Candida albicans Sfl1 Suppresses Flocculation and Filamentation

Janine Bauer and Jürgen Wendland*

Carlsberg Laboratory, Yeast Biology, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark; Department of Microbiology, Friedrich-Schiller-University, Jena, Germany; and Junior Research Group, Fungal Pathogens, Leibniz Institute for Natural Product Research and Infection Biology—Hans-Knöll Institute, Jena, Beutenbergstr., 11a, D-07745 Jena, Germany

Received 29 June 2007/Accepted 17 August 2007

Hyphal morphogenesis in Candida albicans is regulated by multiple pathways which act by either inducing or repressing filamentation. Most notably, Tup1, Nrg1, and Rfg1 are transcriptional repressors, while Efg1, Flo8, Cph1, and Czf1 can induce filamentation. Here, we present the functional analysis of CaSFL1, which encodes the C. albicans homolog of the Saccharomyces cerevisiae SFL1 (suppressor of flocculation) gene. Deletion of CaSFL1 results in flocculation (i.e., the formation of clumps) of yeast cells, which is most pronounced in minimal medium. The flocs contained hyphae already under noninducing conditions, and filamentation could be enhanced with hypha-inducing cues at 37°C. Expression of SFL1 in a heterozygous mutant under the control of the CaMET3 promoter was shown to complement these defects and allowed switching between wild-type and mutant phenotypes. Interestingly, increased expression of SFL1 using a MET3prom-SFL1 construct prior to the induction of filamentation completely blocked germ tube formation. To localize Sfl1 in vivo, we generated a SFL1-GFP fusion. Sfl1-green fluorescent protein was found in the nucleus in both yeast cells and, to a lesser extent, hyphal cells. Using reverse transcription-PCR, we find an increased expression of ALS1, ALS3, HWP1, ECE1, and also FLO8. Our results suggest that Sfl1 functions in the repression of flocculation and filamentation and thus represents a novel negative regulator of C. albicans morphogenesis.

* Corresponding author. Mailing address: Carlsberg Laboratory, Yeast Biology, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark. Phone: 45/3327-5230. Fax: 45/3327-4708. E-mail: jww@crc.dk.

Published ahead of print on 31 August 2007.
evisiae, among them the Ras1-cAMP signaling pathway and the mitogen-activated protein kinase-dependent filamentous growth pathway, as well as the glucose repression pathway and the TOR pathway (33). Downstream of the cAMP-signaling pathway, Tpk2 activates Fip8 and represses Sfl1, which encodes a suppressor of flocculation (11, 15).

We were interested to determine whether flocculation in C. albicans is regulated by a similar mechanism as that for S. cerevisiae and whether flocculation and filamentation can be separated genetically. We identified a SFL1 homolog in the C. albicans genome. The deletion of SFL1 led to a flocculent phenotype best seen in minimal media. This flocculation was accompanied by filamentation under noninducing conditions. SFL1-GFP localizes to the nucleus in both yeast and hyphal cells. Overexpression of SFL1, on the other hand, led to a block in filamentation. This result suggests that Sfl1 is a negative regulator of both flocculation and filamentation in C. albicans. Consistently, hypha-specific genes, particularly ALS1 and ALS3, were found to be expressed in a sfl1 mutant under noninducing conditions.

**MATERIALS AND METHODS**

**Strains and media.** The *C. albicans* and *S. cerevisiae* strains used in this study are listed in Table 1. Cells were grown either in rich medium (YPD, 2% peptone, 2% glucose, 1% yeast extract) or in minimal medium (CSM [2% glucose, 6.7 g/liter yeast nitrogen base with ammonium sulfate and without amino acids], 0.79 g/liter CSM, or SD [2% glucose, 6.7 g/liter yeast nitrogen base with ammonium sulfate and without amino acids]) at 30°C. Minimal media were supplemented with the strain-specific requirements for amino acids, adenine, and uridine. Hyphal induction was done at 37°C by adding 10% serum (calf serum; Sigma) or 0.5g/liter GlcNAc to the media.

Regulation of gene expression was done using the MET3 promoter. Activation of MET3p-SFL1 expression was achieved by growing cells in media lacking methionine and cysteine, and repression of the MET3p promoter could be done by adding 3.5 mM methionine and 3.5 mM cysteine to the medium. For plasmid construction and amplification, *E. coli* DH5α served as a host.

**Disruption of CaSFL1.** The *C. albicans* homolog of the *S. cerevisiae* SFL1 was identified in the *C. albicans* genome sequence (http://www.candidagenome.org) as ORF19.454. Transformation was done by electroporation (24) followed by selective incubation on minimal media lacking the appropriate amino acids or uridine for 2 to 3 days at 30°C. Consecutive complete open reading frame (ORF) deletions of both alleles of CaSFL1 were achieved by using PCR-generated disruption cassettes from pFA plasmids amplified with primers S1-SFL1 and S2-SFL1 as described previously (31). All primers (Table 2) were obtained from biosmers.net GmbH (Ulm, Germany). For each new strain, at least two independent mutants were generated. Verifications of correct integration at the target locus and of the absence of the target ORF in homozygous null mutants were done by diagnostic PCR. The fusion of the MET3 promoter to the SFL1 ORF was done by PCR-based gene targeting using primers S1-SFL1 and S2-MET3p-SFL1. A 3‘-end fusion of SFL1 with green fluorescent protein (GFP) was generated by homologous recombination of a GFP-CdhHS1 cassette in a heterozygous SFL1 mutant, thus tagging the sole allele of SFL1 with GFP.

**Promoter shutdown experiments.** To deplete cells of Sfl1, shutdown experiments of MET3 promoter-controlled SFL1 were performed. To this end, overnight cultures of the strains were grown in SD with the appropriate amino acid supplements. Starting cultures were diluted to an optical density at 600 nm of 0.1 in the same type of fresh medium with or without the addition of 3.5 mM methionine and cysteine. These yeast cultures were then incubated for 4 h at 30°C and then used for imaging. In the case of hyphal induction, 0.5 g/liter GlcNAc was added to the cultures, which were incubated for 4 to 5 h at 37°C prior to photography.

**Embedded growth.** Growth conditions were as described previously (9). Cells of the strains were pregrown in YPD and then poured as dilutions thereof in YPS agar (containing 2% sucrose instead of glucose). These plates were incubated at room temperature for up to 5 days.

**One-hybrid assay.** The SFL1 ORF was amplified using primers CaSFL1-0H1-M1 and CaSFL1-0H2-SphI and cloned into Clp-lexA after digestion with MluI and SphI (New England Biolabs, Germany). This generated a SalexA-SFL1 fusion construct. This plasmid was linearized by Stul and transformed into CaI-8 and CAJ043. Control strains containing NRG1 and GCN4 fusion constructs were kindly provided by Al Brown and are described in reference 30. The strains were pregrown in selective media and then inoculated in YPD and grown for 4 to 6 h at 30°C. Cultures were then adjusted to an optical density at 600 nm of 0.5. For qualitative analysis of lacZ expression, 3 μl of each cell suspension was dropped onto CSM plates and incubated for 2 days at 30°C. Colonies were then subjected to an agarose overlay (0.5% maltose buffer [pH 7.0], 6% N,N-dimethylformamide, 0.5% agarose, 0.1% sodium doceyl sulfate, 20 μg/mL-bis-chloro-3-indolyl-β-D-galactopyranoside [X-Gal; 0.1 mg/mL stock solution]). 2-Nitrophenyl-β-D-galactopyranoside (ONPG) assays were used to quantify β-galactosidase activities for different strains. A 2-μl volume of the cell suspension was pelleted and washed with H2O, resuspended in 300 μl Z buffer (60 mM sodium phosphate monobasic dithydrate, 40 mM sodium phosphate dibasic, 10 mM potassium chloride, 1 mM magnesium sulfate), and divided into three aliquots of 100 μl. Cells were opened by using liquid nitrogen. The enzyme reaction was started by adding 160 μl ONPG-Z buffer solution (4 mg/mg ONPG in Z buffer) to each of the aliquots, followed by incubation at 37°C for 30 min. The reaction was stopped by adding 400 μl of 1 M sodium carbonate. Twofold dilutions were measured at 420 and 550 nm.

**RNA isolation and RT-PCR.** Total RNA was isolated using a RNA试剂总RNA isolation kit (Promega). Reverse transcription (RT)-PCR was performed with an Enhanced Avian RT first-strand synthesis kit (Sigma, Germany) using Oligo(dT)12 according to the manufacturer’s protocol. Primers for RT-PCR were derived from the 3’ end of the genes tested (Table 2). As a control, RH03 was

---

**TABLE 1. Strains used in this study**

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| SC5314 | C. albicans wild type | 17 |
| CAI-8  | ura3::Xmm43 ade2::hisG ade2::hisG | 30 |
| CAI-8::SalexA-lexOP | RPS1::Pflu::SalexA-RPS1-URA3 ade2::hisG ade2::OP-LacZ-ADE2 | 30 |
| CAI-8::SalexA-NRG1-lexOP | RPS1::Pflu::SalexA-NRG1-RPS1-URA3 ade2::hisG ade2::OP-LacZ-ADE2 | 30 |
| CAI-8::SalexA-GCN4-lexOP | RPS1::Pflu::SalexA-GCN4-RPS1-URA3 ade2::hisG ade2::OP-LacZ-ADE2 | 30 |
| SN148  | ura3::Xmm43 ade2::hisG ade2::hisG | 27 |
| CAJ025 | SN148::SFL1::CmLEU | This study |
| CAJ027 | SN148::SFL1::CdhHS1 | This study |
| CAJ029 | SN148::CmLEU2::Cm::CdHS1 | This study |
| CAJ035 | SN148::CmLEU2::Cm::CdHS1 | This study |
| CAJ039 | SN148::CmLEU2::Cm::CdHS1::MET3p-SFL1 | This study |
| CAJ042 | SN148::CmLEU2::Cm::SFL1::GFP-CdhHS1 | This study |
| CAJ049 | CAI-8::RPS1::Pflu::SalexA-SFL1-RPS1-URA3 ade2::hisG ade2::OP-LacZ-ADE2 | This study |
| CAJ066 | CAI-8::RPS1::Pflu::SalexA-SFL1-RPS1-URA3 ade2::hisG ade2::OP-LacZ-ADE2 | This study |
| CAJ071 | SN148::CmLEU2::Cm::URA3 | This study |
**RESULTS**

**Sequence analysis of Sfl1.** Based on sequence similarity, orf19.454 encodes the *C. albicans* homolog of the *S. cerevisiae* **SFL1** gene. *C. albicans* Sfl1 encodes one of the longest ascomycetous Sfl1 proteins, with a length of 805 amino acids. A protein alignment reveals a number of conserved features (Fig. 1). Most notable is a strongly conserved domain within the N terminus that shares sequence similarity with the heat shock factor (HSF) domain. There are two regions in the *C. albicans* Sfl1 that are shared with Sfl1 proteins of other species: *C. albicans* and *Aspergillus fumigatus* Sfl1 proteins share an extended N-terminal sequence, and *C. albicans* and *Kluyveromyces lactis* share an internal Q-rich region. With both AsSfl1p and KiSfl1p *C. albicans* shares the highest degrees of sequence identity (21.5% over the entire proteins), although sequence similarities in the C-terminal halves of the proteins are rather low.

**Deletion of CaSFL1 results in flocculation and filamentation in the absence of inducers.** We deleted both copies of CaSFL1 in the SN148 background (27). To this end, we amplified disruption cassettes from pFA plasmids and generated independent heterozygous and homozygous **sfl1** mutant strains (Table 1). Mutant strains that were of the **sfl1** genotype were phenotypically identical. Subsequently, we present the analysis of the **Ura**- **sfl1** mutant. To analyze cell growth and colony morphology phenotypes, we grew the mutant on solid medium and in liquid full medium at yeast and hyphal growth conditions (Fig. 2). Both the wild-type and the **sfl1** mutant strains formed yeast colonies at 30°C and showed filamentous growth at 37°C in the presence of serum. We noted an increased degree of clumping for the mutant in liquid culture, both in conditions promoting yeast growth and under serum-inducing conditions. We quantified this aggregation behavior during growth in YPD (Table 3). It became evident that in YPD, a large amount of **sfl1** mutant cells were found in aggregates. These aggregates appeared not to be generated due to cytokinesis defects as were observed previously, e.g., for *C. albicans rho4* and *cht3* mutants (12). We went on to analyze cell aggregation in SD (Fig. 3). This showed that **sfl1** cells form macroscopic flocs consisting of large amounts of cells. Microscopic observations revealed that these flocs also contained true hyphal cells.

**An analysis of hyphal induction was done to determine whether the onset of filamentation occurs more rapidly in the **sfl1** mutant.** To this end, cells were induced to form filaments with serum. To this end, cells were induced to form filaments with serum. Aliquots of cells were taken in half-hourly intervals, and the average lengths of germ tubes were determined microscopically.

### Table 2. Oligonucleotide primers used in this study

| Primer | Sequence (5’ to 3’) |
|--------|---------------------|
| #600 U2 | GTTTTACGAATTCATGGCACTACACG |
| #599 U3 | GATTTGTAATTAGATGATGAAGTTGATG |
| #601 H2 | CAAAGAAATGGCCCTCCTTACCAG |
| #602 H3 | GGACATTTGAAGAAAGCTGGTGCAACCG |
| #1031 5'RHO3-cDNA | CTTTAATAATCATTTAATACATGCTTCTTTTGG |
| #1430 L2 | CGTTACCGACGTGACCTGTA |
| #1431 L3 | GTTGTCGACGCGATTGTCGAAGCTG |
| #1744 S1-SFL1 | CATATCCCAATAGAAATGATCAGTCGTAATGTAATATAAAAACGATAACGGTAATAGT |
| #1745 S2-SFL1 | CTATTTGTCGACGCGATTGTCGAAGCTG |
| #1746 G1-SFL1 | CCACTCTAATACATGCTTCTCTTATTTTCCACCTGC |
| #1747 G2-SFL1 | CATTCACTGTGTTGGAAGCAAGTATG |
| #1748 I1-SFL1 | CACCGCACTTCTCATCAATTTG |
| #1749 I2-SFL1 | GCAACGTGAGGATGTTGATGGAAC |
| #1852 S2-SFL1-MET3p | GATTCCAGATGTCGATTTGCAAGTTG |
| #1901 G4-SFL1-MET3p | CATAGTGGGCATTGGTATGATGAGTATG |
| #1960 CaFLO8-I1 | GATGAATGCTGTAGGTGGTGG |
| #1961 CaFLO8-I2 | GACCGGCAAACATCATTCCATTACTC |
| #1982 S1-SFL1-GFP | GATGTTTGCCACCAATCAAAGATAATGATAATAAAAACGATAACGGTAATAGT |
| #3127 ALS1-I1 | GACCCAGGAGGAGAGATGATGAGTATG |
| #3128 ALS1-I2 | ATCATGCAATGGGAACGATCATAAAAAGAGAAAATTAGAA |
| #3129 HWP1-I1 | CCTCAATGCAATGGGAACGATCATAAAAAGAGAAAATTAGAA |
| #3130 HWP1-I2 | CCTCAATGCAATGGGAACGATCATAAAAAGAGAAAATTAGAA |
| #3131 ECE1-I1 | CCTCAATGCAATGGGAACGATCATAAAAAGAGAAAATTAGAA |
| #3132 ECE1-I2 | CCTCAATGCAATGGGAACGATCATAAAAAGAGAAAATTAGAA |
| #3191 G4-CaRHO3 | CATGTGTCGACGCGATTGTCGAAGCTG |
| #3204 ALS3-I1 | CCGAAAATGGCTCTTTATGAAATCAC |
| #3205 ALS3-I2 | CAAACCAATACCAAGTTAGAATGTTG |

* Capital letters correspond to genomic sequences, and lowercase letters correspond to annealing regions of pFA plasmids.

used and was amplified from plasmids containing either a genomic or a cDNA fragment.

**Microscopy.** Cells were grown to exponential phase either in minimal medium or in hyphal-inducing medium for 4 to 6 h at 30°C or 37°C, respectively. Nuclear staining was done by adding 1 μl of 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI; 1 mg/ml, Sigma) to 100 μl of cell suspension. Microscopy was done by using a bifunctional confocal laser scanning and fluorescence microscope on a Zeiss AxioImager platform with the appropriate filter combinations for the acquisition of GFP or DAPI fluorescence. Image acquisition and processing were done using Metamorph software (Universal Imaging Corporation).
Over a time course of 4 h, we did not find significant differences in the average lengths of the generated filaments between the wild type and the \textit{Sfl1} mutant (data not shown).

**Embedded growth induces increased filamentation in a \textit{sfl1/sfl1} strain.** Several mutants in genes encoding proteins within the cAMP pathway were shown to exhibit enhanced filamentation under embedded growth conditions, e.g., the \textit{efg1/efg1} and \textit{flo8/flo8} mutants (10, 18). Interestingly, both of these mutant strains are defective in filamentation under all standard inducing conditions. Thus, we went on to examine the growth phenotype of the \textit{sfl1/sfl1} mutant under embedded conditions in YPS agar at 25°C. \textit{SFL1} strains, including the wild-type SC5314, the SN148, and the heterozygous mutant strains, started to show increased filamentation after more than 2 days under embedded conditions. In contrast, the \textit{sfl1/sfl1} strain formed consistently more abundant filaments under these conditions (Fig. 4). This result suggests that Sfl1p acts as a repressor of filamentation also under microaerophilic conditions when cells are embedded in an agarose matrix and grown at room temperature.

**FIG. 1.** Protein alignment of ascomycetous Sfl1 proteins. Protein sequences of Sfl1 proteins from \textit{A. fumigatus} (Af; GenBank accession number, XP_001481550), \textit{C. albicans} (Ca; XP_715888), \textit{A. gossypii} (Ag; NP_985683), \textit{K. lactis} (Kl; XP_454089), and \textit{Candida glabrata} (Cg) were aligned using the Clustal W algorithm. Matching residues are shaded. The alignment shows the N-terminal part where sequence conservation is highest.

**FIG. 2.** Yeast and hyphal growth of the \textit{sfl1/sfl1} mutant strain. The wild type and the \textit{sfl1/sfl1} mutant were grown on YPD with or without 10% serum at 30°C or 37°C, respectively, in either liquid culture or on solid medium plates. Images were taken after an overnight incubation in liquid medium or after 4 days on solid medium plates. Bars, 30 \textmu m (left) and 100 \textmu m (right).
Upregulated expression of \textit{MET3p-SFL1} inhibits filamentation. To study the effect of depleting cells of Sfl1 or of the overexpression of \textit{SFL1}, we placed the single copy of \textit{SFL1} in a heterozygous \textit{SFL1/sfl1} mutant under the control of the regulatable \textit{CaMET3} promoter. The use of minimal medium supplemented with methionine and cysteine led to transcriptional repression of \textit{SFL1} and thus to the depletion of Sfl1 protein from the cells. In these cells, promoter shutdown resulted in the restoration of the \textit{sfl1} mutant phenotypes of flocculation and increased filamentation (Fig. 5). The growth of cells in SD lacking methionine and cysteine resulted in increased expression of \textit{MET3p-SFL1}, which reduced the clumping phenotype, and all cells remained in the yeast phase. We analyzed the strength of different promoters by using GFP as a reporter. Use of the \textit{Ashbya gossypii TEF} promoter resulted in high-level expression. Induced expression via the \textit{MET3} promoter reached half of that level while \textit{SFL1} promoter-driven expression was only half of that of the \textit{MET3} promoter. Therefore, induced expression of a \textit{MET3p-SFL1} construct results in a twofold overexpression on the protein level compared to endogenous \textit{SFL1} expression (data not shown).

The promoter shutdown and inducing experiments indicate

| No. of cells in aggregates | SC5314 | SN148 | sfl1/sfl1 |
|---------------------------|--------|-------|-----------|
| 1 to 2                    | 166 (39) | 155 (30) | 60 (9)    |
| 3 to 4                    | 134 (32) | 204 (39) | 76 (11)   |
| 5 to 10                   | 96 (23)  | 162 (31) | 98 (15)   |
| >10                       | 24 (6)   | 0 (0)   | 428 (65)  |
| Total                     | 420 (100)| 521 (100)| 662 (100)|

FIG. 3. Deletion of \textit{SFL1} promotes flocculation in minimal medium. The indicated strains were grown overnight in liquid YPD (rich medium) or SD (minimal medium) at 30°C. Images of cell solutions in reagent tubes (upper panels) and microscopic images (lower panels) are shown. Bar, 10 \(\mu\)m.

FIG. 4. A \textit{sfl1/sfl1} mutant shows increased filamentation under embedded growth conditions. Cells of the SC5314 wild-type, SN148, heterozygous \textit{SFL1/sfl1}, and homozygous \textit{sfl1/sfl1} strains were plated submersed in molten YPS agar (2%) and grown for 56 h at 25°C. Bar, 50 \(\mu\)m.
that the mutant phenotypes observed with our sfl1/sfl1 strains were due solely to the deletion of SFL1. Adding GlcNAc to minimal medium and increasing the temperature for incubation to 37°C induces C. albicans cells to form hyphal filaments. This became evident in the wild type, the sfl1/sfl1 mutant strain, and the MET3p-controlled SFL1 strain under repressing conditions. Surprisingly, however, if cells were pregrown in SD (without methionine and cysteine), which allows for full expression of SFL1 via the MET3 promoter, and were then transferred into SD plus GlcNAc medium, germ tube formation was entirely abolished. This block in filamentation required a continued high expression level of SFL1 since cells started to filament when methionine or cysteine was present in the inducing medium. Our results, therefore, indicate that overexpression of SFL1 can lead to a block in hyphal formation in C. albicans (Fig. 5).

Sfl1-GFP localizes to the nucleus. Consistent with the idea of a transcriptional regulator and DNA-binding protein should be a nuclear localization of the Sfl1 protein. Therefore, we wanted to determine the subcellular localization of Sfl1p during yeast and hyphal growth. To this end, we generated an SFL1-GFP/sfl1 strain. This strain was phenotypically like the wild-type strain, suggesting that SFL1-GFP is functional. We observed a Sfl1-GFP signal in the nuclei of yeast cells, which would be consistent with the idea of Sfl1 suppressing flocculation and filamentation under conditions that allow yeast growth. Upon filament induction, Sfl1-GFP could still be observed in the nuclei of hyphal cells but was found to be stronger in yeast or pseudohyphal cells (Fig. 6). This would be consistent with either a diminished expression of SFL1 or a reduced amount of Sfl1p in the nuclei of hyphal cells, which is in line with the notion of a derepression of Sfl1-controlled genes under hypha-inducing conditions.

One-hybrid analysis with SFL1. The data presented suggested that Sfl1p fulfills a role of a transcriptional repressor in C. albicans. To gain further evidence for such a role, we employed a recently developed one-hybrid system with C. albicans (30). To this end, we fused SFL1 to the Staphylococcus aureus lexA DNA-binding domain and integrated this fusion gene at the RPS1 locus in CAI8. The readout of transcriptional regulation was done using a lacZ reporter gene construct. As controls, we used strains provided by the Brown lab that assay the Nrg1 repressor and the Gcn4 activator activity (30). We assayed the level of reporter gene expression by using an X-Gal overlay assay and quantitative analysis of β-galactosidase activity.

FIG. 5. MET3 promoter-controlled expression of SFL1. The wild-type, sfl1/sfl1, and MET3p-SFL1/sfl1 strains were pregrown in minimal media at 30°C with or without methionine/cysteine (+M/C and −M/C, respectively), diluted, and inoculated to the same cell density in fresh medium. Filamentation was induced with the addition of GlcNAc to the medium. Note that filamentation is blocked in cells overexpressing SFL1 (bottom right panel). Bar, 10 μm.
(Fig. 7). This assay confirmed that Gcn4 acts as a transcriptional activator while Nrg1 functions as a repressor. In these assays, we found no activating activity of Sfl1.

**A set of hypha-specific genes is derepressed in the sfl1 mutant.** The flocculation phenotype of the sfl1/sfl1 mutant in minimal medium and the increase in filament formation provide some evidence for an altered transcriptional profile in the mutant. To gain first insight into which genes may be affected on the transcriptional level by the deletion of *SFL1*, we performed RT-PCR to analyze the expression of hypha-specific genes.
FIG. 8. Hypha-specific genes are upregulated in an sfl1 mutant. Cells of the wild type (SC5314, +/+ ) and the sfl1/sfl1 mutant (−/−) were grown in minimal medium for 6 h at RT and then collected for RNA preparation. The quality of the RNA was assessed by using the CaRH03 gene as a control. CaRH03 genomic DNA (lane 1) contains one intron that is missing in its corresponding cDNA (lane 2; PCR derived from a cloned cDNA fragment).

genes (HWP1, ECE1), agglutinin-like sequences (ALS1, ALS3), and also FLO8, which is a known positive regulator of hypha-specific gene expression in C. albicans. Also based on the regulation circuitry in S. cerevisiae, ScFLO8 is known to be regulated by ScSfl1. In this assay, we found increased expression of these genes in the sfl1/sfl1 mutant (Fig. 8).

DISCUSSION

We have identified the C. albicans SFL1 gene by similarity to its S. cerevisiae homolog. Sfl1p contains an N-terminal domain with a high degree of similarity to the HSF DNA-binding domain (15). C. albicans Sfl1p shares an extended N terminus with A. fumigatus Sfl1p, the function of which, however, needs to be elucidated. Sfl1 is a conserved protein and known as a suppressor of flocculation in S. cerevisiae. In S. cerevisiae, Sfl1p acts as transcriptional repressor of flocculation (FLO) genes (15, 28). The deletion of ScSFL1 leads to flocculation and invasive growth (28). We found that CaSFL1 regulates similar processes in C. albicans. The deletion of CaSFL1 resulted in strong flocculation in minimal medium accompanied by increased filamentation in the absence of inducers as well as enhanced filamentous growth under embedded conditions at 25°C. This result suggests that some of the transcriptional circuitry controlled by Sfl1p has been conserved between S. cerevisiae and C. albicans.

The prevailing view in the regulation of C. albicans hyphal morphogenesis is that transcriptional repression by Tup1, Nrg1, Rpg1, and Ssn6 and complexes thereof provide the cells with the means to repress specific gene sets (5–8, 21, 22, 26). The mechanistic difficulties in explaining the complex pattern of regulation of hypha-specific gene expression came from comparative transcript profiling experiments that indicated that in sfl1, tup1, and ssn6 mutant strains, overlapping but extensively different subsets of genes were regulated (16). Our studies now present one additional player in this scheme, Sfl1p. It is noteworthy that deletion of SFL1 does not induce filamentation to an extent seen, for example, in the tup1 mutant. On the other hand, the flocculation phenotype seems to be rather specific for the sfl1 mutant. Formation of cell clumps occurs in the sfl1 mutant strain under different medium conditions but is most obvious in minimal media. As flocs also contain hyphal cells in minimal media, more pronounced floculation might be the result of hypha-specific gene expression in these cells.

In S. cerevisiae, Sfl1p recruits the Snf6-Tup1 complex via an Snf6 interaction domain (11). This domain is only rather weakly conserved on the amino acid level between different fungal Sfl1 proteins. However, it leads to a testable hypothesis in C. albicans, namely, that Sfl1p might repress hypha- and/or flocculation-specific genes via a Sfl1-Ssn6-Tup1 complex. We analyzed the potential of CaSfl1p to act as a repressor of transcription using a recently developed one-hybrid system, which proved useful to identify Gen4 as a transcriptional activator (30). In these assays, CaSfl1 was only weakly downregulating reporter gene transcription and certainly not activating it. Consistent with the idea of a repressor could be a differential localization of CaSfl1 in the nucleus of yeast cells but not of hyphal cells. Even though we found evidence to support this idea, we cannot rule out a nucleocytoplasmic shuttling of Sfl1 that could contribute to its specific activity. Such a shuttling mechanism is quite common and has been described, e.g., for the Msn2/4 global stress response regulator in S. cerevisiae (19).

In S. cerevisiae, Sfl1 is regulated via phosphorylation by Tpk2 (28). By analogy, this could place Sfl1 downstream of the cAMP-signaling pathway in concert with Flo8. Furthermore, in S. cerevisiae, Sfl1p regulates the expression of FLO8 in a negative manner (23). A similar scenario seems likely for C. albicans, since overexpression of SFL1 using the MET3 promoter resulted in the inability of these cells to form hyphae under GlcNAC-induced filamentation. This result is consistent with the role of CaFlo8 as an inducer of hypha-specific genes, as the flob8/flo8 mutant is blocked for hypha-specific gene expression and filamentation (10). First insights into the target genes of Sfl1 came from RT-PCR, which showed expression of the adhesins ALS1 and ALS3 as well as of other hypha-specific genes. FLO8 appears to be upregulated in a sfl1 mutant. Interestingly, the deletion of SFL1 points toward a role of Sfl1 that is not exclusive for filamentation, but it reveals a novel role in flocculation. This function could be important for cell-cell adhesion or cell-surface adhesion, such as that seen in biofilm formation. Determining the Sfl1 regulon, particularly with respect to adhesins and hyphal specific genes, therefore, will be an essential task to understand the role of Sfl1.

In conclusion, our functional analysis of the C. albicans SFL1 gene provides evidence that Sfl1 is a conserved negative regulator of flocculation and filamentation in C. albicans that may regulate a specific gene set required for flocculation but may regulate hypha-specific genes in concert with other known repressors, particularly Tup1 and Nrg1.

ACKNOWLEDGMENTS

We thank Al Brown for making available the C. albicans “one-hybrid-kit.” This research was supported by the EU-Signalpath Marie Curie Training Network.

REFERENCES

1. Bahn, Y.-S., and P. Sundstrom. 2001. CAP1, an adenylate cyclase-associated protein gene, regulates bud-hypha transitions, filamentous growth, and cyclic AMP levels and is required for virulence of Candida albicans. J. Bacteriol. 183:3211–3221.
2. Barelle, C. J., C. L. Priest, D. M. Maccallum, N. A. Gow, F. C. Odds, and A. J. Brown. 2006. Niche-specific regulation of central metabolic pathways in a fungal pathogen. Cell. Microbiol. 8:961–971.
3. Berman, J., and P. E. Sudbery. 2002. Candida albicans: a molecular revolution built on lessons from budding yeast. Nat. Rev. Genet. 2:918–930.

4. Biswas, S., M. Roy, and A. Datta. 2003. N-Acetylglucosamine-inducible CaGAP1 encodes a general amino acid permease which co-ordinates external nitrogen source response and morphogenesis. Microbiology 149:2597–2608.

5. Braun, B. R., W. S. Head, M. X. Wang, and A. D. Johnson. 2000. Identification and characterization of TUP1-regulated genes in Candida albicans. Genetics 156:31–44.

6. Braun, B. R., and A. D. Johnson. 1997. Control of filamentation in Candida albicans by the transcriptional repressor TUP1. Science 277:105–109.

7. Braun, B. R., and A. D. Johnson. 2000. TUP1, CPH1 and EFG1 make independent contributions to filamentation in Candida albicans. Genetics 155:57–67.

8. Braun, B. R., D. Kadosh, and A. D. Johnson. 2001. NRG1, a repressor of filamentous growth in C. albicans, is down-regulated during filament induction. EMBIO J. 26:4753–4761.

9. Brown, D. H., Jr., A. D. Giusani, X. Chen, and C. A. Kumamoto. 1999. Filamentous growth of Candida albicans in response to physical environmental cues and its regulation by the unique CZF1 gene. Mol. Microbiol. 34:651–662.

10. Cao, F., S. Lane, P. P. Raniga, Y. Lu, Z. Zhou, K. Ramon, J. Chen, and H. Liu. 2006. The Flo8 transcription factor is essential for hyphal development and virulence in Candida albicans. Mol. Biol. Cell 17:295–307.

11. Conlan, R. S., and D. Tzamarias. 2001. SFI1 functions via the co-repressor Snf6-Tup1 and the AMP-dependent protein kinase Tpk2. J. Mol. Biol. 309:1007–1015.

12. Dünkel, A., and J. Wendland. 2007. Candida albicans Rho-type GTPase-encoding genes required for polarized cell growth and cell separation. Eukaryot. Cell 6:844–854.

13. Enjalbert, B., D. M. MacCallum, F. C. Odds, and A. J. Brown. 2007. Niche-specific activation of the oxidative stress response by the pathogenic fungus Candida albicans. Infect. Immun. 75:2143–2151.

14. Feng, Q., E. Summers, B. Guo, and G. Fink. 1999. Ras signaling is required for scarum-induced hyphal differentiation in Candida albicans. J. Bacteriol. 181:6339–6346.

15. Fujita, A., Y. Kikuchi, S. Kuhara, Y. Misumi, S. Matsumoto, and H. Kobayashi. 1989. Domains of the SFL1 protein of yeasts are homologous to Myc oncoproteins or yeast heat-shock transcription factor. Gene 85:321–328.

16. García-Sánchez, S., A. L. Mavor, C. L. Russell, S. Argimon, P. Dennison, B. Enjalbert, and A. J. Brown. 2005. Global roles of Snf6 in Tup1- and Nrg1-dependent gene regulation in the fungal pathogen, Candida albicans. Mol. Biol. Cell 16:2913–2925.

17. Gillum, A. M., E. Y. Tsay, and D. R. Kirsch. 1984. Isolation of the Candida albicans gene for orotidine-5′-phosphate decarboxylase by complementation of S. cerevisiae ura3 and E. coli pyrf mutations. Mol. Gen. Genet. 198:179–182.

18. Giusani, A. D., M. Vinces, and C. A. Kumamoto. 2002. Invasive filamentous growth of Candida albicans is promoted by Ces1p-dependent relief of Efg1p-mediated repression. Genetics 160:1749–1753.

19. Gorner, W., E. Durchschlag, M. T. Martínez-Pastor, F. Estruch, G. Ammerer, B. Hamilton, H. Ruis, and C. Schuller. 1998. Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes Dev. 12:586–597.

20. Guo, B., C. A. Styles, Q. Feng, and G. R. Fink. 2000. A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. Proc. Natl. Acad. Sci. USA 97:12158–12163.

21. Hwang, C. S., J. H. Oh, W. K. Huh, H. S. Yim, and S. O. Kang. 2003. Snr6, an important factor of morphological conversion and virulence in Candida albicans. Mol. Microbiol. 47:1029–1043.

22. Kadosh, D., and A. D. Johnson. 2005. Induction of the Candida albicans filamentous growth program by relief of transcriptional repression: a genome-wide analysis. Mol. Biol. Cell 16:2903–2912.

23. Kühler, G. A., T. C. White, and N. Agabian. 1997. Overexpression of a cloned IMP dehydrogenase gene of Candida albicans confers resistance to the specific inhibitor mycophenolic acid. J. Bacteriol. 179:2331–2338.

24. Kojic, E. M., and R. O. Darouiche. 2004. Candida infections of medical devices. Clin. Microbiol. Rev. 17:255–267.

25. Murad, A. M., P. Leng, M. Straffon, J. Wishart, S. Macaskill, D. MacCallum, N. Schnell, D. Talibi, D. Marechal, F. Tekaia, C. D’Enfert, C. Gaillardin, F. C. Odds, and A. J. Brown. 2001. NRG1 represses yeast-lymphoma genesis and hypha-specific gene expression in Candida albicans. EMBIO J. 20:4742–4752.

26. 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.

27. Robertson, L. S., and G. R. Fink. 1998. The three yeast A kinases have specific signaling functions in pseudohyphal growth. Proc. Natl. Acad. Sci. USA 95:13783–13787.

28. Rocha, C. R., K. Schroppel, D. Harcus, A. Marcil, D. Dignard, B. N. Taylor, D. Y. Thomas, M. Whiteway, and E. Leberer. 2001. Signaling through adenylylcyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol. Biol. Cell 12:3631–3643.

29. Russell, C. L., and A. J. Brown. 2005. Expression of one-hybrid fusions with Staphylococcus aureus lexA in Candida albicans confirms that Nrg1 is a transcriptional repressor and that Gcn4 is a transcriptional activator. Fungal Genet. Biol. 42:676–683.

30. Schaub, Y., A. Dünkel, A. Walther, and J. Wendland. 2006. New pFA-cassettes for PCR-based gene manipulation in Candida albicans. J. Basic Microbiol. 46:416–429.

31. Stoldt, V. R., A. Sonneborn, C. E. Leuker, and J. F. Ernst. 1997. Efglp1, an essential regulator of morphogenesis of the human pathogen Candida albicans, is a member of a conserved class of HILH proteins regulating morphogenetic processes in fungi. EMBIO J. 16:1982–1991.

32. Verstrepen, K. J., and F. M. Klis. 2006. Flocculation, adhesion and biofilm formation in yeasts. Mol. Microbiol. 60:5–15.

33. Whiteway, M., and U. Oberholzer. 2004. Candida morphogenesis and host-pathogen interactions. Curr. Opin. Microbiol. 7:350–357.