joining of two non-contiguous fragments of genomic DNA in the previously published sequence. Our results indicate that the ORF is 276 bp longer than previously thought and the predicted amino acid sequence contains a putative hydrophobic signal sequence at the N-terminus.

The predicted amino acid sequence of Cfl1p shows homology with ferric reductase genes from S. cerevisiae.
In particular, it shows high conservation of several specific domains such as those implicated in FAD and NAD(P)H binding, which are also found to be conserved between the wider family of FNR proteins as well as other domains with no known function. The conservation of the positioning of four histidine residues, essential for haem binding in Fre1p, is also interesting and indicates that similarly Cfl1p may also be haem-dependent.

CFL1 transcription is negatively regulated by both iron and copper. This is not unexpected, since previous biochemical evidence showed that the C. albicans ferric reductase activity is regulated by both copper and iron. A cell that is unable to acquire iron can be seen as a reflection of intracellular iron accumulation by the cell. This would imply that CFL1 is indeed being directed to the cell surface as the presence of a hydrophobic signal sequence suggests. Thus, to date there are two parallel lines of evidence indicating that C. albicans uses a ferric reductase in iron acquisition in a similar manner to S. cerevisiae. Previous biochemical evidence (Morrissey et al., 1996) showed that it possesses a cell-surface ferric reductase activity whilst genetic evidence presented here demonstrates that C. albicans CFL1 is capable of rescuing a S. cerevisiae ferric reductase mutant and shows homology to other known ferric reductase genes. Furthermore, the Candida genome sequencing project has revealed the presence of a FET3-like gene in C. albicans. FET3 is a S. cerevisiae gene encoding a ferrous oxidase that forms part of the iron-transporter complex that internalizes iron after it has been reduced by the reductase (Askwith et al., 1994). This provides additional support for the hypothesis that it uses a similar reductive mechanism to S. cerevisiae in iron acquisition. All studies carried out to date have been performed in vitro and it is not clear how this may relate to the in vivo situation. It is known that C. albicans can acquire iron from siderophores and haem, both sources that are likely to be available in vivo. The mechanism by which it may acquire iron from such sources and the role of the reductase in this have yet to be assessed. However, with the isolation of genes implicated in iron acquisition, we are now in a position to be able to construct mutant strains with deletions of these genes and assay for the effects on growth and ability to acquire iron from a range of different sources.

REFERENCES


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