The opportunistic pathogen *Candida albicans* is the most common causative agent of fungal infections in humans (Wenzel, 1995). It possesses a number of traits that make it a successful commensal organism and pathogen, including an ability to adhere to a variety of host tissues and to grow in the diverse conditions encountered in the human body (reviewed by Calderone & Fonzi, 2001). The ability of *C. albicans* to persist in a wide range of niches within the human body suggests that this fungus is highly adaptable and phenotypically plastic. One developmental programme that contributes to the adaptability of *C. albicans* is known as ‘white–opaque switching’ (reviewed by Soll, 2004), in which budding yeast cells switch between two cell types: the white phase, which grows as round to oval-shaped cells that produce smooth white colonies, and the opaque form, which has elongated cells with surface pimples and produces flat, grey colonies (Slutsky et al., 1987). *C. albicans* was long thought to be asexual, but it is now known that this species is capable of mating (Magee & Magee, 2000) and that opaque cells, the mating-competent form, arise only from cells that are homozygous at the mating-type locus (*MTL*) (Lockhart et al., 2002; Miller & Johnson, 2002). The white form has been shown to be more virulent in a systemic-infection animal model whereas the opaque form is better at colonizing and infecting the skin (Kvaal et al., 1997, 1999), showing that the two forms are adapted for different niches within the host. Therefore, the ability to undergo this reversible switching probably contributes to the versatility of *C. albicans* as a colonizer and pathogen within the human body.

In addition to phenotypic switching, *C. albicans* is capable of undergoing a morphological transition between the single-cellular budding yeast form and multicellular filamentous forms of growth (reviewed by Sudbery et al., 2004). The ability to switch between yeast and filamentous morphologies (hyphae and pseudohyphae) is also believed to be important for *C. albicans* to cause disease (Kumamoto &Vinces, 2005).

Though yeast-to-hyphae morphogenesis is thought to be distinct from phenotypic switching, they both share a common regulatory component. A major regulator of hyphal morphogenesis, *Efg1p* (Stoldt et al., 1997), also regulates white–opaque switching (Lachke et al., 2003a; Sonneborn et al., 1999). Deletion of *EFG1* results in cells that possess gene expression patterns and morphology similar to those of opaque cells. Overexpression of *EFG1* in opaque cells forces conversion of these cells to the white phase. Therefore, *Efg1p* is a regulator of both types of cellular switching.

## INTRODUCTION

The opportunistic pathogen *Candida albicans* is the most common causative agent of fungal infections in humans (Wenzel, 1995). It possesses a number of traits that make it a successful commensal organism and pathogen, including an ability to adhere to a variety of host tissues and to grow in the diverse conditions encountered in the human body (reviewed by Calderone & Fonzi, 2001). The ability of *C. albicans* to persist in a wide range of niches within the human body suggests that this fungus is highly adaptable and phenotypically plastic. One developmental programme that contributes to the adaptability of *C. albicans* is known as ‘white–opaque switching’ (reviewed by Soll, 2004), in which budding yeast cells switch between two cell types: the white phase, which grows as round to oval-shaped cells that produce smooth white colonies, and the opaque form, which has elongated cells with surface pimples and produces flat, grey colonies (Slutsky et al., 1987). *C. albicans* was long thought to be asexual, but it is now known that this species is capable of mating (Magee & Magee, 2000) and that opaque cells, the mating-competent form, arise only from cells that are homozygous at the mating-type locus (*MTL*) (Lockhart et al., 2002; Miller & Johnson, 2002). The white form has been shown to be more virulent in a systemic-infection animal model whereas the opaque form is better at colonizing and infecting the skin (Kvaal et al., 1997, 1999), showing that the two forms are adapted for different niches within the host. Therefore, the ability to undergo this reversible switching probably contributes to the versatility of *C. albicans* as a colonizer and pathogen within the human body.

In addition to phenotypic switching, *C. albicans* is capable of undergoing a morphological transition between the single-cellular budding yeast form and multicellular filamentous forms of growth (reviewed by Sudbery et al., 2004). The ability to switch between yeast and filamentous morphologies (hyphae and pseudohyphae) is also believed to be important for *C. albicans* to cause disease (Kumamoto & Vinces, 2005).

Though yeast-to-hyphae morphogenesis is thought to be distinct from phenotypic switching, they both share a common regulatory component. A major regulator of hyphal morphogenesis, *Efg1p* (Stoldt et al., 1997), also regulates white–opaque switching (Lachke et al., 2003a; Sonneborn et al., 1999). Deletion of *EFG1* results in cells that possess gene expression patterns and morphology similar to those of opaque cells. Overexpression of *EFG1* in opaque cells forces conversion of these cells to the white phase. Therefore, *Efg1p* is a regulator of both types of cellular switching.

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**Abbreviations:** EMSA, electrophoretic mobility shift assay; GST, glutathione S-transferase; HA, haemagglutinin.

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**The morphogenetic regulator Czf1p is a DNA-binding protein that regulates white–opaque switching in *Candida albicans***

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Czf1p has been demonstrated to regulate the switch between the yeast-cell morphology and filamentous morphologies of the human fungal pathogen *Candida albicans*. The predicted amino acid sequence of Czf1p contains a zinc-cluster motif similar to the DNA-binding domains of proteins such as *Saccharomyces cerevisiae Gal4p*, suggesting that Czf1p is a DNA-binding protein. Czf1p also demonstrates genetic interaction and a two-hybrid interaction with a second regulator of *C. albicans* cellular morphology, Efg1p. During growth in contact with an agar matrix, Efg1p has a negative effect on filamentation and Czf1p antagonizes this effect. In addition to regulating cellular morphology, Efg1p plays a role in regulating the cell-type switch between the commonly observed white phase of *C. albicans* and the opaque, mating-competent phase. While overexpression of *EFG1* stimulates the switch from opaque to white, the results reported here demonstrate that overexpression of Czf1p promotes the reverse switch, from white to opaque. We also demonstrate that Czf1p binds CZF1 promoter DNA *in vitro*. Therefore, for the regulation of both contact-dependent filamentation and white–opaque switching, Czf1p and Efg1p have opposing functions.

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Another regulator of hyphal morphogenesis, Czf1p, is important for contact-dependent filamentous growth when cells are grown in contact with a matrix such as agar (Brown et al., 1999). Czf1p antagonizes the negative effect of Efg1p on contact-dependent filamentous growth, thereby promoting filamentation under these conditions (Giusani et al., 2002). In this report, we demonstrate direct DNA binding of Czf1p to its own promoter as well as a role for Czf1p in the regulation of white-opaque switching. Overexpression of Czf1, like overexpression of the white-opaque switch regulator Wor1/Tos9/Eap2 (Huang et al., 2006; Srikantha et al., 2006; Zordan et al., 2006), results in conversion of MTL-homozygous cells to the opaque phase. This effect of CZF1 overexpression is dependent on the presence of Wor1p/Tos9p/Eap2p. CZF1 overexpression also results in altered expression of the Wor1p/Tos9p/Eap2p gene.

### METHODS

**Strains and growth conditions.** The strains of C. albicans used in this study are listed in Table 1. Transformation of C. albicans was performed using the lithium acetate method (Ausubel et al., 1989). Escherichia coli strains used were XL-1 Blue (Stratagene) for plasmid propagation, BL21 (Novagen) for protein production, and TOP10F* for TOPO cloning (Invitrogen).

C. albicans cells were routinely grown in YPD media (1% yeast extract, 2% bacto peptone, 2% dextrose). For selective media, C. albicans cells were routinely grown in YPD media (1% yeast extract, 2% bacto peptone, 2% dextrose). For selective media, C. albicans was grown on YPD agar with phloxine B (5 or 50 μg ml⁻¹) and uridine (61.2 μg ml⁻¹). Colonies in the opaque phase, which stain red (Anderson & Soll, 1987), were picked and purified.

For RNA analysis, liquid cultures were observed microscopically to confirm that they were composed of only one phase, and cells were harvested and frozen at −80 °C in RNA later (Ambion).

**Plasmids.** Plasmid pYPB-ADH1pt-CZF1, encoding the CZF1 ORF under the control of the strong ADH1 promoter, was constructed as described previously (Vinces et al., 2006). The pGST–CZF1 plasmid was constructed by PCR amplification of CZF1 using primers POM1F and POM1R (Table 2), followed by digestion and ligation into BamHI/EcoRI-cut pGEX-6-P3 (Pharmacia).

**Protein preparation.** For purification of GST–Czf1p, cells were grown to OD₆₀₀ 0.7 and induced with 0.5 mM IPTG for 1 h. Cells were harvested and resuspended in 50 mM sodium phosphate, pH 8.0, 2 mM PMSF and aprotinin (1:10,000; Sigma), and passed through a French pressure cell twice at 20,000 p.s.i. (138,000 kPa). The lysate was clarified by centrifugation at 18,000 r.p.m. (38,000 g) for 15 min in a Sorvall SS34 rotor. The cleared lysate was loaded onto a pre-equilibrated glutathione Sepharose column (5 ml bed volume).

### Table 1. Strains used in this study

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<tr>
<th>Strain</th>
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<tr>
<td>CAI-4</td>
<td>SC5314 Δura3::λ.imm434Δura3::λ.imm434</td>
<td>Fonzi &amp; Irwin (1993)</td>
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<tr>
<td>WO-1</td>
<td>Δura3 strain derived from strain WO-1</td>
<td>P. T. Magee*</td>
</tr>
<tr>
<td>TOHO3</td>
<td>Δura3::FRT1/tos9-Δa::FRT1 ura3</td>
<td>Srikantha et al. (2006)</td>
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<td>This study</td>
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<td>This study</td>
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</table>

*University of Minnesota.

### Table 2. Primers used in this study

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<td>POM1R†</td>
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<tr>
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</tr>
<tr>
<td>CZF3endR</td>
<td>CCCCTCAACACGAGAAGC</td>
</tr>
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</table>

*BamHI site underlined; ATG start codon in bold type. †EcoRI site underlined.
and incubated at 4 °C for 30 min. The resin was washed three times with 50 mM sodium phosphate, pH 8.0. Protein was eluted with 50 mM sodium phosphate, pH 8.0/10 mM glutathione, and eluates were concentrated using a 10K Vivaspin filter to about 2 ml.

**Electrophoretic mobility shift assays (EMSAs).** Primer pairs CZF1AF/CZF1AR, TATAF/TATAR and EMSA5/EMSA6 (Table 2) were used to amplify 565, 271 or 117 bp fragments of the CZF1 promoter (fragments E, T and E3, respectively; Fig. 1a). DNA probes were gel-purified and labelled with [γ-32P]ATP using T4 polynucleotide kinase (New England Biolabs) and the manufacturer’s protocol. EMSAs were modified from published protocols (Ausubel et al., 1989). Binding reactions contained 12% (v/v) glycerol, 12 mM HEPES-NaOH (pH 7.9), 4 mM Tris-Cl (pH 7.9), 60 mM KCl, 1 mM EDTA, 1 mM DTT, 2 μg poly(dI-dC), 5 μg BSA, 0.7–3.5 ng 32P-labelled oligonucleotide probe, and varying amounts of protein. After incubation for 30 min at 30 °C, the mixture was separated on 4% polyacrylamide, 0.06% bisacrylamide non-denaturing gels with low-ionic-strength buffer at 4 °C. Dried gels were visualized by autoradiography. Supershifts were performed by incubation with antibody [anti-glutathione S-transferase (GST) rabbit IgG (Molecular Probes), anti-haemagglutinin (HA) mouse monoclonal IgG (Babco), or anti-Xpress mouse monoclonal IgG (Invitrogen)] for an additional 15 min at 30 °C.

**Measurement of phenotypic switching.** White cells were grown overnight at 37 °C, observed microscopically to confirm white phase purity and then diluted into YPD to OD600 0.1. Cells were grown for various times at 25 °C in liquid medium or on plates, then plated onto YPD agar with 50 μg phloxine B ml−1 and grown for 7 days at 25 °C. To calculate the proportion of colonies that switched to the opaque phase, the number of red or red-sectored colonies and the total number of colonies were counted.

**Northern analyses.** Total RNA was extracted using an RNeasy Midi kit (Qiagen) with mechanical disruption using glass-zirconia beads. Probes were generated with the following primer pairs (Table 2): for WOR1/TOS9/EAP2, WOR1F and WOR1R; for WH11, WH11F and WH11R; for OP4, OP4F and OP4R; for CZF1, CZFS29 and CZFendR. The 18S rRNA probe was as described elsewhere (Bahn & Sundstrom, 2001). Probes were labelled with [α-32P]dATP (NEN-Perkin Elmer) using a Primer-It II Random Primer Labelling kit (Stratagene). Northern blot hybridization was performed as described elsewhere (Sambrook et al., 1989). Results were quantified using ImageQuant TL (Amersham).

**RESULTS**

**Czf1p is a DNA-binding protein that binds its own promoter**

Czf1p regulates expression from its own promoter. In vivo, Czf1p binds to a regulatory region far upstream of its ORF and can be detected by chromatin immunoprecipitation

![Fig. 1. EMSA of Czf1p binding to the CZF1 promoter.](http://mic.sgmjournals.org)
(Vinces et al., 2006). To determine whether Czf1p binds DNA directly, EMSA was performed using radiolabelled DNA fragments corresponding to regions upstream of the CZF1 ORF (Fig. 1a).

As shown in Fig. 1(b), GST–Czf1p shifted the E probe (Fig. 1b, lane 2), corresponding to sequences upstream of a previously characterized transcription start site at –2065 relative to the start of the CZF1 ORF (Vinces et al., 2006), but failed to shift the T probe, corresponding to sequences near the start of transcription (Fig. 1b, lane 5). One major complex was formed, with a minor higher-molecular-mass complex seen when higher concentrations of protein were used. Formation of the E probe complex was subject to competition by specific competitors but not by unrelated sequences (Fig. 1b, lanes 8–10). An excess amount of purified unfused GST protein was unable to form a complex with the CZF1 promoter probe (Fig. 1b, lane 11).

To identify a smaller Czf1p-binding region, fragments derived from the E probe were tested in the EMSA experiment. The highest binding of GST–Czf1p was to a 117 bp fragment termed E3 (Fig. 1c, lane 1).

To confirm that complex formation was due to the GST–Czf1p fusion protein, an antibody supershift was performed. Radiolabelled probe E3 was incubated with GST–Czf1p in the presence of anti-GST, anti-HA or anti-Xpress antibodies. A further reduction in the mobility of the protein–DNA complex was observed when anti-GST was added (Fig. 1c, lane 2) but not when anti-HA or anti-Xpress antibodies were added (Fig. 1c, lanes 3 and 4). These results demonstrate that Czf1p has DNA-binding activity, and that it binds to the promoter region of the CZF1 gene.

Overexpression of CZF1 results in conversion of white phase cells to the opaque phase

CZF1 is more highly expressed in the opaque phase than in the white phase (Lan et al., 2002; Tsong et al., 2003; Vinces et al., 2006) and is down-regulated in the stationary phase of opaque cells but upregulated in opaque cells during mating (Zhao et al., 2005). To investigate the role of CZF1 in white–opaque switching, a construct encoding CZF1 under the control of the strong ADH1 promoter (Vinces et al., 2006) was integratively transformed into MTL-heterozygous (switching-incompetent) and MTL-homozygous (switching-competent) strains. Switching to the opaque phase was not observed in the MTL-heterozygous a/α strains, despite high expression of CZF1 (data not shown). Thus, CZF1 expression was not sufficient to overcome the previously observed repressive effects of a1–α2 on switching (Lockhart et al., 2002; Miller & Johnson, 2002). In contrast, when cultures of white cells of the MTL-homozygous strain MVY108 (CAI-4/pYPB-ADH1pt-CZF1) were incubated in liquid medium for 32 h and plated on YPD agar containing phloxine B, opaque colonies or sectors were eightfold more frequent than for MVY107 (CAI-4/pYHB-ADH1pt) (Fig. 2a; P<0.02 by t test). In the switching strain WO-1, the percentage of opaque colonies or sectors was threefold higher when CZF1 was overexpressed (Fig. 2a; P<0.004 by t test).

To demonstrate that CZF1 overexpression promoted the formation of bona fide opaque colonies, cells from red colonies were further characterized. The cells exhibited the expected elongated morphology characteristic of opaque cells (data not shown). In addition, Northern blotting showed that following growth in YPD at 25 °C for 18 h, cells identified as white expressed the white-specific gene WH11, while cells identified as opaque expressed the opaque-specific gene OP4 (Fig. 2b). Therefore, we conclude that ectopic expression of CZF1 promoted the formation of opaque cells.

To characterize CZF1 expression in strains carrying pYPB-ADH1pt-CZF1, white or opaque cells of strains MVY107 (CAI-4/pYHB-ADH1pt) and MVY108 (CAI-4/pYPB-ADH1pt-CZF1) were grown in YPD medium at 25 °C for 18 h and RNA was analysed by Northern blotting. As shown in Fig. 2(c), the expression of CZF1 from the PADH1 promoter was substantially higher than the expression from the native locus. Expression of CZF1 from its native promoter was higher in opaque cells than in white cells, consistent with previous results (Tsong et al., 2003; Vinces et al., 2006), and was not reduced by ectopic expression of CZF1.

Because CZF1 promotes filamentation only when cells are growing in contact with semi-solid medium, the effect of growth conditions on the ability of overexpressed CZF1 to promote the switch to opaque was investigated. We found that pre-growth in liquid medium (Fig. 3a, grey bars) or on the surface of agar plates (Fig. 3a, black bars) led to the formation of similar numbers of opaque colonies; consistent differences were not observed. However, under both pre-growth conditions, there was a trend toward higher numbers of opaque colonies after longer incubation. In fact, following incubation on phloxine B-containing YPD agar plates for long periods of time (e.g. 10 days), white colonies of MVY108 (CAI-4/pYPB-ADH1pt-CZF1) developed red rings, suggesting that accumulation of cells in the opaque phase was occurring (Fig. 3b). In contrast, white cells of strain MVY107 (CAI-4/pYHB-ADH1pt) produced normal white colonies with occasional red colonies or sectors following incubation on YPD phloxine B plates for the same length of time (data not shown). Cells from the red rings exhibited opaque cell morphology, while cells from the central white portion of the same colonies exhibited white cell morphology (data not shown).

In contrast to the results obtained with CZF1-overexpressing strains, the czf1 null mutant exhibited an extremely low frequency of switching to opaque (data not shown). These results demonstrated a role for CZF1 in regulating the switch from the white to the opaque phase in both CAI-4-derived and WO-1-derived strains.
Effects of CZF1 overexpression on WOR1/TOS9/EAP2 expression in MTL homozygotes

Recent results demonstrate that Wor1p/Tos9p/Eap2p is a master regulator of white–opaque switching, that is preferentially expressed in opaque cells and not expressed in MTL a/z heterozygotes (Huang et al., 2006; Srikantha et al., 2006; Zordan et al., 2006). Ectopic expression of WOR1/TOS9/EAP2 in white cells results in their mass conversion to opaque (Huang et al., 2006; Srikantha et al., 2006; Zordan et al., 2006). Therefore, the effect of overexpression of CZF1 on transcription of WOR1/TOS9/EAP2 was studied. Northern blotting revealed that when CZF1 was ectopically expressed in white cells (Fig. 4, lanes 2 and 4), levels of WOR1/TOS9/EAP2 expression increased to 16 % or more of the level in opaque cell cultures (Fig. 4, lanes 7 and 9). The proportion of opaque cells in these cultures was <1 %. In contrast, in an MTL a/z strain, CZF1 overexpression had no effect on WOR1/TOS9/EAP2 expression (Fig. 4, lane 5). Consistent effects of CZF1 on expression levels of WOR1/TOS9/EAP2 in opaque cells were not observed (Fig. 4, lanes 6–9). Thus, Czf1p exerts an effect on the expression of the white–opaque regulator gene WOR1/TOS9/EAP2.

To determine whether Wor1p/Tos9p/Eap2p was required for the effects of Czf1p, switching in a wor1/tos9/eap2 null mutant strain that overexpressed CZF1 was analysed using the methods described in Fig. 2(a). While overexpression of CZF1 in a WOR1/TOS9/EAP2+ strain resulted in 10.6 ± 1.1 % red colonies or sectors on phloxine B plates (mean and SD determined with triplicate cultures), overexpression of CZF1 in TOHO3, a WO-1-derived strain lacking Wor1p/Tos9p/Eap2p, resulted in <0.1 % red colonies or sectors. Overexpression of CZF1 in a CAI-4-derived wor1/tos9/eap2 null mutant yielded similar results (data not shown).

DISCUSSION

CZF1 was originally identified in a heterologous genetic screen for C. albicans sequences that interfere with pheromone-induced cell cycle arrest in Saccharomyces cerevisiae (Whiteway et al., 1992), and was subsequently identified in a screen for regulators of contact-dependent filamentation (Brown et al., 1999). The predicted sequence of the protein encoded by the CZF1 ORF suggested that Czf1p would have DNA-binding activity, and direct
usually high.

WOR1/TOS9/EAP2 expression in the CZF1 homozygous white cells resulted in higher expression of WOR1/TOS9/EAP2 transcript (data not shown). Since misexpression of WOR1/TOS9/EAP2 in white cells results in switching to the opaque phase (Huang et al., 2006; Srikantha et al., 2006), Czf1p may exert its effect on phenotypic switching via altered expression of CZF1.

Interestingly, ectopic expression of CZF1 in MTL heterozygotes did not result in higher expression of WOR1/TOS9/EAP2. In MTL heterozygotes, WOR1/TOS9/EAP2 expression is repressed through binding of α1–α2 at a site or sites more than 5 kb upstream of the start of the ORF (Srikantha et al., 2006; Tsong et al., 2003; Zordan et al., 2006). CZF1 expression may not be sufficient to relieve this repression, resulting in the failure to express WOR1/TOS9/EAP2 and the inability to undergo white-to-opaque switching. In contrast, when WOR1/TOS9/EAP2 is ectopically expressed in MTL homozygotes, bypassing positively regulate its own transcription (Huang et al., 2006; Zordan et al., 2006), resulting in an all or nothing pattern of expression in individual cells (Huang et al., 2006). Therefore, it is likely that overexpression of CZF1 leads to WOR1/TOS9/EAP2 expression in a subset of white cells. The proportion of WOR1/TOS9/EAP2-expressing white cells did not appear to increase upon longer incubation, as cultures grown for 18 h did not contain higher levels of WOR1/TOS9/EAP2 transcript (data not shown). Since misexpression of WOR1/TOS9/EAP2 in white cells results in switching to the opaque phase (Huang et al., 2006; Srikantha et al., 2006; Zordan et al., 2006), Czf1p may exert its effect on phenotypic switching via altered expression of WOR1/TOS9/EAP2.

Consistent with this model, ectopic expression of CZF1 did not increase switching in the absence of Wor1p/Tos9p/Eap2p.

Fig. 3. Effects of CZF1 overexpression on white–opaque switching. (a) Cells were pre-grown in liquid YPD (grey bars) or on YPD plates (black bars) at 25 °C for various times. Following growth, cells were plated on YPD plates containing 5 or 50 μg phloxine B ml⁻¹ and incubated at 25 °C. The fraction of red (opaque) colonies or red sectors obtained after pre-growth for the indicated time is shown. The strains were CAI-4-derived MVY108 (CAI-4/pYPB-ADH1pt-CZF1) and WO-1-derived MVY106 (WO-1/pYPB-ADH1pt-CZF1). (b) White cells of strain MVY108 (CAI-4/pYPB-ADH1pt-CZF1) incubated for 10 days on YPD agar containing 50 μg phloxine B ml⁻¹. White colonies developed red rings after several days of growth, suggesting accumulation of cells in the opaque form.

Fig. 4. Overexpression of CZF1 affects the expression of the white–opaque phase-switching regulator gene WOR1/TOS9/EAP2. Cells in the white phase (lanes 1–5) or the opaque phase (lanes 6–9) were grown for 10 h in liquid YPD at 25 °C, and RNA was extracted as described in Methods. Total RNA (20 μg per lane) from each sample was analysed by Northern blotting with WOR1 and 18S rRNA probes. Strains were as follows: lane 1, MVY107, white (CAI-4/pYPB-ADH1pt); lane 2, MVY108, white (CAI-4/pYPB-ADH1pt-CZF1); lane 3, MVY105, white (WO-1/pYPB-ADH1pt); lane 4, MVY106, white (WO-1/pYPB-ADH1pt-CZF1); lane 5, MVY109, white (CAI-4 MTLα/α pYPB-ADH1pt-CZF1); lane 6, MVY107, opaque (CAI-4/pYPB-ADH1pt); lane 7, MVY108, opaque (CAI-4/pYPB-ADH1pt-CZF1); lane 8, MVY105, opaque (WO-1/pYPB-ADH1pt); lane 9, MVY106, opaque (WO-1/pYPB-ADH1pt-CZF1). All lanes are from the same gel, but intervening irrelevant lanes have been removed for clarity. The WOR1/TOS9/EAP2 transcript (approx. 4 kb) and 18S rRNA are indicated on the right.
a1–a2-mediated repression, opaque cell formation occurs (Huang et al., 2006; Srikantha et al., 2006; Zordan et al., 2006).

Prolonged incubation of CZF1-overexpressing cells resulted in the accumulation of higher numbers of opaque cells. Previous studies show that the ADH1 promoter is down-regulated in the post-exponential phase (Bertram et al., 1996), and therefore the percentage of opaque cells in a culture may level off at later time points because CZF1 is no longer overexpressed. Cells incubated on plates or in liquid medium gave rise to similar numbers of opaque colonies. However, on plates, CZF1-overexpressing colonies developed distinctive red rings after prolonged incubation. The formation of these rings may reflect the regulation of the ADH1 promoter, as cells in the centre of the colony may be in stationary phase and not expressing high levels of CZF1, while cells at the periphery may be actively growing and overexpressing CZF1.

Both Czf1p and Efg1p regulate white–opaque switching (Lachke et al., 2003a; Sonneborn et al., 1999) and contact-dependent invasive filamentation (Giusani et al., 2002). During growth within agar, Czf1p antagonizes the repressive effect of Efg1p on filamentation. Czf1p and Efg1p also have opposing effects on the transcription of CZF1 (Vinces et al., 2006): Efg1p is necessary for expression of CZF1, while ectopic overexpression of CZF1 reduces transcription from the native CZF1 promoter. Efg1p regulates the transcription of large numbers of genes, including cell wall proteins, virulence factors and metabolic genes (Doedt et al., 2004; Lane et al., 2001; Nantel et al., 2002; Sohn et al., 2003; Staub et al., 2002), and Czf1p may modify the effects of Efg1p on the expression of some of these genes. Thus, transcription of WOR1/TOS9/EAP2 in MTL homozygotes may be regulated through the opposing activities of Efg1p and Czf1p.

It is interesting that Czf1p and Efg1p regulate hyphal morphogenesis in MTL heterozygotes and white–opaque switching in MTL homozygotes. These two processes may be related because mating projection formation resembles filamentation. Indeed, one recent study has shown that a number of genes associated with filamentous growth are regulated during mating (Zhao et al., 2005). Contact-dependent cues also promote both filamentation and mating (Brown et al., 1999; Lachke et al., 2003b).

The effects of Czf1p on contact-dependent invasive filamentation are particularly apparent at lower temperatures (Brown et al., 1999). As shown here, Czf1p promotes opaque cell formation in MTL homozygotes and white–opaque switching is also regulated by temperature; opaque cells are most readily obtained at lower temperature (Slutsky et al., 1987). During growth of C. albicans on the skin, fungi might receive environmental cues indicating both contact and low temperature. Thus, it is intriguing that opaque cells are more virulent in skin infections (Kvaal et al., 1999) and that mating, an opaque-cell-dependent response, is promoted during growth on skin (Lachke et al., 2003b). Czf1p contributes to the biology of C. albicans during growth on certain types of surfaces at reduced temperature.

In contrast, Efg1p promotes virulence during systemic infection, and a mutant lacking Efg1p and a second regulator of filamentation, Cph1p, is completely avirulent in the intravenous model (Lo et al., 1997). In MTL homozygotes, Efg1p promotes the formation of white cells (Lachke et al., 2003a; Sonneborn et al., 1999), the form that is more virulent in systemic infection (Kvaal et al., 1997). These observations support the hypothesis that Efg1p is a key regulator that promotes systemic virulence. By possessing a collection of regulators that optimize its behaviour in different locations within the host, C. albicans is well adapted for efficiently exploiting its host.

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