Role of Candida albicans Aft2p transcription factor in ferric reductase activity, morphogenesis and virulence

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INTRODUCTION

Candida albicans is one of the most frequently isolated among opportunistic fungal pathogen species in humans. It can cause various forms of candidiasis including mucosal infections and systemic diseases, especially in immunocompromised patients (Klepser, 2006). One of the important properties of C. albicans is its ability for morphological transition, which is related to its pathogenicity and virulence. C. albicans can grow as single-celled, budding yeast forms, pseudohyphal or hyphal forms. Moreover, the process of morphological transition is reversible (Berman, 2006). Several environmental conditions are known to trigger a switch from yeast to filamentous growth. Mutants which grow only in yeast cell forms under examined laboratory conditions are avirulent in mouse models (Banerjee et al., 2008; Cao et al., 2006).

Morphogenesis in C. albicans is controlled by both positive and negative transcription factors. Many positive regulators have been characterized, including the Cph1p, Efg1p, Flo8p, Rim101p and Ume6p (Banerjee et al., 2008; Cao et al., 2006; Carlisle et al., 2009; Davis et al., 2000b; Doedt et al., 2004; Liu et al., 1994). A null mutant of any of these transcription factors affects filamentous growth on filament-inducing media (Spider or serum). The negative regulation is mediated mainly by Tup1p through Rfg1p and Nrg1p (Murad et al., 2001). A lack of any one of these negative regulators results in derepression of hyphal-specific genes and constitutive filamentous growth, even when the mutant grows under non-filament-inducing conditions. As understanding of the virulence mechanism in C. albicans is enhanced through more investigation into the regulation of morphogenesis, it will be possible to bring new approaches to identify novel antifungal agents.

Iron is an important nutrient required by almost all organisms, especially as a cofactor in some metabolic functions, but excess iron in cells is toxic (Byers & Arceneaux, 1998). In Saccharomyces cerevisiae, iron shortage in the environment leads to transcriptional activation of many genes under the control of the iron-dependent transcription factors Aft1p and Aft2p (Philpott & Protchenko, 2008). Aft1p mainly activates the cell surface iron uptake systems in iron depletion. Aft2p and Aft1p have overlapping roles in the control of genes related to iron metabolism. Aft2p can activate the transcription of genes involved in intracellular iron use in the absence of Aft1p (Courel et al., 2005). Many differences exist in the regulation of iron acquisition and homeostasis between S. cerevisiae and C. albicans. C. albicans has also adopted many complex strategies to scavenge the depleting iron from the host environment for its survival (Almeida et al., 2010).
2009) and different kinds of iron acquisition systems are related to its virulence (Ramanan & Wang, 2000). In C. albicans, Sfu1p (a GATA-type factor) negatively regulates iron-responsive gene expression (Lan et al., 2004). The CCAAT binding factors complex also plays a significant role in iron homeostasis (Baek et al., 2008). Recently, Sef1p was identified as a new positive regulator of iron acquisition in C. albicans (Homann et al., 2009). Up to now, the C. albicans ORF with closest similarity to S. cerevisiae AFT1 or AFT2, ORF19.2272, has not been characterized.

The focus of this paper is the role of ORF19.2272 in ferric reductase activity, morphogenesis and virulence in C. albicans. Only one Aft-type homologous sequence, CaAFT2 (ORF19.2272), exists in the C. albicans genome sequence database. Here, we cloned CaAFT2; this could compensate for the S. cerevisiae aft1A mutant growth defect under low-iron conditions and with a non-fermentable carbon source in the medium. AFT2 deletion in C. albicans affected colony morphology and invasive growth. Deletion of AFT2 significantly attenuated the cell surface ferric reductase activity and virulence of C. albicans in a mouse model. Our data suggest that CaAft2p is a new positive transcription factor and that it plays an important role in ferric reductase activity, colony morphogenesis and virulence in C. albicans.

METHODS

Strains, media and growth conditions. All strains are listed in Table 1. C. albicans and S. cerevisiae strains used in this study are derivatives of BWP17 strain and W303a strain, respectively. Strains were routinely grown in YPD (Oxoid) supplemented with 80 µg uridine ml⁻¹. C. albicans transformants were selected on synthetic complete (SC; Sangon Biotech) medium lacking arginine, tryptophan or uracil. SC supplemented with 1 mg 5-fluoroorotic acid (5-FOA; Sangon Biotech) ml⁻¹ and 80 µg uridine ml⁻¹ was used to counterselect for CaURA3. Hyphal induction was done at 37 °C in Spider medium and YPG + 10% fetal calf serum (FCS; Gibco). C. albicans was starved for iron by growth at 30 °C in M199 media (Invitrogen), buffered at pH 4 containing 50 µM bathophenanthroline disulfonate (BPS; Sigma).

Generation of aft2Δ/aft2Δ mutant and complemented (aft2Δ/aft2Δ+AFT2) strains. Primers used in this study are listed in Table 2. To construct the aft2Δ/aft2Δ mutant (NKF25), BWP17 strain was transformed with PCR products amplified from the pRS-ARG4ΔSpeI template (Wilson et al., 1999) and the AFT2-5DR and AFT2-3DR primers. After this heterozygous mutant NKF24 was constructed, NKF24 was then transformed with PCR products amplified from the pDDB57 template and AFT2-5DR and AFT2-3DR primers, to generate the aft2 null mutant NKF25 strain. Correct homologous recombination was confirmed by PCR using AFT2-5detect and AFT2-3detect primers and Southern blot analysis. Probes used for Southern blot analysis were generated by PCR, gel-purified and DIG-labelled using random primers. Probe labelling and hybridization were performed using a DIG high prime DNA labelling and detection starter kit I (Roche) according to the manufacturer’s protocols. To complement the aft2 mutation in strain NKF25, NKF25 was first plated on solid medium containing 5-FOA, which led to the loss of aft2Δ/aft2Δ mutant and complemented (aft2Δ/aft2Δ+AFT2) strain NKF40. Then NKF40 was transformed with plasmid pCR4-CaAFT2 to create CaAFT2 complemented strain NKF46.

Plasmid construction. Primers AFT2-5comp and AFT2-3comp were designed to amplify a DNA fragment containing the 990 bp upstream region, the 2397 bp ORF and the 270 bp downstream

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>C. albicans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWP17</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG</td>
<td>Wilson et al. (1999)</td>
</tr>
<tr>
<td>NKF21</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ftr1::ARG4/ftr1::URA3-dpl200</td>
<td>Knight et al. (2005)</td>
</tr>
<tr>
<td>NKF25</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG aht2::ARG4/aht2::URA3-dpl200</td>
<td>This study</td>
</tr>
<tr>
<td>NKF40</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG aht2::ARG4/aht2::dpl200</td>
<td>This study</td>
</tr>
<tr>
<td>NKF46</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG aht2::ARG4/aht2::dpl200, pCR4+AFT2</td>
<td>This study</td>
</tr>
<tr>
<td>NKF47</td>
<td>ure3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG aht2::ARG4/aht2::dpl200, pCR4</td>
<td>This study</td>
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<tr>
<td><strong>S. cerevisiae</strong></td>
<td></td>
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<tr>
<td>W303a</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100</td>
<td>Thomas &amp; Rothstein (1989)</td>
</tr>
<tr>
<td>NKF24</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100, YEplac195</td>
<td>This study</td>
</tr>
<tr>
<td>NKF50</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100 aht1::TRP1</td>
<td>This study</td>
</tr>
<tr>
<td>NKF52</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100 aht1::TRP1, YEplac195</td>
<td>This study</td>
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<tr>
<td>NKF53</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100 aht1::TRP1, YEplac195-CaAFT2</td>
<td>This study</td>
</tr>
<tr>
<td>NKF55</td>
<td>MATa leu2-3,112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100 aht1::TRP1, YEplac195-PGK1-CaAFT2</td>
<td>This study</td>
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region of CaAFT2. The PCR products were digested with BamHI/KpnI and cloned into pCR4 and YEplac195 plasmids at the BamHI/KpnI sites, yielding plasmids pCR4-CaAFT2 and YEplac195-CaAFT2, respectively. The YEplac195-PGK1-CaAFT2 plasmid was constructed as follows. A 750 bp PGK1 promoter region of S. cerevisiae was amplified by PCR using primers PGK1-F and PGK1-R, and the resulting product was digested with XbaI/BamHI and ligated into XbaI/BamHI-digested YEplac195 to generate YEplac195-PGK1. The 2397 bp ORF and the 270 bp downstream region of CaAFT2 were amplified using primers AFT2-F and AFT2-3comp; the product was digested with BamHI and ligated into BamHI-digested YEplac195 to generate YEplac195-PGK1-CaAFT2.

S. cerevisiae aft1Δ construction. To disrupt AFT1 in S. cerevisiae W303a, the aft1:: TRP1 disruption cassette was generated by amplification of plasmid pGBK7 by PCR with primers ScAFT1-3det and ScAFT1-5det; the product was digested with BamHI and ligated into XbaI/BamHI-digested YEplac195 to generate YEplac195-PGK1-AFT1. The resulting construct was transformed into S. cerevisiae W303a, and the transformants were selected for the presence of plasmid pGBK7. The resulting strain was then transformed with the plasmid YEplac195-AFT1 and YEplac195-PGK1-AFT1, respectively. The YEplac195-AFT1 plasmid was constructed as follows. A 750 bp PGK1 promoter region of S. cerevisiae was amplified by PCR using primers PGK1-F and PGK1-R, and the resulting product was digested with XbaI/BamHI and ligated into XbaI/BamHI-digested YEplac195 to generate YEplac195-PGK1. The 2397 bp ORF and the 270 bp downstream region of CaAFT2 were amplified using primers AFT2-F and AFT2-3comp; the product was digested with BamHI and ligated into BamHI-digested YEplac195 to generate YEplac195-PGK1-CaAFT2.

**RESULTS**

Identification of CaAFT2

The yeast S. cerevisiae contains a pair of homologous iron-responsive transcription activators, Aft1p and Aft2p. To identify an homologous gene(s) of ScAFT1 and ScAFT2 in C. albicans, a BLAST search of S. cerevisiae Aft1p and Aft2p against the C. albicans genome was carried out, using BLASTP (protein/protein) at the CandidaDB web server (http://www.candidagenome.org). BLAST analysis of the ScAft1p sequence against the C. albicans genome sequence did not identify any homologues of Aft1p. This search only revealed a closest homologue of the ScAft2p protein and it was named CaAft2p. CaAFT2 encodes a protein of 798 aa, which is considerably longer than the 416 aa of ScAft2p. The N-terminal parts of CaAft2p and ScAft2p were the most similar regions in the proteins. The predicted product of CaAFT2 exhibits 32 % identity to ScAft2p from residues 21 to 177. The high degree of identity within the N terminus of both proteins supports the prediction that CaAFT2 codes for a DNA-binding protein. The C-terminal region of CaAft2p is quite rich in glutamine and 21.5 % of amino acids between residues 465 and 656 are glutamines. Glutamine-rich domains have been found in a number of transcriptional activators (Mitchell & Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989). pSORT analysis shows that there are two nuclear localization signals (KKREDVDAPKISSKKAR at 170 site and Tjian, 1989).

**CaAFT2 can rescue S. cerevisiae aft1Δ mutant growth defect**

The growth of a S. cerevisiae strain lacking AFT1 is impaired under low-iron conditions. Moreover, a non-fermentable carbon source in the medium could aggravate this defect (Casas et al., 1997). To determine whether CaAft2p has the same function as ScAft1p, the CaAFT2 gene ORF, with 990 bp of its own promoter, was cloned into YEplac195 vector. However, this construct could not rescue the Scaft1Δ growth defect on the iron-limited plate or the medium containing glycerol as a carbon source (Fig. 1). We predict that the native promoter of the CaAFT2 gene may have no or a weak ability to promote AFT2 gene expression in S. cerevisiae, and a low CaAFT2 expression level leads to no observed growth-promoting
function. So we used 700 bp of the strong phosphoglycerate kinase (PGK1) promoter from \textit{S. cerevisiae} to substitute the native promoter of \textit{CaAFT2}. As expected, when driven by the PGK1 promoter from YEplac195 vector, it could effectively rescue the defect of Scaft1Δ. The results confirmed that \textit{CaAFT2} has a similar function to \textit{ScAFT1} and is able to rescue the growth defect of the \textit{aft1} mutation in \textit{S. cerevisiae}.

**Deletion of AFT2 has no effect on cell growth under iron-limited conditions**

In \textit{S. cerevisiae}, the \textit{aft1} mutant was sensitive to iron deprivation, while the \textit{aft2} mutant exhibited no iron-dependent phenotype. But the \textit{aft1Δaft2Δ} double mutant did not grow completely under iron-deprived conditions. Moreover, the growth defect of the \textit{aft1Δaft2Δ} mutant could be reversed when the \textit{AFT2} gene was reinserted into the \textit{aft1Δaft2Δ} mutant (Blaiseau et al., 2001). To explore whether deletion of \textit{AFT2} could affect growth in \textit{C. albicans} cells, we deleted both \textit{AFT2} alleles by using a PCR-directed gene disruption. Successful deletion of \textit{C. albicans AFT2} was confirmed by PCR detection and Southern blot (Fig. 2). We used M199 (pH 4) media adding 50 μM BPS to generate iron-limited conditions. The growth curves of the wild-type and \textit{aft2Δ/aft2Δ} strains were measured. These strains had similar growth rates under both iron-sufficient (M199 pH 4) and iron-limited (M199 pH 4 + 50 μM BPS) conditions in liquid media (Fig. 3a, b). The same results were observed on solid media (Fig. 3d, e). So, the \textit{aft2Δ/aft2Δ}
aft2Δ strain was not sensitive to iron-limited conditions. The results indicated, like AFT2 in S. cerevisiae, that CaAFT2 is not essential for cell growth under iron-limited condition in C. albicans.

**Effect of AFT2 on ferric reductase activity**

In S. cerevisiae, ScAFT1 regulates cell surface reductase activity (Yamaguchi-Iwai et al., 1995); the results described above suggest that CaAFT2 has a similar function to ScAFT1. We next carried out a cell surface reductase activity assay to determine whether deletion of AFT2 has an effect on C. albicans cell surface reductase activity. The wild-type and aft2 null mutant strains were grown to exponential phase and assayed for ferric reductase activity. The aft2 null mutant exhibited very low activity compared with the activity of the wild-type strain in M199 buffered at pH 4 (Fig. 4). The cell surface ferric reductase activity of the aft2Δ/aft2Δ strain was the same as that of the wild-type following culture in M199, pH 4, plus 50 μM BPS. When cultured in pH 8 media, cell surface ferric reductase activity showed 63% of the activity of wild-type strain (Fig. 4). These results show that CaAft2p can regulate cell ferric reductase activity under some specific growth conditions (such as pH 4 or pH 8, but not pH 4 + 50 μM BPS).

**Influence of AFT2 on colony morphology and invasive growth**

To study the function of AFT2 in C. albicans further, we wanted to test the effect of an aft2 deletion on morphology. To achieve this, the aft2 null mutant strain was constructed. We also reintroduced the wild-type AFT2 gene into the aft2 mutant for complementation. Successful complementation of C. albicans AFT2 was confirmed by PCR (Fig. 2). Deletion of AFT2 did not cause any differences in growth rates in the liquid medium or on the solid medium tested. We then tested if an aft2 deletion affected colony morphology. Overnight cultures of C. albicans strains (5 μl) were spotted on solid Spider and YPG + 10% FCS media. Colonies were examined macroscopically after incubation at 37 °C for 4 days. The results showed that the aft2Δ/aft2Δ strain formed smooth colonies with no filamentous growth, whereas the wild-type showed wrinkled colonies with peripheral hyphae (Fig. 5). The capability to form peripheral hyphae could be reversed by reintroducing wild-type AFT2 back into the aft2Δ/aft2Δ strain under control of its own promoter. These results...
suggest that CaAft2p plays a role in governing colony morphology and invasive growth in \textit{C. albicans}.

**Disruption of the AFT2 gene attenuates virulence in a systemic infection model of mice**

In \textit{C. albicans}, colony morphology is important for its interaction with a human host. Therefore, we tested the virulence of the \textit{C. albicans} \textit{aft2} null mutant strain compared with wild-type and \textit{AFT2} reinsertion strains in a mouse model of intravenous infection. All of the mice injected with the wild-type strains died by day 11 (Fig. 6). No statistical difference was found in virulence between wild-type strains and \textit{AFT2} reinsertion strains ($P>0.139$). In contrast, the mice injected with \textit{aft2}D/\textit{aft2}D strain did not all die until day 28. So the virulence of the \textit{aft2}D/\textit{aft2}D strain was distinctly lower than that of wild-type strain ($P<0.01$). These results indicate that the virulence defect of the \textit{aft2}D/\textit{aft2}D strain was specifically due to a loss of the \textit{AFT2} gene in this mouse model of intravenous infection.

**DISCUSSION**

In \textit{S. cerevisiae}, Aft1p activates the cell surface iron uptake systems and siderophore transport systems following iron depletion (Casas \textit{et al.}, 1997; Yamaguchi-Iwai \textit{et al.}, 1995); Aft2p and Aft1p have overlapping roles in the control of the iron-regulated pathway(s). Aft2p also activates the transcription of genes involved in intracellular iron use in the absence of Aft1p (Blaiseau \textit{et al.}, 2001). Following BLAST analysis, we identified a protein homologous to an Aft-type transcription factor in \textit{C. albicans}. In this study, we characterized \textit{CaAFT2} (ORF19.2272), which is homologous to \textit{ScaFT2}. Glutamine-rich domains in the C-terminal region and nuclear localization signals in the N-terminal region indicate that Aft2p is a novel transcriptional regulator in \textit{C. albicans}.

Previous studies have indicated that strains lacking the \textit{AFT1} gene are more sensitive to iron deprivation than \textit{aft2}Δ mutants in \textit{S. cerevisiae}, and a non-fermentable carbon source such as glycerol in the medium could aggravate this defect. The \textit{Scaft1}Δ strain was not able to grow on YPG, whereas \textit{Scaft2}Δ grew to a similar level as the wild-type strain (Rutherford \textit{et al.}, 2001). Our results also show that \textit{Scaft1}Δ displays no growth not only on YPG but also on SC-uracil plus 50 $\mu$M BPS. The growth defect of the \textit{Scaft1}Δ strain could be reversed through transformation with pCR4-\textit{P}_{\text{PGK1}}-\textit{CaAFT2} containing the wild-type \textit{CaAFT2} under control of the \textit{ScPGK1} promoter, indicating that CaAft2p can effectively function to activate iron acquisition gene expression by its ability to complement the \textit{aft1}Δ strain of \textit{S. cerevisiae}. But, interestingly, deletion
of AFT2 in C. albicans had no effect on the growth of strains when grown in iron-limited media or media buffered at pH 8. Actually, C. albicans has a more complicated iron acquisition mechanism than S. cerevisiae (Almeida et al., 2009; Philpott & Protchenko, 2008). Moreover, gene expression has a niche specificity in C. albicans (Barelle et al., 2006). That growth of the aft2Δ/aft2Δ mutant is not affected under these experimental conditions is probably due to the niche specificity or the compensatory mechanisms of other iron acquisition pathways which may compensate for the absence of AFT2 in C. albicans.

Although the aft2Δ/aft2Δ strain had no significant differences in growth rate from wild-type cells under yeast growth conditions at 30 °C, it was shown that CaAft2p plays a positive role in regulating ferric reductase activity. We observed that in the M199 media buffered at pH 4 and pH 8, the cell surface ferric reductase activity of the aft2 mutant was only 24 and 63% of that of the wild-type, respectively. The data suggest that CaAft2p acts as a positive transcriptional factor and regulates ferric reductase gene expression. We also found that there is no difference between aft2Δ/aft2Δ and wild-type strains when grown under M199 buffered at pH 4 plus 50 μM BPS. This may be because different growth conditions lead to different expression of ferric reductase genes. To date, studies on the regulation of ferric reductase genes in C. albicans are rare (Baek et al., 2008; Liang et al., 2009). Rim101p and CCAAT binding factors can regulate ferric reductase gene expression. Here, we showed that CaAft2p is another transcription factor capable of regulating ferric reductase gene expression. In S. cerevisiae, both Aft1p and Aft2p regulate gene expression through a similar promoter element, PyPuCACCCPu (Rutherford et al., 2003). We found that the same promoter element exists in promoters of some C. albicans ferric reductase genes (such as FRE2 and FRP1). Research will be done to further confirm the relationship between CaAft2p and regulation of ferric reductase genes.

In C. albicans, various environmental conditions (such as growth at 37 °C, in serum, pH-neutral media, starvation and so on) can trigger the yeast-to-hyphae transition. Different environmental responses are mediated by many transcriptional regulatory factors (Biswas et al., 2007). These factors may integrate signals from different signalling pathways and regulate morphology change. In this article, we identified CaAft2p, a new regulator of colony morphology and invasive growth in C. albicans. The aft2Δ/aft2Δ strain only displayed smooth colonies and failed to form peripheral hyphae. The defects of aft2Δ/aft2Δ could be rescued by introducing a wild-type AFT2 gene. Therefore, CaAFT2 governs colony morphology and invasive growth. The ability to form hyphal filaments has been correlated with virulence in C. albicans (Lo et al., 1997). Our results showed that the virulence of the aft2Δ/aft2Δ strain was reduced in comparison with wild-type and reininsertion strains. The attenuated virulence of the aft2 null mutant is consistent with reports of reduced or non-filament C. albicans strains, including null mutants in which transcriptional activators encoded by C. albicans RIM101, FLO8 and UME6 were disrupted (Banerjee et al., 2008; Cao et al., 2006; Davis et al., 2000a). Nevertheless, it was observed that the aft2Δ/aft2Δ strain exhibited reduced cell surface ferric reductase activity, which means that the ability of iron acquisition was affected. As we know, virulence of C. albicans is also related to the ability to acquire iron (Ramanan & Wang, 2000), so we are not able to distinguish from our data whether the attenuated virulence is a result of changed morphogenesis, reduced iron acquisition ability or a combination of mechanisms.

A recent study suggests that transcriptional rewiring in the regulation of iron acquisition has been identified in comparisons between C. albicans and S. cerevisiae (Homann et al., 2009). Different regulatory networks controlling iron acquisition in these species indicate that the iron acquisition circuit has undergone a major variation. It is possible that CaAft2p is not involved in iron homeostasis. But, interestingly, the results in this article indicated that CaAft2p plays a role in regulating ferric reductase activity and is a potential positive regulator of ferric reductase genes, suggesting that CaAft2p still holds the ancestral function of the Aft-type positive regulator family. Continued studies on CaAft2p will provide more information and help us further understand the iron regulatory network.

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Aft2p, a novel regulator in \textit{C. albicans}


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