The Transcription Factor Homolog CTF1 Regulates β-Oxidation in Candida albicans

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Carbon starvation is one of the many stresses to which microbial pathogens are subjected while in the host. Pathways necessary for the utilization of alternative carbon sources, such as gluconeogenesis, the glyoxylate cycle, and β-oxidation of fatty acids, have been shown to be required for full virulence in several systems, including the fungal pathogen Candida albicans. We have investigated the regulatory network governing alternative carbon metabolism in this organism through characterization of transcriptional regulators identified based on the model fungi, Saccharomyces cerevisiae and Aspergillus nidulans. C. albicans has homologs of the ScCAT8/AnFacB and ScADR1/AnAmdX transcription factors that regulate induction of genes encoding the proteins of gluconeogenesis, the glyoxylate cycle, and ethanol utilization. Surprisingly, C. albicans mutants lacking CAT8 or ADR1 have no apparent phenotypes and do not regulate genes for key enzymes of these pathways. Fatty acid degradation and peroxisomal biogenesis are controlled by nonhomologous regulators, OAF1/PIP2 in S. cerevisiae and FarA/FarB in A. nidulans; C. albicans is missing OAF1 and PIP2 and, instead, has a single homolog of the Far proteins, CTF1. We have shown that CTF1 is required for growth on lipids and for expression of genes necessary for β-oxidation, such as F0X2. ctf1Δ/ctf1Δ (ctf1Δ/Δ) strains do not, however, show the pleiotropic phenotypes observed for fox2Δ/Δ mutants. The ctf1Δ/Δ mutant confers a mild attenuation in virulence, like the fox2Δ/Δ mutant. Thus, phenotypic and genotypic observations highlight important differences in the regulatory network for alternative carbon metabolism in C. albicans compared to the paradigms developed in other model fungi.

Candida albicans is both a ubiquitous commensal of the human microbial flora and the most important fungal pathogen of humans (9, 43). While C. albicans can infect nearly any site in the body, the most serious manifestation, disseminated bloodstream infections, particularly affects immunocompromised individuals and is fatal in about 40% of cases (67). Candida species are responsible for ~9% of cases of hospital-acquired sepsis and up to 12% of central line-associated bloodstream infections, with C. albicans causing about half of these infections (23, 67). Studying the biology of C. albicans in its natural niche, the mammalian host, provides insights into how this intriguing species has adapted to become such a successful pathogen and, as a result, is crucial to the development of new drug targets and treatment strategies.

An increasing body of literature indicates that some host niches are carbon limited and that mutations that abrogate utilization of nonfermentable carbon sources are compromised in virulence models for many (but not all) fungal pathogens of both plants and animals, including C. albicans, Magnaporthe grisea, Leptosphaeria maculans, Stagonospora nodorum, and Colletotrichum lagenarium (2, 5, 27, 33, 46, 48, 59, 66). In particular, studies have focused on the pathways of gluconeogenesis, the glyoxylate cycle and β-oxidation of fatty acids, as these pathways are specifically upregulated during contact with host immune cells in a variety of human fungal pathogens (5, 14, 18, 32, 33, 47, 51, 63). The glyoxylate cycle, which assimilates two-carbon compounds into the tricarboxylic acid cycle, is also required for virulence in some bacterial pathogens, notably Mycobacterium tuberculosis (35, 36). It therefore seems likely that C. albicans and other pathogens utilize multiple nonfermentable carbon sources during infection. We and others have also shown that mutations in key genes of the pathways of alternative carbon metabolism confer pleiotropic phenotypes, suggesting that the regulation and function of these pathways in C. albicans have diverged from the paradigms developed in model systems (46, 48). The fungal regulatory networks for alternative carbon metabolism are best understood in Saccharomyces cerevisiae and Aspergillus nidulans. In both species, regulation of genes involved in alternative carbon metabolism pathways occurs in two distinct stages: glucose repression and induction by specific carbon sources. While the molecular mechanisms are slightly different, the presence of glucose inactivates genes for alternative carbon metabolism via the ScMIG1/AnCreA transcriptional repressors (where Sc represents S. cerevisiae and An represents A. nidulans). Under glucose-limiting conditions, this repression is relieved, and alternative carbon genes are expressed at a basal level (10, 53, 54). Subsequently, specific carbon sources activate subsets of genes required for their utilization. In S. cerevisiae, the primary transcriptional regulators involved are CAT8, ADR1, OAF1, and PIP2; a few others play minor roles. CAT8 induces expression of genes involved in the glyoxylate cycle and gluconeogenesis, while ADR1 regulates ethanol utilization and OAF1/PIP2 regulate peroxisomal biogenesis and β-oxidation (11, 13, 22, 50, 56). All four of these proteins exert combinatorial control on a variety of carbon-related promoters (57, 61, 68).

Regulation of carbon metabolism is more complex in filamentous fungi such as A. nidulans. Beyond CreA-mediated

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glucose derepression. FacB induces genes required for acetate metabolism, including those involved in gluconegenesis and the glyoxylate cycle (28, 64). These pathways, however, are also induced by other nonfermentable carbon sources in a FacB-independent manner. In the presence of long-chain fatty acids, this induction is mediated by FarA and FarB, homologous transcription factors, while a third protein, ScfA, responds to short-chain fatty acids (24). Two other transcription factors, AcuK and AcuM, also regulate gluconegenic genes and are required for growth on nonfermentable carbon sources (26). AmdX, the closest A. nidulans sequence homolog to ADR1, regulates the acetamidase AmdS, but is not required for growth on a variety of nitrogen or carbon sources (39). Thus, while S. cerevisiae has an integrated transcriptional network, A. nidulans possesses multiple circuits that regulate carbon metabolism (26).

In this work, we have characterized the C. albicans homologs of some of these transcriptional regulators. The putative C. albicans CAT8 and ADR1 proteins show high homology to the cognate regulators in both S. cerevisiae and A. nidulans. Surprisingly, we found no phenotypes for C. albicans strains lacking CAT8 or ADR1, and they do not appear to regulate the expression of the core genes required for alternative carbon metabolism, such as those encoding fructose-1,6-bisphosphatase (FBP1), isocitrate lyase (ICL1), or the multifunctional protein of β-oxidation (FOX2). These findings are substantially different from those for the corresponding S. cerevisiae CAT8 or ADR1 mutants or for the A. nidulans facB mutant (22, 28, 64, 68).

C. albicans lacks identifiable homologs of the S. cerevisiae β-oxidation/peroxisome biogenesis regulators OAF1 and PIP2. Instead, as we report here, C. albicans has a single homolog of the A. nidulans transcription factors FarA/FarB, which regulate genes for β-oxidation enzymes, gluconegenesis, and the glyoxylate cycle in the presence of long-chain fatty acids in that species (24). We demonstrate that CTF1 is necessary for growth on fatty acids, regulates expression of several genes encoding enzymes of β-oxidation, including FOX2, and when mutated, confers a mild attenuation of virulence, similar to that reported for fox2α/fox2Δ (fox2α/Δ) strains (46, 48). Thus, at a phenotypic and genotypic level, the regulation of alternative carbon assimilation pathways in C. albicans appears to share more similarity to that of filamentous fungi than to that of budding yeast, but there are clearly Candida-specific adaptations in these regulatory networks.

MATERIALS AND METHODS

Strains and media. The C. albicans strains used are listed in Table 1 and are based on SC5314 and its auxotrophic derivatives CAH4-F2 and RM1000 (17, 40). Standard yeast media were used (55), including YPD (1% yeast extract, 2% peptone, 2% dextrose) and YNB (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose). To test carbon utilization, the glucose was replaced by peptone, 2% dextrose) and YNB (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose). To test carbon utilization, the glucose was replaced by peptone, 2% dextrose) and YNB (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose). The resulting plasmids were pMR5 (cat8α::hisG-URA3-hisG), pMR16 (cat8α::HIS1), pMR12 (cat8::hisG-URA3-hisG), and pMR14 (ctf1Δ::HIS1). For transformation, the disruption construct was liberated from the plasmid backbone by using HindIII/SacII. These were used to transform CAH4-F2 or RM1000. Correct heterozygotes were confirmed by PCR. For adr1 heterozygotes, Ura− recombinants were selected on media containing 5-fluoroorotic acid (5). The second allele was disrupted in the same manner, and homozygous mutants were confirmed by PCR for the presence of the disruption alleles and the absence of the wild-type allele. For cat8 and ctf1 mutants, the first allele was disrupted using the hisG-URA3-hisG cassette, and the second allele was disrupted using the HIS1 disruption cassette.

Complementation. Complementing constructs for ADR1, CAT8, and CTF1 were made by PCR-amplifying the open reading frame flanked by ∼500 bp of the 5′ UTR and ∼300 bp of the 3′ UTR. PCR products were cloned into Cpi10, which targets integration to the phenotypically neutral RPS10 locus (7, 38). Ura− homozygous mutant strains were transformed with either the complementation plasmids pMR1, CIp10-ADR1; pMR6, CIp10-ADF1; or pMR10-(CTF1)- or empty Cpi10. Integration at the RPS10 locus was verified by PCR.

In vitro growth assays. Strains used in the spot dilution assay were grown to mid-log phase in YPD, collected by centrifugation, washed with water, and transferred to 96-well plates at an optical density at 600 nm of 1.0. The cells were then serially diluted fivefold and spotted onto solid YNB medium containing 2% glucose, potassium acetate, ethanol, oleate, olive oil, glycerol, or Tween as the sole carbon source by using a multichannel micropipette. Plates were incubated at 30°C for 3 to 7 days, depending on the carbon source, as indicated in the figure legends.

Northern blotting. C. albicans strains were grown overnight in YNB medium with 2% glucose and then diluted into the same medium and grown for 4 h at 30°C to mid-log phase. Cells were then harvested by centrifugation, washed twice with water, and resuspended in YNB medium containing 2% (w/v) glucose, 2% (w/v) potassium acetate, or 2% (v/v/v) oleic acid. The cultures were grown for 1 h at 30°C, then collected by centrifugation. RNA was isolated using hot acidic phenol (3). Fifteen nanograms of RNA per sample was run on a 1% MOPS (morpholinepropanesulfonic acid) agarose gel, then transferred to nitrocellulose. Gene-specific probes were amplified by PCR, labeled with the KudPrime DNA labeling system (Invitrogen), and purified using Quick Spin column (Pierce). Blots were incubated in prehybridization solution consisting of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50% formamide, 5× Denhardt’s solution, 0.1% sodium dodecyl sulfate, and 100 mg/ml single-stranded DNA for 2 h at 42°C and then hybridized with labeled probe overnight. RNA was used as a loading control either by ethidium bromide staining or by stripping the transfer membranes in boiling 0.1% sodium dodecyl sulfate and then reprobed with an 18S rRNA probe for all subsequent experiments.

GFP peroxisomal targeting sequence (PTS) assays. All green fluorescent protein (GFP) plasmids were based on pACT1-GFP (4). Primers were designed to amplify GFP from this plasmid and incorporate the last five amino acids of the C terminus of CaCL1 (where Ca represents C. albicans), CaFOX2, or SCML1 at the C terminus of GFP. The resulting PCR product was digested with HindIII and NheI, and GFP was excised from pACT1-GFP by using the same restriction enzymes. The digested PCR product and vector were ligated to create pMR23, pMR24, and pMR26 (Table 2). Each plasmid was transformed into CAH4-F2, MRC113 (per5ΔΔΔ), MRC3 (fox2Δ/Δ), and MRC40 (ctf1Δ/Δ). Integration at RPS10 was confirmed by PCR. The resulting GFP strains were grown overnight in YNB, washed and diluted in fresh media, and grown to log phase. Cells were then washed with water and resuspended in YNB with 2% glucose or 2% oleate and grown for 1 h at 30°C. GFP localization was visualized by fluorescence microscopy.

In vivo virulence assays. C. albicans cultures were grown to mid-log phase in YPD overnight. The next day, cells were collected by centrifugation, washed, and resuspended in phosphate-buffered saline. Ten female ICR mice per strain were infected via tail vein injection with 106 C. albicans yeast-form cells as described previously (48). The mice were subsequently monitored for signs of infection and
TABLE 1. C. albicans strains

| Strain               | Description                                                                 | Reference |
|----------------------|-----------------------------------------------------------------------------|-----------|
| Control strains      |                                                                             |           |
| SC5314               | Wild type                                                                   | 17        |
| CA14-F2              | ura3::Xmn434::ura3::Xmn434                                                   | 17        |
| RM1000               | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG                              | 40        |
| MRC6                 | uaa::Xmn434::ura3::Xmn434 fox2::HisG fox2::HisG RP10::RP10::URA3             | 48        |
| MRC10                | uaa::Xmn434::ura3::Xmn434 ic1::HisG ic1::HisG RP10::RP10::URA3              | 48        |
| Transcription factor mutants |                                                                             |           |
| MRC24                | uaa::Xmn434::ura3::Xmn434 adr1::HisG adr1::HisG RP10::RP10::URA3             | This study|
| MRC25                | uaa::Xmn434::ura3::Xmn434 adr1::HisG adr1::HisG RP10::RP10::URA3             | This study|
| MRC89                | uaa::Xmn434::ura3::Xmn434 adr1::HisG adr1::HisG RP10::RP10::URA3             | This study|
| MRC90                | uaa::Xmn434::ura3::Xmn434 adr1::HisG adr1::HisG RP10::RP10::URA3             | This study|
| MRC79                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG cat8::HisG cat8::His1 RP10::RP10::URA3 | This study|
| MRC80                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG cat8::HisG cat8::His1 RP10::RP10::URA3 | This study|
| MRC77                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG cat8::HisG cat8::His1 RP10::RP10::URA3 | This study|
| MRC78                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG cat8::HisG cat8::His1 RP10::RP10::URA3 | This study|
| MRC41                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1 RP10::RP10::URA3 | This study|
| MRC42                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1 RP10::RP10::URA3 | This study|
| MRC43                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1 RP10::RP10::URA3 | This study|
| MRC49                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1 RP10::RP10::URA3 | This study|
| PTS GFP strains      |                                                                             |           |
| MRC91                | uaa::Xmn434::ura3::Xmn434 RPS10::RP10::pACT1p-GFP-SKAKA-COOH (CaICL1)       | This study|
| MRC93                | uaa::Xmn434::ura3::Xmn434 RPS10::RP10::pACT1p-GFP-DKAKI-COOH (CaFOX2)       | This study|
| MRC94                | uaa::Xmn434::ura3::Xmn434 RPS10::RP10::pACT1p-GFP-DLSKL-COOH (ScMLS1)       | This study|
| MRC113               | uaa::Xmn434::ura3::Xmn434 pe5::HisG pe5::HisG                              | This study|
| MRC114               | uaa::Xmn434::ura3::Xmn434 pe5::HisG pe5::HisG                              | This study|
| MRC116               | uaa::Xmn434::ura3::Xmn434 pe5::HisG pe5::HisG                              | This study|
| MRC117               | uaa::Xmn434::ura3::Xmn434 pe5::HisG pe5::HisG                              | This study|
| MRC3                 | uaa::Xmn434::ura3::Xmn434 fox2::HisG fox2::HisG                             | This study|
| MRC122               | uaa::Xmn434::ura3::Xmn434 fox2::HisG fox2::HisG                             | This study|
| MRC124               | uaa::Xmn434::ura3::Xmn434 fox2::HisG fox2::HisG                             | This study|
| MRC125               | uaa::Xmn434::ura3::Xmn434 fox2::HisG fox2::HisG                             | This study|
| MRC40                | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1       | This study|
| MRC133               | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1       | This study|
| MRC134               | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1       | This study|
| MRC135               | uaa::Xmn434::ura3::Xmn434 his1::HisG his1::HisG ctf1::His1 ctf1::His1       | This study|

Euthanized when moribund according to protocols approved by the Animal Welfare Committee of the University of Texas Health Science Center at Houston, TX. Survival data were plotted using Prism 5 (GraphPad Software) and analyzed using the log-rank test.

RESULTS

Identification of putative transcriptional regulators of carbon utilization. Work from our lab and from others has previously demonstrated a role for the pathways of alternative carbon metabolism in the virulence of C. albicans (5, 46, 48). We sought to further understand the roles of these processes in C. albicans by analyzing the regulatory networks that control these pathways. These networks are best understood in the model yeast, S. cerevisiae, involving the interplay of several transcriptional regulators, including MIG1 (mediating glucose repression), CAT8 (gluconeogenesis and glyoxylate cycles), ADR1 (alcohol dehydrogenases and fatty acid degradation), and the OAF1/PIP2 heterodimer (fatty acid degradation and peroxisome biogenesis). MIG1 was shown to have a role in glucose repression in C. albicans via transcript profiling, though one more subtle than that for the S. cerevisiae homolog (37, 69). The other transcription factors have not been studied in detail; CAT8 (orf19.5097) was shown to be a minor regulator of the JEN1 lactate permease (58) while the ADR1 homolog (orf19.2752) was identified during genome annotation (8) and is otherwise uncharacterized. The C. albicans CAT8 is 38.0% identical to the S. cerevisiae protein and 28.2% identical to AnFaB, while CaADR1 shares 24.7% and 23.8% identity with the S. cerevisiae and A. nidulans homologs, respectively. Interestingly, despite the overall similar homologies between
C. albicans and the other two species, a pairwise comparison of these proteins of S. cerevisiae and A. nidulans reveals very high divergence—only 6.2% identity for CAT8/FacB and 5.7% for ADR1/AmdX.

In contrast, C. albicans homologs of the peroxisome/β-oxidation regulators OAF1 and PIP2 were not previously identified. We attempted to use a bioinformatic approach to find these genes, but the best hit was a distant match to C. albicans CTA4 (Fig. 1B), a transcriptional regulator involved in the response to nitrosative stress (11). A mutant of CTA4 was tested for its ability to assimilate a variety of nonfermentable carbon sources and found to have no defects (12; our unpublished observations), indicating that it has no apparent role in carbon metabolism in C. albicans.

The regulation of carbon metabolism has also been thoroughly studied in A. nidulans, for which Hynes and coworkers identified FarA and FarB, homologous transcription factors required for growth on fatty acids (24). C. albicans has a single homolog of the Far proteins (Fig. 1A), CTF1, which is 39.1% identical to FarA and 27.4% identical to FarB. S. cerevisiae does not have a Far/CTF1 homolog, and there is no sequence similarity between Far/CTF1 and OAF1/PIP2 (Fig. 1 and data not shown). CTF1 (orf19.1499) was named by the genome annotation effort (8) based on its homology to the cutinase transcription factor of Fusarium solani (Nectria haematococca), the first of this family of transcription factors to be identified (30).

**Effects of transcription factor mutants on carbon assimilation.**

Mutant strains deleted for each transcription factor were made in C. albicans (see Materials and Methods). The mutants were made in CAI4-F2 (adr1/H9004) or RM1000 (cat8/H9004 ctf1/H9004) and complemented by integration of the gene at the

### Table 2. Plasmids

| Plasmid | Description | Reference |
|---------|-------------|-----------|
| pCtp10  | RPS10-integrating plasmid | 38 |
| pMR1    | pCtp10-CaCAT8 | This study |
| pMR6    | pCtp10-CaADR1 | This study |
| pMR17   | pCtp10-CaTF1 | This study |
| pMR21   | pCtp10-CaPEX5 | This study |
| pMR3    | pBSK-adr1::hisG-URA3-hisG | This study |
| pMR5    | pBSK-cat8::hisG-URA3-hisG | This study |
| pMR12   | pBSK-ctf1::hisG-URA3-hisG | This study |
| pMR14   | pBSK-ctf1::HIS3 | This study |
| pMR16   | pBSK-cat8::HIS3 | This study |
| pMR19   | pBSK-pex5::hisG-URA3-hisG | This study |
| pACT1-GFP | pACT1-GFP in Ctp10 | 4 |
| pMR23   | pACT1p-GFP-SKAKA-COOH (CaICL1) | This study |
| pMR24   | pACT1p-GFP-DKAKI-COOH (CaFOX2) | This study |
| pMR26   | pACT1p-GFP-DLSKL-COOH (ScMLS1) | This study |

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![FIG. 1. Two distinct families of transcription factors regulating fatty acid catabolism and peroxisome biogenesis in ascomycetes. Homologs were identified based on BLAST similarity to C. albicans CTF1 (A) or S. cerevisiae OAF1 (B). Only hits with a BLAST e value of <10 to 50 were included, except for C. albicans CTA4, which is the closest C. albicans homolog of ScOAF1. Proteins were aligned using ClustalW, and the phylogenetic tree was built using the neighbor-joining algorithm. A subset of homologs was included, but those chosen represent the species diversity of these family members. Boldface type indicates a homolog from a species pathogenic to humans. F. oxysporum, Fusarium oxysporum; Y. lipolytica, Yamowia lipolytica; D. hansenii, Debaryomyces hansenii; L. elongisporus, Lodderomyces elongisporus; C. guilliermondii, Candida guilliermondii; C. immitis, Coccidioides immitis; A. niger, Aspergillus niger; C. glabrata, Candida glabrata; V. polyspora, Vanderwaltozyma polyspora; A. gossypii, Ashbya gossypii; K. lactis, Kluyveromyces lactis.**

| Plasmid | Description | Reference |
|---------|-------------|-----------|
| pCIp10  | RPS10-integrating plasmid | 38 |
| pMR1    | pCtp10-CaCAT8 | This study |
| pMR6    | pCtp10-CaADR1 | This study |
| pMR17   | pCtp10-CaTF1 | This study |
| pMR21   | pCtp10-CaPEX5 | This study |
| pMR3    | pBSK-adr1::hisG-URA3-hisG | This study |
| pMR5    | pBSK-cat8::hisG-URA3-hisG | This study |
| pMR12   | pBSK-ctf1::hisG-URA3-hisG | This study |
| pMR14   | pBSK-ctf1::HIS3 | This study |
| pMR16   | pBSK-cat8::HIS3 | This study |
| pMR19   | pBSK-pex5::hisG-URA3-hisG | This study |
| pACT1-GFP | pACT1-GFP in Ctp10 | 4 |
| pMR23   | pACT1p-GFP-SKAKA-COOH (CaICL1) | This study |
| pMR24   | pACT1p-GFP-DKAKI-COOH (CaFOX2) | This study |
| pMR26   | pACT1p-GFP-DLSKL-COOH (ScMLS1) | This study |
RPS10 locus, as described previously (7, 38). We constructed at least two independent deletion strains for each gene. The mutant strains were viable and had no apparent phenotype on glucose-containing media (Fig. 2).

We tested the resulting mutant and complemented strains for their ability to utilize a variety of nonfermentable compounds as sole sources of carbon by spotting serial dilutions onto solid media. None of these strains had growth defects on media containing the nonfermentable carbon sources acetate, ethanol, or glycerol (Fig. 2A). However, the $ctf1^{\Delta}/H9004^{\Delta}/MRC41$ mutant failed to utilize oleic acid (oleate) or linolenic acid, both unsaturated long-chain fatty acids, and was significantly impaired on media containing extra-virgin olive oil, a mixture of triglycerides, as a carbon source; this was complemented by the reintegration of a wild-type copy of the gene (Fig. 2B). A second, independently constructed, $ctf1^{\Delta}/H9004^{\Delta}^{\Delta}$ mutant strain behaved identically (data not shown). Our demonstration that $ctf1^{\Delta}/H9004^{\Delta}^{\Delta}$ mutants fail to grow on oleate is in contrast to an earlier report in which no such defect was seen (12). A strain lacking the glyoxylate cycle enzyme isocitrate lyase ($icl1^{\Delta}/H9004^{\Delta}/MRC10^{\Delta}$) is defective on a variety of alternative carbon sources (48) and was included as a control. FOX2, the multifunctional enzyme of $/H9252$-oxidation, had not been previously tested on linoleic acid or olive oil, but is required for the utilization of both lipids (Fig. 2B).

Surprisingly, we did not observe any phenotypes for the $cat8^{\Delta}/H9004^{\Delta}$ and $adr1^{\Delta}/H9004^{\Delta}$ mutants. Both mutants grew at rates comparable to those for the wild type on all of the carbon sources tested (Fig. 2). In contrast, the $S.\ cerisevisei$ $cat8^{\Delta}$ strain is unable to utilize any nonfermentable carbon source (22), while the $S.\ cerisevisei$ $adr1^{\Delta}$ strain has growth defects in the presence of ethanol, glycerol, and citrate, but not on acetate or lactate (68). Interestingly, the $A.\ nidulans$ homologs of these transcription factors, FacB and AmdX, have phenotypes more closely matching our observations in $C.\ albicans$. FacB is required for growth on acetate, but not on other carbon sources (28), while $amdX$ mutants have no reported nutrient-based growth defects (39). We tested these mutants for hyphal morphogenesis, resistance to oxidative stress, and growth at elevated temperatures and found no defects compared to the wild-type control (our unpublished observations).

FIG. 2. $ctf1^{\Delta}/H9004^{\Delta}$ and $adr1^{\Delta}/H9004^{\Delta}$ mutant strains have growth defects on specific carbon sources. Fivefold serial dilutions of the indicated strains were plated onto solid YNB media with various carbon sources and incubated at 30°C. Carbon sources were present at 2%. (A) Growth on glucose (2 days), ethanol, glycerol, or acetate (4 days). (B) Growth on glucose (2 days), oleate, linolenic acid, or olive oil (6 days). The strains used were the wild-type (SC5314), $icl1^{\Delta}/H9004^{\Delta}$ (MRC10), $cat8^{\Delta}/H9004^{\Delta}$ (MRC79), $cat8^{\Delta}/H9004^{\Delta} + CAT8$ (MRC77), $adr1^{\Delta}/H9004^{\Delta}$ (MRC24), $adr1^{\Delta}/H9004^{\Delta} + ADR1$ (MRC89), $ctf1^{\Delta}/H9004^{\Delta}$ (MRC41), $ctf1^{\Delta}/H9004^{\Delta} + CTF1$ (MRC49), and $fox2^{\Delta}/H9004^{\Delta}$ (MRC6) strains. The $ADR1$- and $CAT8$-complemented strains were omitted from panel B for space reasons, but behaved identically to the parent mutants and the wild type (data not shown).

FIG. 3. (A) $ctf1^{\Delta}/H9004^{\Delta}$ strains cannot metabolize Tween 20. Fivefold serial dilutions of the indicated strains were plated onto solid YNB media with the indicated carbon source at 2% and incubated at 30°C for 2 days (glucose) or 6 days (others). Strains used were as indicated in the legend for Fig. 2. (B) Chemical structure of the Tween compounds. (C) Predominant fatty acid side chains of the different Tween compounds.
fatty acid utilization. Our data indicate that *C. albicans* can use Tween 20, 40, 60, 80, and 85 with roughly equal avidity (Fig. 3A and data not shown), in contrast to an earlier report in which a panel of *C. albicans* clinical isolates were tested and found to assimilate Tween 40, 60, and 85, but not Tween 20 or 80 (52). Use of any Tween as a carbon source requires the β-oxidation protein FOX2 and the glyoxylate enzyme ICL1 (Fig. 3A). Surprisingly, CTF1 is required for the assimilation of Tween 20 and, to a lesser degree, Tween 40, but not for that of Tween 60 (52). Of note, CTF1 is required for the assimilation of Tween 20, in which the fatty acid moiety is predominantly the saturated C12 lauric acid, in the absence of CTF1. We did not find any phenotypes for the cat8Δ or adr1ΔΔ mutations in the presence of Tween 20 (data not shown).

**Effects of carbon source and regulator mutations on expression of carbon metabolic structural genes.** The phenotypic data shown above combined with the homology of CTF1 to the known transcription factors FarA and FarB suggested that CTF1 might regulate genes involved in fatty acid degradation and peroxisome biogenesis and might itself be regulated by carbon source. To test this, we performed Northern analysis to assay the expression of CTF1 and of FOX2, ICL1, and FBP1. CTF1 is strongly induced in the presence of the fatty acid oleate (Fig. 4A). We were unable to detect mRNA for this gene under any other condition, further linking this transcription factor homolog to fatty acid metabolism. CTF1 mRNA was difficult to detect even in the presence of fatty acids and generally appeared as a diffuse smear on Northern blots, suggesting an unstable mRNA, though we have not addressed this specifically.

To determine whether CTF1 contributes to the carbon source-based gene regulation, we assayed the expression of FOX2 between the ctf1ΔΔ mutant and wild-type strains grown in media containing glucose, acetate, or oleate as the sole carbon source (Fig. 4B). As shown previously, FOX2 mRNA is undetectable when cells are grown in glucose, derepressed in the presence of acetate, and highly induced by oleate (48). Induction by oleate is completely dependent on CTF1 (Fig. 4B and C), indicating that transcriptional expression of this gene is positively regulated by CTF1. Interestingly, expression of ICL1 was greatly reduced in the ctf1ΔΔ strain in oleate, but was unaffected in this mutant on acetate, indicating that, like in *A. nidulans*, the transcriptional regulators that induce ICL1 expression differ based on the carbon source (28, 64).

Given the known roles of CAT8 and ADR1 homologs in gene expression in response to changing carbon conditions in *S. cerevisiae* and *A. nidulans*, we investigated the effect of these mutants on the gene expression in *C. albicans* (Fig. 4C). The ctf1ΔΔ mutant was also included in this analysis. Neither CAT8 nor ADR1 had an effect on the induction of FOX2, ICL1, or FBP1 in the presence of alternative carbon sources (a slight reduction of FOX2 mRNA in the adr1ΔΔ strain was not reproducible). While this is consistent with the absence of any growth phenotype for these mutants, it is in contrast to the roles of these genes in other fungi.

**CTF1 regulates a broad set of genes required for β-oxidation and peroxisomal biogenesis.** OAF1 and PIP2 in *S. cerevisiae* control expression of a diverse array of genes whose products are involved in β-oxidation or peroxisomal biogenesis (68). We expanded our analysis of gene regulation by CTF1 to four additional genes; POT1, POX1, and EC11 encode other enzymes of β-oxidation, and PEX5 encodes the peroxisomal import receptor for proteins containing a type 1 PTS (PTS1), the classical “SKL” motif found at the extreme carboxy termini of proteins found in the peroxisomal matrix (reviewed in reference 60). All four of these genes are induced in the presence of fatty acids, and this induction is dependent either entirely (POT1) or in part (POX1, PEX5) on CTF1, except for EC11, which is unaffected by the deletion of CTF1 (Fig. 5). Thus, CTF1 plays a role similar to that of OAF1/PIP2 in regulating a broad set of genes required for fatty acid degradation, despite
Without this sequence, deletion of CTF1 causes a mild attenuation of virulence, of a magnitude similar to that of the farA or farB mutants, in vivo. We therefore tested this mutant in the standard mouse model of systemic candidiasis as described previously (48). The ctf1Δ/Δ mutant had a mild but not statistically significant attenuation in virulence compared to the wild type in vivo, while the adr1Δ/Δ mutant had a mild but not statistically significant attenuation in virulence compared to the wild type (data not shown).

**DISCUSSION**

In this work, we have characterized CTF1, a transcriptional regulator of fatty acid catabolism and peroxisome biogenesis in *C. albicans*. CTF1 is a sequence and functional homolog of FarA and FarB. *A. nidulans* transcription factors required for growth on lipids (24). As for *A. nidulans farA/farB* mutants, deletion of *CTF1* blocks growth when fatty acids are the sole carbon source and abrogates or reduces the induction of genes encoding key components of β-oxidation by lipids. Despite this, peroxisome-tagged proteins localize in a typical punctate pattern, indicating that *ctf1Δ/Δ* cells maintain peroxisomal structures. Mutation of *CTF1* causes a mild attenuation of virulence, of a magnitude similar to that of *fox2Δ/Δ* strains (Fig. 7) (48). The *cat8Δ/Δ* mutant had no discernible attenuation in virulence, while the *adr1Δ/Δ* mutant had a mild but not statistically significant attenuation in virulence compared to the wild type (data not shown).

While our findings regarding CTF1 are consistent with the known functions of FarA and FarB from *A. nidulans* (24), they contrast with a previous study that touched on *C. albicans* CTF1. Coste and coworkers identified three *C. albicans* zinc
finger transcription factors in an *S. cerevisiae* screen for suppressors of mutants in PDR1 and PDR3, regulators of pleiotropic drug resistance, CTA4, ASG1, and CTF1 (12). A CTF1 mutant was not found to have any phenotype for drug resistance or carbon utilization; in contrast, asgl mutants failed to grow on acetate as a carbon source (12), and this gene is thus a candidate regulator of glyoxylate cycle genes.

We also characterized the closest *C. albicans* homologs of two additional regulators of carbon metabolism, CAT8 and ADR1 (FacB and AmdX in *A. nidulans*). Despite significant sequence similarity to these well-characterized transcription factors, *C. albicans* strains deleted for CAT8 or ADR1 had no apparent phenotype under any conditions tested and did not affect expression of genes encoding enzymes required for β-ox-
idation, the glyoxylate cycle, or gluconeogenesis, though CAT8 has been previously shown to have a slight effect on the expression of the JEN1 lactate permease (58). We tested the cat8ΔΔ mutant using lactate as a sole carbon source and found no growth defect compared to the wild-type strains (our unpublished observations). The function of these proteins remains to be uncovered, but their role in carbon metabolism, if any, is subtle. Recently, several reports have shown that there has been a substantial realignment of function for some transcription factors between S. cerevisiae and C. albicans; a notable example is GAL4, which regulates galactose utilization in S. cerevisiae but regulates glycolysis in C. albicans (34).

A simplified schematic of the regulation of carbon catabolic pathways in C. albicans, S. cerevisiae, and A. nidulans is shown in Fig. 8. While MIG1/CreA modulates glucose repression in each species, the induction of different pathways by carbon source is quite different. In S. cerevisiae, CAT8 regulates the induction of glyoxylate cycle genes in the presence of acetate or oleate and has no effect on the regulation of β-oxidation genes such as FOX2 (22, 68). In contrast, in A. nidulans, FarA and FarB respond to fatty acids to regulate both β-oxidation and glyoxylate functions (24). The CAT8 homolog FacB induces the glyoxylate cycle only in the presence of acetate (28, 64). C. albicans appears to be more similar to A. nidulans, in which CTF1 regulates both fatty acid and glyoxylate genes in the presence of fatty acids, and other regulators must be involved on other carbon sources, but our results indicate that neither CAT8 nor ADR1 play significant roles. We cannot exclude that the regulation by CTF1 of genes such as FOX2, POT1, and POX1 may be indirect.

C. albicans efficiently utilizes diverse lipids as carbon sources, including saturated and unsaturated long-chain fatty acids, complex lipids such as olive oil, and polyethylene glycol sorbate (Tween) compounds, though it cannot use fatty acids of fewer than 10 carbons (our unpublished observations). C. albicans assimilated all the Tween compounds we tested (Tweens 20, 40, 60, 80, and 85), in contrast to an earlier report (52), and this was dependent on the activity of the glyoxylate cycle. As judged by the phenotype of the ctf1ΔΔ mutant strain, peroxisomal β-oxidation is required only for degradation of Tween 20, the version with the smallest side chain lipid (mostly C_{12} lauric acid), and has a mild defect on Tween 40, which has the second-smallest side chain (mostly C_{16} palmitate). It is not readily apparent why the degradation of these complex ester-lipid molecules would differ based on the lipid side chain.

It was previously noted that several putative peroxisomal proteins of C. albicans do not have recognizable PTS1s at their C termini but, nevertheless, localized to the peroxisomes (45). There is some flexibility in this sequence, with a consensus in the three C-terminal amino acids of S/C/A–K/H/R–L (21). The C. albicans ICL1 (AKA), MLS1 (ERL), and FOX2 (AKI) show some resemblance to this consensus, but all have important differences. It was thus formally possible that the PTS was elsewhere in the protein. However, fusion proteins of GFP with these C-terminal sequences were effectively transported to the peroxisome in a PEX5-dependent manner (inefficiently for the ICL1 fusion), indicating that C. albicans tolerates a wider variation in the PTS1 than do other well-characterized species. Localization of these reporters was not dependent on the presence of FOX2 or CTF1. Peroxisomal proliferation was not grossly impaired in the ctf1ΔΔ strain, as this mutant still had a mild defect on Tween 40, which has the second-smallest side chain (mostly C_{16} palmitate). It is not readily apparent why the degradation of these complex ester-lipid molecules would differ based on the lipid side chain.

There is ample evidence that the function of CAT8 has been adapted to suit the particular needs of different species. While this protein is required for growth on any nonfermentable carbon source in S. cerevisiae, a facB mutant of A. nidulans has growth defects only in the presence of acetate as the sole carbon source and retains the ability to utilize ethanol and fatty acids. In the yeast Kluyveromyces lactis, CAT8 regulates the glyoxylate cycle, but not gluconeogenesis (20). It also controls
genes for two acetyl coenzyme A synthetases (ACS) and a lactate permease (31), as does \textit{S. cerevisiae} CAT8 (29). Common in all three species, however, is a role for CAT8 in alternative carbon regulation; in stark contrast, we find that the \textit{C. albicans} CAT8 gene has no obvious role in carbon metabolism at either the phenotypic or gene expression level. The function of this very highly conserved transcriptional regulator remains an open question for \textit{C. albicans}.

Several lines of evidence indicate that \textit{C. albicans} experiences nutritional stress within the host, including the induction of genes required for utilization of nonfermentable carbon sources in \textit{ex vivo} and \textit{in vivo} models and the mild to severe attenuation of virulence conferred by deletion of genes required for growth on these less-favored nutrients (5, 18, 32, 33, 41, 46, 48, 65). The importance of these processes is not limited to \textit{C. albicans}; many of the same pathways have been found to be required for the virulence and persistence of \textit{Mycobacterium tuberculosis} in animals (35, 36). The glyoxylate cycle, in particular, is also required for full virulence in several fungal pathogens of plants (2, 27, 59, 66). This is not universally true, however, as \textit{Cryptococcus neoformans} and \textit{Aspergillus fumigatus} appear to not require this pathway in the host (44, 51), though these genes are induced in vivo in \textit{C. neoformans} (51). Many investigators have used an in vitro macrophage phagocytosis model to understand gene expression changes induced by immune cell contact and have found the glyoxylate cycle genes to be upregulated in \textit{C. albicans, S. cerevisiae, C. neoformans, A. fumigatus, and Paracoccidioides brasiliensis} (14–16, 18, 19, 32, 33, 51, 62). The molecular mechanisms by which cells sense and signal carbon starvation during host contact are not clear for any of these species; our results suggest that there may be significant differences between the host environment and paradigms developed in vitro.

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REFERENCES

1. Alani, E., L. Cao, and N. Kleecker. 1987. A method for gene disruption that allows repeated use of URA3 selection in the construction of multiply disrupted yeast strains. Genetics 116:541–545.

2. Asakura, M., T. Okuno, and Y. Takano. 2006. Multiple contributions of \textit{Paracoccidioides brasiliensis} to attenuation of virulence conferred by deletion of genes required for utilization of nonfermentable carbon sources. \textit{Eukaryot. Cell} 7:268–275.

3. Asakura, M., T. Okuno, and Y. Takano. 2008. Divergent functions of three \textit{Candida albicans} zinc-cluster transcription factors (CAT4A, ASG1 and CTF1) complementing pleiotropic drug resistance in \textit{Saccharomyces cerevisiae}. \textit{Microbiology} 154:1491–1501.

4. Barelle, C. J., C. L. Manson, D. M. MacCallum, F. Odds, N. A. Gow, and J. Iouk. 2008. UPR glycolytic gene expression upon \textit{Paracoccidioides brasiliensis} internationalization by murine macrophages and in vitro nutritional stress condition. \textit{Med. Mycol.} 46:123–134.

5. Asakura, M., T. Okuno, and Y. Takano. 2007. Transcriptional control of gluconeogenesis in \textit{Aspergillus fumigatus} in the filamentous fungus \textit{Aspergillus nidulans}. \textit{Genetics} 176:1557–1569.

6. Asakura, M., T. Okuno, and Y. Takano. 2006. Analysis of the regulation, expression, and localisation of the isoform lyase from \textit{Aspergillus fumigatus}, a potential target for antifungal drug development. \textit{Fungal Genet. Biol.} 43:476–489.

7. Asakura, M., T. Okuno, and Y. Takano. 2004. \textit{Cryptococcus neoformans} gene expression during murine macrophage infection. \textit{Eukaryot. Cell} 3:4120–1433.

8. Asakura, M., T. Okuno, and Y. Takano. 2003. \textit{Cryptococcus neoformans} gene expression during murine macrophage infection. \textit{Eukaryot. Cell} 3:4120–1433.
of Candida albicans upon internalization by macrophages. Eukaryot. Cell. 3:1076–1087.

33. Lorenz, M. C., and G. R. Fink. 2001. The glyoxylate cycle is required for fungal virulence. Nature 412:83–86.

34. Martchenko, M., A. Levitin, H. Hogues, A. Nantel, and M. Whiteway. 2007. Transcriptional rewiring of fungal galactose metabolism circuitry. Curr. Biol. 17:1007–1013.

35. McKinney, J. D., K. Honer zu Bentrup, E. J. Munoz-Elias, A. Miczak, B. Chen, W. T. Chan, D. Swenson, J. C. Sacchettiini, W. R. Jacobs, Jr., and D. G. Russell. 2000. Persistence of Mycobacterium tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 406:735–738.

36. Munoz-Elias, E. J., and J. D. McKinney. 2005. Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. Nat. Med. 11:638–644.

37. Murad, A. M., C. d’Enfert, C. Gaillardin, H. Tournu, F. Tekaia, D. Talibi, D. M. Roermund, D. Maccallum, F. Odds, and B. Distel. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Microbiology 151 Pt 2:297–302.

38. Neti, J. E., A. J. Lepuk, K. Marchillo, and D. R. Andes. 2009. Time course global gene expression analysis of an in vivo Candida biofilm. J. Infect. Dis. 200:307–313.

39. Noble, S. M., and A. D. Johnson. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot. Cell. 4:298–309.

40. Odds, F. C. 1988. Candida and candidosis. Bailliere Tindall, Philadelphia, PA.

41. Olivas, L., M. Royuela, B. Romero, M. C. Monteiro, J. M. Minguez, F. Laborda, and J. R. De Lucas. 2008. Ability to grow on lipids accounts for the fully virulent phenotype in neutropenic mice of Aspergillus fumigatus null mutants in the key glyoxylate cycle enzymes. Fungal Genet. Biol. 45:45–60.

42. Piekarska, K., G. Hardy, E. Mol, J. van den Burg, K. Strijbis, C. van Roermund, M. van den Berg, and B. Distel. 2008. The activity of the glyoxylate cycle in peroxisomes of Candida albicans depends on a functional beta-oxidation pathway: evidence for reduced metabolite transport across the peroxisomal membrane. Microbiology 154:3061–3072.

43. Piekarska, K., E. Mol, M. van den Berg, G. Hardy, J. van den Berg, C. van Roermund, D. Maccallum, F. Odds, and B. Distel. 2006. Peroxisomal fatty acid beta-oxidation is not essential for virulence of Candida albicans. Eukaryot. Cell. 5:1847–1856.

44. Prigueau, O., A. Porta, A. Poutrier, S. Colonna-Romano, T. Noel, and B. Maresca. 2003. Genes involved in beta-oxidation, energy metabolism and glyoxylate cycle are induced by Candida albicans during macrophage infection. Yeast 20:723–730.

45. Ramirez, M. A., and M. C. Lorenz. 2007. Mutations in alternative carbon utilization pathways in Candida albicans attenuate virulence and confer pleiotropic phenotypes. Eukaryot. Cell. 6:280–290.

46. Reuss, O., A. Vik, R. Kolter, and J. Morschhauser. 2004. The SAT1 flipper, an optimized tool for gene disruption in Candida albicans. Gene. 341:119–127.

47. Rottensteiner, H., A. J. Kal, B. Hamilton, H. Ruis, and H. F. Tabak. 1997. A heterodimer of the Zn2Cys6 transcription factors Pbp2p and Oaf1p controls induction of genes encoding peroxisomal proteins in Saccharomyces cerevisiae. Eur. J. Biochem. 247:776–783.

48. Rude, T. H., D. L. Toffaletti, G. M. Cox, and J. R. Perfect. 2002. Relationship of the glyoxylate pathway to the pathogenesis of Cryptococcus neoformans. Infect. Immun. 70:5684–5694.

49. Rudek, W. 1978. Esterase activity in Candida species. J. Clin. Microbiol. 8:756–759.

50. Ruijter, G. J., and J. Visser. 1997. Carbon repression in aspergilli. FEBS Microbiol. Lett. 151:103–114.

51. Schaller, H. J. 2003. Transcriptional control of nonfermentative metabolism in the yeast Saccharomyces cerevisiae. Curr. Genet. 43:139–160.

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

53. Simon, M., G. Adam, W. Rapatz, W. Spevak, and H. Ruis. 1991. The Saccharomyces cerevisiae ADR1 gene is a positive regulator of transcription of genes encoding peroxisomal proteins. Mol. Cell. Biol. 11:669–704.

54. Smith, J. J., S. A. Ramsey, M. Marelli, B. Marzolf, D. Hwang, R. A. Saleem, R. A. Rachubinski, and J. D. Aitchison. 2007. Transcriptional responses to fatty acid are coordinated by combinatorial control. Mol. Syst. Biol. 3:115.

55. Soares-Silva, I., L. Saiva, P. Kutter, K. D. Entian, and M. Casal. 2004. The disruption of JEN1 from Candida albicans impairs the transport of lactate. Mol. Membr. Biol. 21:403–411.

56. Solomon, P. S., R. C. Lee, T. J. Wilson, and R. P. Oliver. 2004. Pathogenicity of Stagonospora nodorum requires malate synthase. Mol. Microbiol. 53:1065–1073.

57. Subramani, S., A. Koller, and W. B. Snyder. 2000. Import of peroxisomal matrix and membrane proteins. Annu. Rev. Biochem. 69:399–418.

58. Tachibana, C., J. Y. Yoo, J. B. Tagne, N. Kacherovsky, T. I. Lee, and E. T. Young. 2005. Combined global localization analysis and transcriptome data identify genes that are directly coregulated by ADR1 and Cdt8. Mol. Cell. Biol. 25:2138–2146.

59. Tavares, A. H., S. S. Silva, V. V. Bernardes, A. Q. Maranhao, C. M. Kayw, M. Pocas-Fonseca, and I. Silva-Pereira. 2005. Virulence insights from the Paracoccidioides brasiliensis transcriptome. Genet. Mol. Res. 4:372–389.

60. Thirach, S., C. R. Cooper, Jr., and N. Vanitatanakom. 2008. Molecular analysis of the Penicillium marneffei glyoxaldehyde-3-phosphate dehydrogenase-encoding gene (gpdA) and differential expression of gpdA and the isocitrate lyase-encoding gene (acuD) upon internalization by murine macrophages. J. Med. Microbiol. 57:1332–1338.

61. Todd, R. B., R. L. Murphy, H. M. Martin, J. A. Sharp, M. A. Davis, M. E. Katz, and M. J. Hynes. 1997. The acetate regulatory gene sacB of Aspergillus nidulans encodes a Zn(II)Cys6 transcriptional activator. Mol. Gen. Genet. 254:495–504.

62. Walker, L. A., D. M. Maccallum, G. Bertram, N. A. Gow, F. C. Odds, and A. J. Brown. 2009. Genome-wide analysis of Candida albicans gene expression patterns during infection of the mammalian kidney. Fungal Genet. Biol. 46:210–219.

63. Wang, Z. Y., C. R. Thornton, M. J. Kershaw, L. Dehao, and N. J. Talbot. 2003. The glyoxylate cycle is required for temporal regulation of virulence by the plant pathogenic fungus Magnaporthes grisea. Mol. Microbiol. 47:1601–1612.

64. Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39:309–317.

65. Young, E. T., K. M. Dombek, C. Tachibana, and T. Ideker. 2002. Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. J. Biol. Chem. 278:26146–26158.

66. Zaragoza, O., C. Rodriguez, and C. Gancedo. 2000. Isolation of the MIG1 gene from Candida albicans and effects of its disruption on catabolite repression. J. Bacteriol. 182:320–326.