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Original Article

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The zinc-finger transcription factor, Ofi1, regulates white–opaque switching and filamentation in the yeast *Candida albicans*

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Abstract

Candida albicans is a major fungal pathogen of humans. The most striking biological feature of *C. albicans* is its phenotypic plasticity, allowing it to undergo morphological transitions in response to various environmental cues. Transcription factors play critical roles in the regulation of morphological transitions. Here, we report the role of opaque and filamentation inducer 1 (Ofi1), a previously uncharacterized zinc-finger-containing protein encoded by the gene *orf19.4972*, in the regulation of white–opaque switching and filamentous growth. Over-expression of *OFI1* not only induced white-to-opaque switching but also promoted filamentation and invasive growth in *C. albicans*. Deletion of *OFI1* had no obvious effect on filamentation under the culture conditions tested, while deletion of *OFI1* reduced the frequency of white-to-opaque switching. We propose that Ofi1 functions downstream of Wor1, the master regulator of white–opaque switching. However, over-expression of *OFI1* in the *wor1*/*wor1* mutant could not induce the opaque phenotype, suggesting that Ofi1 does not work alone and other transcription factors downstream of Wor1 are also involved in this regulation. Given the importance of Ofi1 in the regulation of white–opaque switching and filamentation, the present study establishes a new link between these two processes.

Key words: *Candida albicans*, zinc-finger transcription factor, opaque and filamentation inducer 1, morphological transition, white–opaque switching, filamentation

Introduction

Transcription factors are central to the regulation of gene expression in eukaryotic organisms. In the human fungal pathogen *Candida albicans*, zinc-finger transcription factors are involved in a variety of biological processes including filamentation, white–opaque switching, biofilm formation, virulence, antifungal resistance, and sexual reproduction [1–7]. Filamentation and white–opaque switching are two important features of *C. albicans* that play critical roles not only in pathogenesis but also in sexual mating [1,2,8,9].

In response to environmental changes, *C. albicans* often undergoes filamentation or white–opaque phenotypic switching [1,5,10–12]. For example, the physiological temperature of human hosts (37°C), neutral pH conditions, and serum can induce filamentous growth in *C. albicans* [1,5]. High levels of CO_2 and *N*-acetylglucosamine (GlcNAc) can also promote filamentation and white-to-opaque switching [11,12]. These environmental cues first act on sensor proteins present on the cell surface. The signals are then transduced to different downstream pathways (e.g. the cAMP/PKA pathway and

the MAPK cascade) which activate or repress their downstream transcription factors to regulate phenotypic transitions [5]. The master regulator of white–opaque switching, Wor1, is a transcription factor that functions downstream of the cAMP/PKA signaling pathway [12–15]. Wor1, together with a number of other transcription factors including Wor2, Wor3, Czf1, and Efg1, forms an interlocking feedback loop that governs transitions between the white and the opaque phases [3]. Moreover, Wor1 is the central regulator of white–opaque switching and binds to the promoter regions of over 200 genes [3].

In the present study, we focus on the role of opaque and filamentation inducer 1 (Ofi1), a zinc-finger transcription factor, in the regulation of white–opaque switching and filamentation in *C. albicans*. Zordan *et al.* [3] reported that Wor1 binds to the promoter region of the *OFI1* gene (*orf19.4972*). Here, we show that over-expression of *OFI1* promotes filamentous growth and opaque cell formation under several culture conditions. Although deletion of *OFI1* had no obvious effect on filamentation under the culture conditions tested, the *ofi1*/*ofi1* mutant exhibited reduced switching frequencies from the white to the opaque phase in Lee's GlcNAc medium.

Materials and Methods

Strains and growth conditions

The strains used in this study are listed in **Supplementary Table S1**. YPD (20 g/l yeast extract, 10 g/l peptone, and 20 g/l glucose) was used for routine growth of *C. albicans* cells. Yeast extract and peptone were purchased from BD Company (Franklin Lakes, USA). Modified Lee's medium [12] and Spider medium (10 g/l Difco nutrient broth, 10 g/l mannitol, and 2 g/l dibasic potassium phosphate) [16] were used for the morphological analyses. All constituent parts of Lee's medium were purchased from Sigma-Aldrich (St Louis, USA). We used 12.5 g/l glucose or 12.5 g/l GlcNAc as the carbon source for Lee's medium; and 20 g/l agar (BD Company) was added to corresponding liquid media to make solid agar plates. Cells were grown on Lee's glucose plates for 4 days. Colonies were collected and diluted in water, and then replated onto different media. For white–opaque switching assays, the colony number on each plate was 40–100. For filamentation assays, ~80 cells of different strains were plated on each plate.

Construction of the *OFI1* over-expression plasmid

To construct the over-expression plasmid pACT1-OFI1, a fragment containing the *OFI1* ORF and a 550-bp 3′-UTR region of *OFI1* were amplified by polymerase chain reaction (PCR) with primers OFI1F and OFI1R (**Supplementary Table S2**). PCR reactions were performed using TaKaRa Ex Taq® DNA Polymerase (TaKaRa, Dalian, China) according to the manufacturer's instructions. PCR reaction condition: 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 2 min, and concluded at 72°C for 10 min. PCR products were digested with *Eco*RV and *Hin*dIII and then subcloned into the *Eco*RV/*Hin*dIII site of plasmid pACT1 [12], generating the over-expression plasmid pACT1-OFI1.

Strain constructions

The over-expression plasmid pACT1-OFI1 was linearized with *Asc*I and used for transformation of the WT strains (CAI4 and WUM5A) of *C. albicans*, generating the *OFI1* over-expression strains (DH1116 and DH1121).

The *OFI1* reconstituted strain was constructed using a fusion PCR strategy as previously reported [17]. Briefly, the ORF of *OFI1* and 3′-UTR fragment was amplified from genomic DNA of the WT strain and the *ARG4* marker was amplified by PCR from the plasmid pSN69 [17]. The three fragments (the *OFI1* ORF, *OFI1* 3′-UTR, and *ARG4* marker) served as templates for fusion PCR. Fusion PCR reaction condition: 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 7 min, and concluded at 72°C for 10 min. The fusion PCR products were used for transformation of the *ofi1*/*ofi1* mutant (TF38), generating the *OFI1* reconstituted strain (DH1126). The primers used are listed in **Supplementary Table S2**.

To generate *MTL***a**/Δ and *MTL*Δ/α strains, one of the *MTL* alleles was disrupted in the *MTL***a**/α strains of the WT (SN152) or *ofi1*/*ofi1* mutant (TF38) by targeted disruption with *Sph*I and *Sac*I linearized plasmid L23.14 according to our previous report [18]. *MTL***a**/Δ strains of the WT and *ofi1*/*ofi1* mutant (SN152a and DH1138) were used for white–opaque switching assays.

White–opaque switching assays

White–opaque switching assays were performed as described previously [12]. Briefly, cells were first grown on Lee's glucose medium plates for 4 days at 25°C. Cells from pure white or opaque colonies were replated onto Lee's glucose medium or Lee's GlcNAc medium plates, and then incubated at 25°C for 4 days. The total number of colonies and colonies exhibiting different phenotypes were counted.

Filamentous and invasive growth assays

All strains used for filamentous and invasive growth assays were *MTL* heterozygous (**Supplementary Table S1**). Lee's glucose medium, Lee's GlcNAc medium, and Spider medium were used for filamentous growth assay. Spider medium was used for the invasive growth experiments. Three microliters of Spider medium containing ~2000 cells was dropped onto agar plates containing the same medium and then incubated at 25°C for 3 days. The plates were imaged before and after washing with ddH_2O.

RNA extraction and reverse transcriptase–qPCR

Cells were grown on Lee's glucose medium plates for 3 days and collected for RNA extraction. Total RNA was extracted using the GeneJET RNA Purification kit (Thermo Fisher Scientific Inc., Waltham, USA) according to the manufacturer's instruction. cDNA was synthesized using Thermo Scientific RevertAid Reverse Transcriptase (Thermo Fisher Scientific Inc.). Quantitative PCR reactions were prepared with THUNDERBIRD™ SYBR® qPCR Mix (Toyobo, Osaka, Japan) and operated by CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, USA). *ACT1* served as the reference.

Results

Over-expression of *OFI1* promotes white-to-opaque switching

Ofi1 is encoded by the *C. albicans* gene *orf19.4972* (Candida Genome Database, http://www.candidagenome.org). The putative protein sequence of Ofi1 contains three zinc-finger DNA-binding motifs (ZnF_C2H2, aa 97–119, aa 125–147, and aa 153–173), which indicates that Ofi1 can potentially bind to DNA sequences. Global gene expression profile analysis indicates that the relative expression level of *OFI1* was much higher in opaque cells than in white cells [18–21].

We first constructed an *OFI1* over-expression plasmid, pACT1-OFI1, by inserting the ORF region of the *OFI1* gene into

plasmid pACT1 [12]. To examine the effect of over-expression of *OFI1* on white–opaque switching, the plasmid was transformed into strain WUM5A, a derivative of WO-1 [22]. The expression levels of *OFI1* in the over-expression strains were examined using quantitative RT-PCR analysis. As shown in **Fig. 1**, the expression level of *OFI1* in white cells of the over-expression strain (DH1116) was ~15 folds higher than in white cells of the control (DH1114). This result indicates that *OFI1* was successfully expressed in the over-expression strain. Of note, the expression level of *OFI1* in opaque cells of the control was much higher than in white cells. Therefore, we predicted that Ofi1 could be involved in the regulation of white–opaque switching in *C. albicans*.

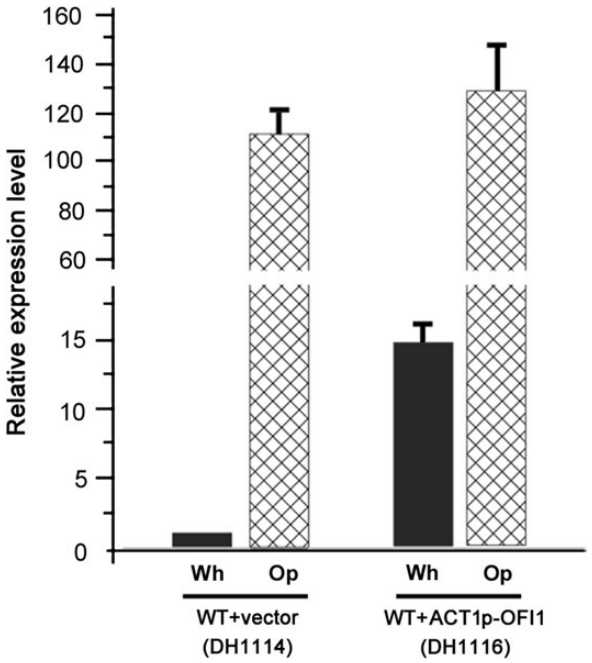


Figure 1. Relative expression levels of *OFI1* gene in white (Wh) and opaque (Op) cells of the WT control (DH1114) and over-expression (DH1116) strains Quantitative RT-PCR analysis was performed to examine the relative expression levels of *OFI1* in different cell types. The expression level of *ACT1* was used as a reference. The value of the relative expression level of *OFI1* in white cells of the control was set as '1'.

We further analyzed the effect of the over-expression of *OFI1* on the switching frequency. As shown in **Fig. 2**, the frequency of white-to-opaque switching of the over-expression strain (DH1116) was increased over 10 folds on Lee's glucose medium plates compared with that of the control (DH1114). On Lee's GlcNAc medium plates, the white–opaque switching frequency of the control was 61.1% ± 0.6%, while over-expression of *OFI1* increased the switching frequency to 100.0% ± 0.0%. However, over-expression of *OFI1* had no notable effect on the opaque-to-white transition. Comparison of the cultures on Lee's glucose and Lee's GlcNAc medium plates revealed that both the control and the over-expression strain exhibited higher switching frequency on Lee's GlcNAc medium plates. This result is consistent with previous reports indicating that GlcNAc has an inducing effect on opaque cell formation [12,18]. However, it was found that in the *wor1*/*wor1* mutant of *C. albicans* the over-expression of *OFI1* did not promote opaque cell formation, but induced filamentous growth (**Supplementary Fig. S1**), indicating that Ofi1 does not work alone and other unidentified factors downstream of Wor1 are also required for the formation of opaque cells. These results also suggest that the master regulator Wor1, but not Ofi1, is essential for the opaque phenotype.

Effect of deletion of *OFI1* on white–opaque switching in *C. albicans*

Since the expression of *OFI1* is enriched in opaque cells, we then examined the effect of deletion of the *OFI1* gene on white–opaque switching. Because *MTL* heterozygous derivatives of the laboratory strain SC5314 are incompetent for white-to-opaque switching [18], the *MTLα* locus was disrupted in the *ofi1*/*ofi1* mutant with plasmid L23.14. As shown in **Fig. 3**, when cultured on Lee's glucose medium plates, the white-to-opaque and opaque-to-white switching frequencies in the *ofi1*/*ofi1* mutant (DH1138) were comparable with those of the control (SN152a) and the *OFI1* reconstituted strain (DH1142). On Lee's GlcNAc medium plates, the white-to-opaque

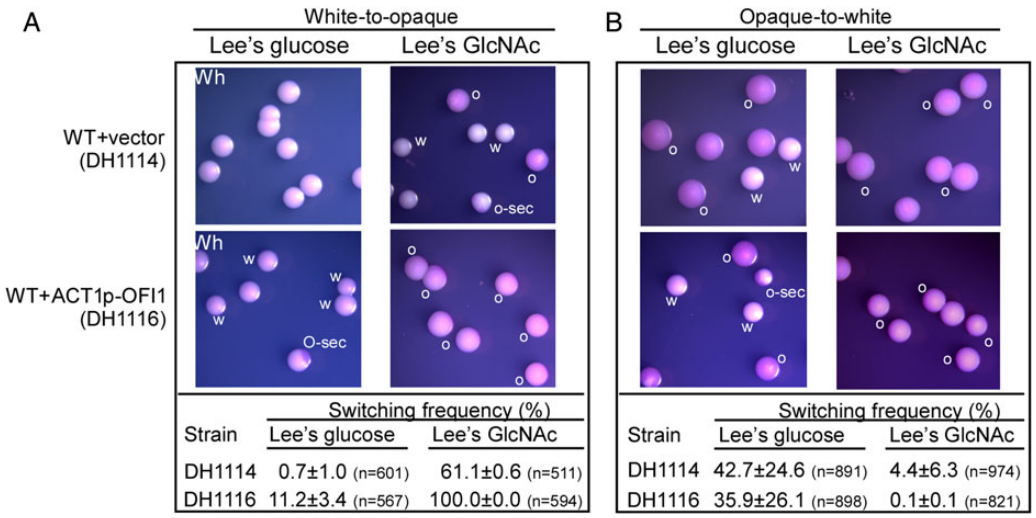
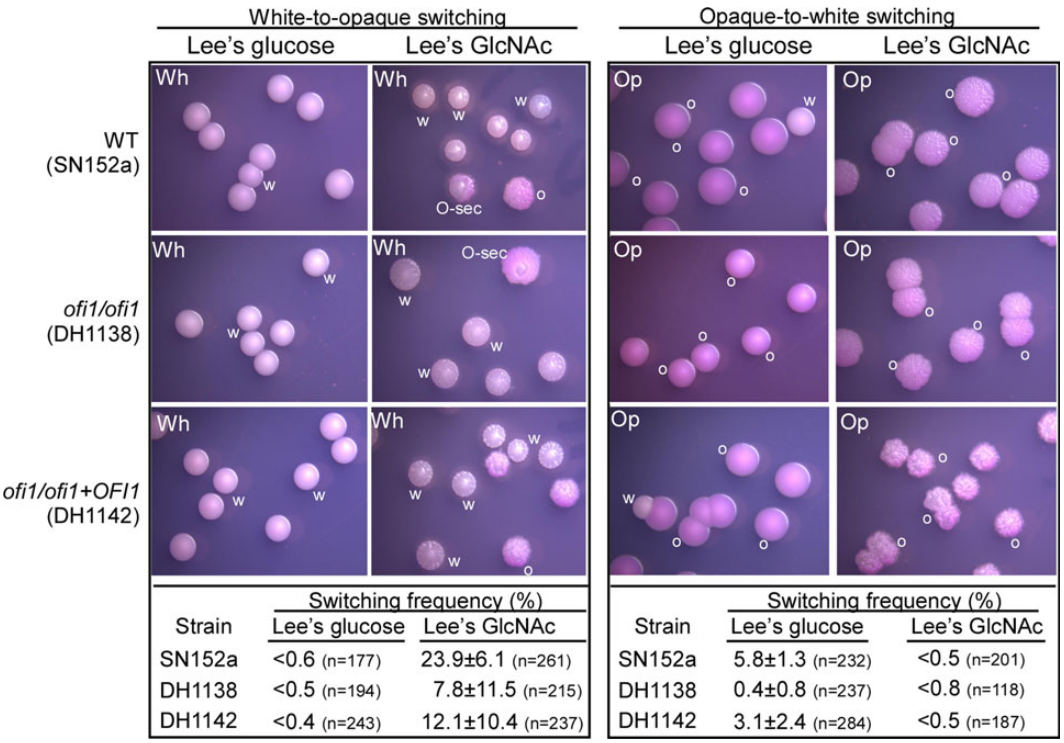


Figure 2. Effects of over-expression of *OFI1* on white–opaque switching Cells from a pure white colony or an opaque colony were plated on Lee's glucose or Lee's GlcNAc medium plates and incubated at 25°C for 4 days. (A) White-to-opaque switching. The switching frequency of DH1116 is significantly higher that of DH1114 on Lee's GlcNAc medium ($P < 0.05$, Student's *t*-test). (B) Opaque-to-white switching. *n*, total number of colonies; Wh or w, white colony; o, opaque colony. WT, WUM5A.



Strain	Switching frequency (%) Lee's glucose	Lee's GlcNAc
SN152a	<0.6 (n=177)	23.9±6.1 (n=261)
DH1138	<0.5 (n=194)	7.8±11.5 (n=215)
DH1142	<0.4 (n=243)	12.1±10.4 (n=237)

Strain	Switching frequency (%) Lee's glucose	Lee's GlcNAc
SN152a	5.8±1.3 (n=232)	<0.5 (n=201)
DH1138	0.4±0.8 (n=237)	<0.8 (n=118)
DH1142	3.1±2.4 (n=284)	<0.5 (n=187)

Figure 3. Effect of deletion of *OFI1* on white–opaque switching Cells from a pure white colony or an opaque colony were plated on Lee's glucose or Lee's GlcNAc medium plates and incubated at 25°C for 4 days. The white-to-opaque switching frequency of DH1138 is significantly lower that of the WT (SN152a) on Lee's GlcNAc medium ($P < 0.05$, Student's *t*-test). *n*, total number of colonies; Wh or w, white colony; Op or o, opaque colony. WT, SN152a.

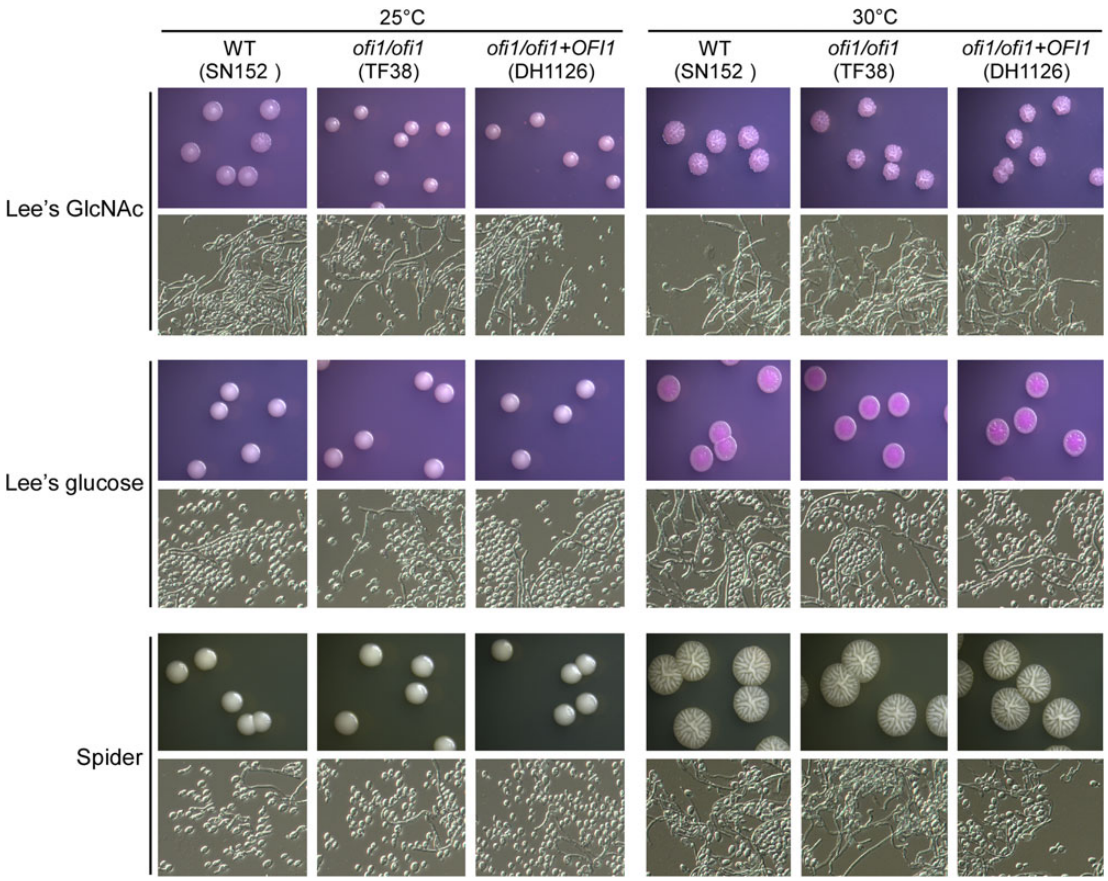


Figure 4. Deletion of *OFI1* has no obvious effect on filamentous growth in *C. albicans* Cells were cultured on Lee's glucose medium, Lee's GlcNAc medium, and Spider medium plates, and incubated at 25°C or 30°C for 3 days. Colony and cellular morphologies are shown. WT, SN152.

switching frequencies of all these three strains were extremely low, while the *ofi1*/*ofi1* mutant exhibited a relatively lower white-to-opaque switching frequency than the control and the *OFI1* reconstituted strain. These results are consistent with the over-expression results (**Fig. 2**). As previously mentioned, Wor1 can bind to the promoter of *OFI1* [3]. Therefore, Ofi1 could function as an inducer of the opaque phenotype downstream of the Wor1 master regulator.

Role of Ofi1 in filamentation in *C. albicans*

Filamentation is another striking feature of the pathogenic yeast *C. albicans*. Many zinc-finger transcription factors, such as Czf1 and Bcr1, regulate filamentous growth [7,23]. Some of these transcription factors are involved in the regulation of both filamentation and white–opaque switching [5]. We were interested in whether Ofi1 regulates filamentation in *C. albicans*. *MTL***a**/α strains were used for the filamentation assays. To study the effect of the deletion of *OFI1* on filamentation, the *ofi1*/*ofi1* mutant (TF38), control (SN152), and *OFI1* reconstituted strain (DH1126) underwent filamentous growth at similar levels on plates with three different media (Lee's glucose medium, Lee's GlcNAc medium, and Spider medium), respectively, at 25°C or 30°C (**Fig. 4**). Therefore, the deletion of *OFI1* did not obviously affect filamentation under the conditions tested. However, the over-expression of *OFI1* promoted filamentous growth on Lee's glucose medium, Lee's GlcNAc medium, and Spider medium at 25°C (**Fig. 5**). At 30°C (**Fig. 5**) and 37°C (data not shown), all of the strains underwent filamentation. Therefore, it was difficult to discriminate the robustness of filamentation at high temperatures (30°C and 37°C).

Role of Ofi1 in invasive growth of *C. albicans*

The capacity for invasive growth is required for *C. albicans* to penetrate host tissues to initiate infections. Because filamentation is

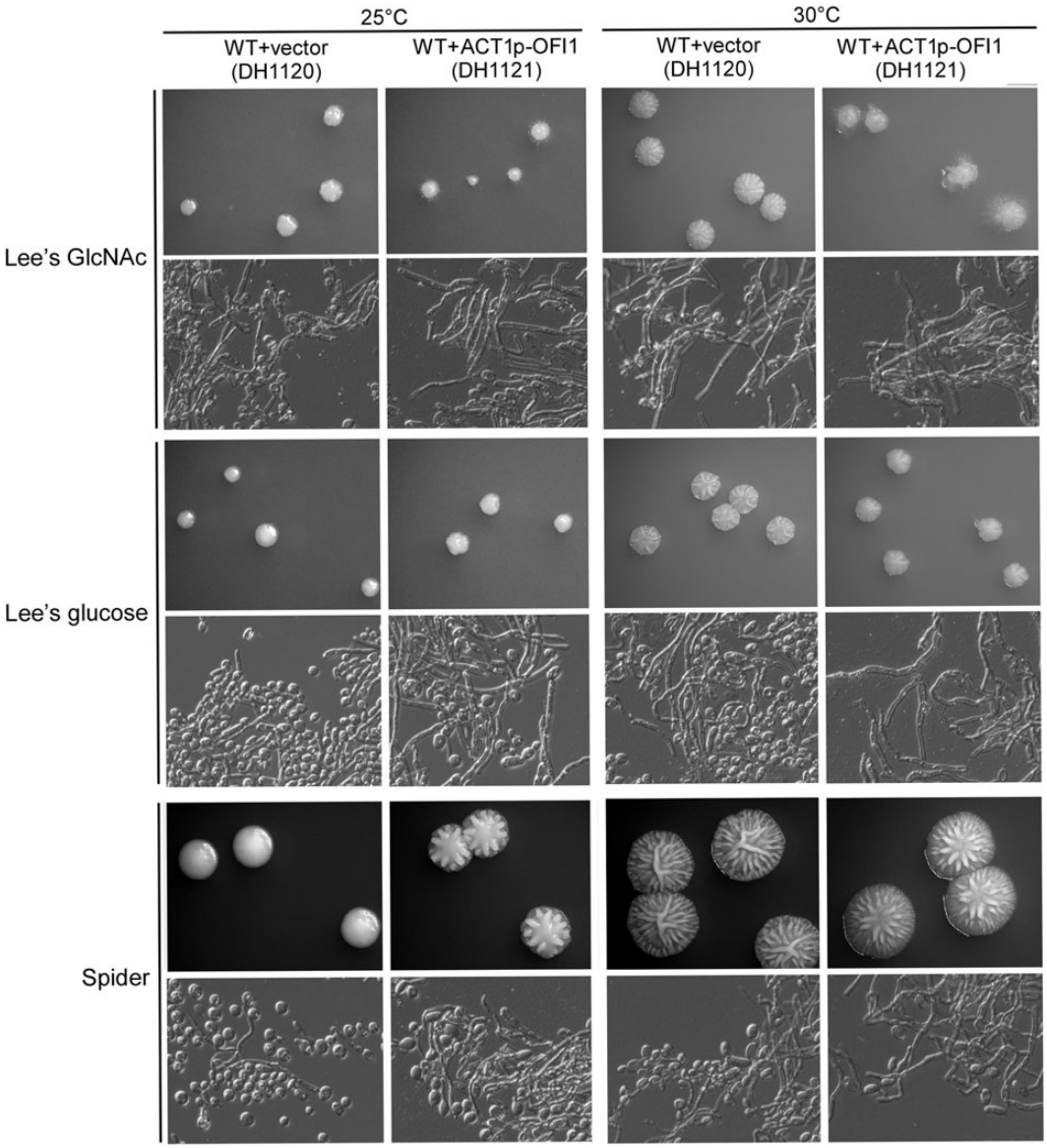


Figure 5. Over-expression of *OFI1* promotes filamentous growth in *C. albicans* Cells were plated on Lee's glucose medium, Lee's GlcNAc medium, or Spider medium plates, and incubated at 25°C or 30°C for 3 days. Colony and cellular morphologies are shown. WT, CAI4.

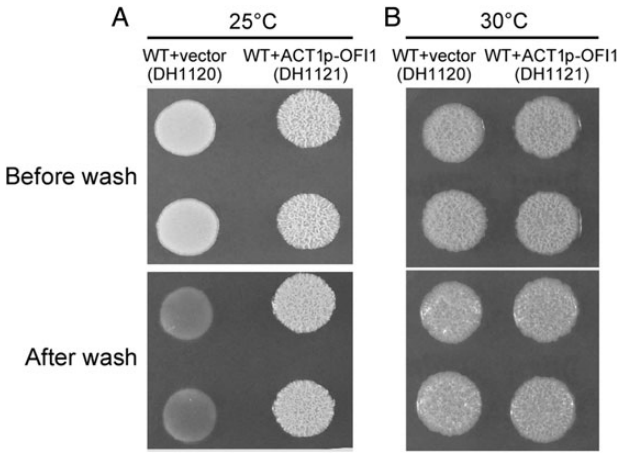


Figure 6. Over-expression of *OFI1* promotes invasive growth in *C. albicans*
Three microliters of liquid medium containing ~2000 cells was dropped onto Spider medium plates and then incubated at 25°C (A) or 30°C (B) for 3 days. The plates were imaged before and after washing. WT, CAI4.

strongly associated with this ability, we further tested whether Ofi1 regulated invasive growth in *C. albicans*. Cells were cultured on Spider medium for 3 days and washed with ddH_2O. At 25°C, over-expression of *OFI1* in a WT strain clearly enhanced the invasive growth capacity of the fungus (**Fig. 6A**). Consistently, spots of the over-expression strain were more wrinkled than those of the control at 25°C, which indicated that the over-expression strain had undergone more robust filamentation. At 30°C, both the over-expression strain and the WT control underwent strong invasive growth. None of the spots could be removed by washing (**Fig. 6B**).

Discussion

Transcription factors regulate a number of biological aspects in *C. albicans*, such as phenotypic transitions, virulence, stress and antifungal resistance, and biofilm development [5]. In the present study, we report the role of Ofi1, a previously uncharacterized zinc-finger transcription factor, in the regulation of white–opaque switching and filamentous growth in *C. albicans*. White–opaque switching and filamentation are two important features of this fungal pathogen and are tightly linked with pathogenesis. Over-expression of *OFI1* not only induced white-to-opaque switching but also promoted filamentation and invasive growth in *C. albicans* under some culture conditions. But over-expression of *OFI1* in the *wor1/wor1* mutant had no inducing effect on white-to-opaque switching, suggesting that Ofi1 and other unidentified transcription factors may regulate this switching process in a coordinative manner. Deletion of *OFI1* significantly reduced the frequency of white-to-opaque switching on Lee's GlcNAc medium. These results are consistent with the fact that *OFI1* is enriched in opaque cells. Zordan *et al.* [3] reported that deletion of *OFI1* (*orf19.4972*) had no obvious effect on the frequency of white-to-opaque switching. They only tested the frequency of the mutant on synthetic defined medium with glucose as the carbon source and did not test that of *OFI1* over-expression strains. Consistently, we also found that deletion of *OFI1* had no significant effect on white-to-opaque switching on Lee's glucose medium (**Fig. 3**). However, when GlcNAc was used as a carbon source, the case is different.

White–opaque switching and filamentation have been traditionally regarded as unrelated because opaque cells typically do not undergo filamentation under regular culture conditions [7,24,25]. Until recently, it was proposed that the regulation of white–opaque switching and filamentation is tightly linked [5,7,25]. Interestingly, a number of environmental cues (e.g. CO_2 and GlcNAc) and many transcription factors (e.g. Efg1, Czf1, and Flo8) regulate both white–opaque transitions and filamentation. Here, we report that Ofi1 induces both opaque cell formation and filamentation only when over-expressed and thus establish a new link between these two processes. This regulatory mechanism of phenotypic plasticity may favor both commensal and pathogenic properties of *C. albicans* in humans.

Supplementary data

Supplementary data is available at *ABBS* online.

Acknowledgements

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