Identification of Candida albicans Genes that Induce Saccharomyces cerevisiae Cell Adhesion and Morphogenesis

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Morphogenesis and adhesion to host tissues and medical devices contribute to the virulence of Candida albicans, the most common fungal pathogen isolated from humans. However, identification of molecular mechanisms of C. albicans adhesion and morphogenesis has been impaired by the lack of effective molecular and genetic tools available for this organism. Saccharomyces cerevisiae provides an attractive model system for studying C. albicans adhesion and morphogenesis because of its well-characterized genetics and gene expression systems. To gain insight into the genetic mechanisms of C. albicans adhesion and morphogenesis, we used a parallel plate flow chamber to screen and quantitatively characterize attachment to polystyrene of an adhesion-deficient nonfilamentous flo8A S. cerevisiae strain expressing a C. albicans genomic library. We identified six C. albicans genes that are capable of promoting cell adhesion and pseudohyphal development in S. cerevisiae. We also analyzed the ability of these adhesion-promoting genes to regulate the expression of FLO11, which encodes an endogenous S. cerevisiae adhesin. One C. albicans gene, EAP1, appears to directly mediate adhesion and morphogenesis while the remaining five (EAP2, SWI1, MSB1, AAF1, and TEC1) upregulate expression of endogenous S. cerevisiae adhesins. These results suggest that S. cerevisiae is a useful system for molecular characterization of factors that regulate C. albicans adhesion and morphogenesis and that parallel plate flow chamber-based adhesion assays can be used in conjunction with genetic screens to identify molecular mechanisms regulating fungal cell adhesion.

Introduction

Candida albicans is the most frequently isolated human fungal pathogen and one of the four most common causes of microbial bloodstream infections (1). The attributable mortality of bloodstream candidemia ranges from 30% to 40%, with the majority of these cases caused by C. albicans (2). Candidemia is usually associated with biofilms formed on indwelling devices, such as catheters, endotracheal tubes, and pacemakers (3). In addition to harboring a reservoir of infectious cells, biofilms also confer resistance to antifungal agents, including amphotericin B, azoles, and caspofungin (4).

Discovering the molecular mechanisms of C. albicans virulence is critical for developing and improving targeted antifungal therapies. However, C. albicans cannot be easily studied by molecular biological methods and genetics due to the lack of effective molecular tools, such as stable plasmid expression systems, limited mating ability, and the absence of known sporulation. In contrast, Saccharomyces cerevisiae, baker’s or brewer’s yeast, has been extensively studied and developed as a model genetic system. The similarity between genetic and biochemical pathways in S. cerevisiae and C. albicans has assisted in the understanding of pathways regulating virulence factors in C. albicans. In addition, the availability of a wide range of genetic tools and approaches for the study of S. cerevisiae greatly facilitates research on C. albicans by bypassing many experimental difficulties associated with work on C. albicans. A commonly used strategy to identify C. albicans genes involves complementation of corresponding mutations in S. cerevisiae.

Adhesion of C. albicans to mammalian tissues is seen as a very important determinant of pathogenesis (5). A family of cell wall glycoproteins, known as adhesins, mediates the adhesion of fungi to host ligands. Many of these adhesins, termed glycosyolphosphatidylinositol-dependent cell wall proteins (GPI-CWP), are characterized by the presence of an N-terminal signal peptide and a C-terminal sequence signaling GPI anchor attachment (6). The GPI anchor is partially cleaved, and then the protein is cross-linked to β(1,6)-glucan and incorporated into cell wall (6). A number of C. albicans and Candida glabrata genes encoding GPI-CWP adhesins, including CaALS1, CaALAI, CaHWPI, and CgEPAI, have been identified and found to mediate adhesion to extracellular matrix proteins and human endothelial and epithelial cells (7–10). These adhesins are also thought to mediate the initial attachment of C. albicans cells to plastic...
prostheses and implanted devices and contribute to biofilm development (3).

Candida albicans can switch between the yeast form and various filamentous states: germ tubes, pseudohyphae, and hyphae (11). The ability to switch between yeast form and filamentous states is necessary for virulence, although the exact contributions of each of these morphogenic forms have not been conclusively identified (12). Two different signaling pathways, a MAP kinase cascade and a cAMP-dependent pathway, have been shown to regulate the morphogenetic switch in both S. cerevisiae and C. albicans (11). In S. cerevisiae, these pathways regulate the expression of FLO11, a gene essential for haploid invasive growth and diploid pseudohyphal growth (13). Among the factors that regulate FLO11 transcription are STE12 and TEC1 activated by the MAPK cascade and SFL1 and FLO8 regulated by the cAMP pathway (14). In C. albicans, homologues to most of the genes involved in the pseudohyphal growth of S. cerevisiae are present (15), suggesting that S. cerevisiae can be used as a model to identify components of signal transduction pathways required for morphogenesis in C. albicans.

In this study, we developed an approach to identify C. albicans genes that induce adhesion of S. cerevisiae cells to polystyrene by using shear flow in a parallel plate flow chamber to select adherent clones. In addition, we tested the ability of these selected adhesion-promoting genes to activate the transcription of FLO11 and to enhance adhesion to epithelial cells. Finally, we demonstrated that expression of these selected adhesion-promoting genes can also restore invasive growth to a haploid flo8A mutant strain and pseudohyphal formation to a diploid S. cerevisiae flo8/flo8 mutant strain.

Materials and Methods

Yeast Strains and Media. Saccharomyces cerevisiae haploid strain SPY308 (a ura3-52 his3::hisG Leu2::hisG flo8::kan) was used to select adhesion-promoting genes from a genomic library of C. albicans strain SC5314 kindly provided by Yue Fu and Scott Filler (8). S. cerevisiae diploid strain SPY311 (a/a ura3-52/ura3-52 his3::hisG his3::hisG Leu2::hisG LEU2 flo8::kan/flo8:: HIS3) defective in filamentation was used to test the ability of the selected adherence-promoting genes to restore the pseudohyphal growth of the mutant. S. cerevisiae haploid strain SPY309 (a ura3-52/ his3::hisG flo8::hisG) and diploid strain SKY2021 (a/a ura3-52/ura3-52 flo11::hisG LEU2::hisG) were used to analyze whether the ability of the selected adhesion-promoting genes to restore adhesion and pseudohyphal growth to the flo8 mutants was FLO11-dependent. All strains used in this study were derived from the S1278b genetic background (16) using PCR disruption (17).

Synthetic complete media lacking specific nutrients and filamentous growth media have been described previously (18). Synthetic low ammonium medium (SLAD) contained 50 μM ammonium sulfate. Uracil was added to SLAD medium to a concentration of 0.2 mM to make SLAD + Ura. Galactose was added to media to replace glucose in order to express genes within plasmids containing S. cerevisiae GAL1 promoter. Yeast cells were transformed using lithium acetate transformation (19).

Parallel Plate Flow Chamber Cell Adhesion Assay. Yeast cell adhesion to polystyrene surfaces was quantified using a parallel plate flow chamber (GlycoTech, Rockville, MD) as previously described (20). The parallel plate flow chamber consists of a flow deck that fits inside a 60-mm Petri dish. A silicone rubber gasket (width = 0.25 cm, thickness = 0.01 in.) is placed between the flow deck and the 60-mm Petri dish to form the flow chamber. The Petri dish was attached to the flow deck and the gasket by holding the deck inverted and vacuum was applied to hold the Petri dish on the deck. Yeast cell suspension was sonicated briefly to break cell lumps and instilled into the flow chamber with a syringe from the outlet of the flow chamber. A peristaltic pump was pre-run with the sodium phosphate buffer (0.1 M pH 6.0) to remove all air in the pump and tubing system and connected to the inlet of the flow chamber. The cell suspension was incubated for 3 h to allow the cells to settle on the surface of the Petri dish.

The shear stress generated by the flow at the bottom surface of the flow chamber detaches yeast cells from the surface. The detachment assay was performed by increasing the flow rate of the sodium phosphate buffer (0.1 M pH 6.0), and thus the shear stress, in a stepwise manner. The shear stress is defined by $\tau = 3Q/2wh^2$ where $Q$ is the volumetric flow rate, $\mu$ is the viscosity, $2h$ is the height of the flow field, and $w$ is the width of the flow field. The volumetric flow rate was varied to obtain the desired shear stress. The flow chamber was placed on a motorized X-Y stage (Prior) of an Olympus IX70 inverted microscope during the assay. Three fields were selected under the microscope and the positions were memorized by the motorized stage. For each applied shear stress, images of the three fields were captured using a digital camera (Nikon Spot) and Metavue software package. The number of cells remaining attached to the surface was automatically identified and counted by Metavue on the basis of contrast of the cells and cell sizes. The adhesion of cells was quantified as the ratio of the mean fraction of cells in each of the selected three fields remaining attached after exposure to an applied shear force for 15 min.

Centrifugation Cell Adhesion Assay. A centrifugal cell detachment assay was performed as previously described, with modifications (21). Yeast cells were cultured and treated as indicated in the parallel plate flow chamber assay. After brief sonication to break cell lumps, the cell suspension was filled into the center wells of 96-well plates and incubated for 3 h to allow the cells to settle on the surface of the plates. The wells were filled with sodium phosphate buffer (0.1 M pH 6.0) and sealed with packing tape to avoid air bubbles. The detachment assay was performed by inverting the plates and spinning in a swing bucket rotor of a benchtop Beckman centrifuge for 15 min at 1000g. Three fields of each well were selected under the microscope and images were captured using a digital camera (Nikon Spot) and Metavue software package. The number of cells remaining attached to the surface was automatically identified and counted by Metavue on the basis of contrast of the cells and cell sizes. The adhesion of cells was quantified as the ratio of the mean fraction of cells in each of the selected three fields remaining attached after exposure to an applied centrifugal force for 15 min.

Selecting for Adherent Clones of S. cerevisiae. Adhesion-promoting genes were selected from a genomic library of C. albicans strain SC5314 as previously described, with modifications (20). The genomic library of C. albicans SC5314 was constructed in the plasmid pYesR (8, 22). Expression of the genes within the library is regulated by the S. cerevisiae GAL1 promoter. This genomic library was transformed into S. cerevisiae SPY308. Cells carrying the genomic library were cultured
in synthetic complete medium lacking uracil (SC-ura) and containing galactose as a carbon source, overnight at 30 °C. Cells were pelleted and resuspended in 0.1 M sodium phosphate buffer, pH 6.0. After being sonicated for 30 s, the cell suspension was added to the parallel plate flow chamber and incubated at room temperature for 3 h. Nonadherent cells were removed by applying shear stress of 2.5 dyn/cm² and the fraction of attached cells was measured by image analysis. Adherent cells were recovered by placing solid medium to cover the flow path of the parallel plate flow chamber and incubating the plate overnight at 30 °C. Cells were scraped from the solid medium, repoolled, and cultured in liquid medium again. The selection for adherent clones was repeated four times to purify the pool. At the end of this selection procedure, cells were plated to obtain individual colonies. Yeast cells from randomly selected colonies were cultured in minimal medium containing either galactose to induce expression of the C. albicans genes or glucose to suppress expression of the C. albicans genes. Centrifugation cell adhesion assay was used to identify colonies that showed enhanced adhesion to plates when cultured in minimal medium containing galactose but showed no enhanced adhesion when grown in minimal medium containing glucose. Approximately 50 individual colonies were selected and plasmids were isolated from these colonies and sequenced. Six distinct plasmids were identified from sequencing and further studied. An oligonucleotide corresponding to a region in the GAL1 promoter was used as the primer to obtain the sequence of the insert DNA adjacent to the GAL1 promoter (22). The obtained sequences were compared to the sequence of the C. albicans genome (http://www-sequence.stanford.edu/group/candida).

**Adhesion to Human Kidney Epithelial Cells.** 293 human kidney epithelial cells were grown to confluence in six-well tissue culture plates in minimum essential medium (Invitrogen) containing 10% horse serum (Invitrogen). The cells were washed twice in PBS containing Mg²⁺ and Ca²⁺ (PBS⁺⁺) at 37 °C. Yeast adhesion to 293 cells was measured essentially as described previously (20). Briefly, yeast cells (500 cells/µL) suspended in PBS⁺⁺ were sonicated for 30 s and added to a confluent monolayer of 293 human kidney epithelial cells incubated for 1 h at 37°C. The initial number of yeast cells in this inoculum was confirmed by colony counting. Nonadherent yeast cells were rinsed using PBS⁺⁺. Next, 0.05% trypsin was added to the wells and the cells were suspended in water and plated on YPD agar. The number of adherent cells was determined by colony counting, and adhesion was expressed as the fraction of cells remaining attached.

**Agar Invasion Assay.** Strains to be tested were patched on synthetic complete agar plates lacking uracil and containing 2% galactose. Cells were grown at 30 °C for 2 days and the plates were photographed. Next, the plates were rinsed with running water to remove non-adherent cells and the plates were photographed again (20).

**Pseudohyphal Growth Assay.** The pseudohyphal growth assay was performed essentially as described previously (20). Strains to be tested were streaked on SLAD + Ura plates containing 2% galactose to obtain single cells. Cultures were grown at 30 °C for 2 days and representative colonies were photographed.

**Northern Blot Analysis.** S. cerevisiae strains to be analyzed were cultured in minimal medium containing glucose overnight at 30 °C. Cultures were diluted 10-fold into fresh minimal medium containing galactose and incubated to an OD₆₀₀ of 0.8 at 30 °C. Cells were washed and total RNA was extracted from cells by phenol/chloroform followed by ethanol precipitation. For each sample, 20 µg of total RNA was separated by electrophoresis on a formaldehyde gel and transferred by capillary action to a nylon membrane. DNA probes (1000-bp region of FLO11 and ACT1 open reading frames) were amplified and radiolabeled by PCR. Hybridization and washes were performed according to Palecek et al. (23).

**Results**

**Identification of C. albicans Genes That Enhance S. cerevisiae Adhesion to Polystyrene.** FLO11 is a S. cerevisiae gene encoding a cell surface protein involved in adhesion to agar and cell filamentation (13, 24). FLO8 encodes a transcription factor required for FLO11 expression (25). flo8Δ and flo11Δ strains adhere poorly to polystyrene (20, 26), suggesting that Flo11p directly mediates the adhesion of S. cerevisiae to plastic. We designed a method utilizing heterologous expression of C. albicans genes from a genomic library constructed from the C. albicans SC5314 strain (8) in a nonadherent flo8Δ strain to identify C. albicans genes capable of conferring the ability to adhere to polystyrene either by upregulation of endogenous adhesins or by expression of a C. albicans adhesin. haploid S. cerevisiae flo8Δ cells expressing C. albicans genomic library under control of the S. cerevisiae GAL1 promoter were first plated prior to growth in liquid media; 10⁶ colonies (independent transformants) were collected and cells from these colonies were collected and pooled. The pooled transformants were cultured in liquid minimal medium plus galactose overnight at 30 °C. A cell suspension containing approximately 10⁷ cells was added to the parallel plate flow chamber and incubated at room temperature in sodium phosphate buffer (0.1 M pH 6.0) for 3 h. Nonadherent cells were removed by flowing sodium phosphate buffer through the chamber at a shear stress of 2.5 dyn/cm² and the fraction of attached cells was measured. The adherent population was collected, subcultured, and recycled through the flow chamber as in the initial round of selection. This selection procedure was repeated four times, and the fraction of cells remaining attached at 2.5 dyn/cm² shear stress increased after each round of selection (Figure 1), suggesting that an adherent sub-population was enriched.

At the end of the selection procedure, individual colonies were isolated and their adhesion to polystyrene was quantitatively characterized using a centrifugation adhesion assay. Fifty individual colonies were selected, and plasmids were isolated from these colonies and sequenced. Six distinct plasmids were identified, each of which was duplicated in these 50 colonies. Strains expressing these six plasmids exhibited significantly greater adhesion than S. cerevisiae cells harboring empty plasmids. The plasmids contained in these clones were designated as pYE-1 to pYE-6. The plasmids were isolated from the corresponding S. cerevisiae transformants, amplified in E. coli, and transformed into a haploid S. cerevisiae flo8Δ strain. Each of the C. albicans genes in these plasmids increased cell adhesion to polystyrene, measured by cells that remained adherent after exposure to a centrifugal force of 1000g. Enhanced adhesion was observed when cells were cultured in minimal medium containing galactose, but not when cells were cultured in the presence of glucose, suggesting that the expression of the selected candidate genes was required for the enhanced adhesion to polystyrene (Figure 2).
Enhance activity or expression of an endogenous contained in these plasmids could encode proteins that plasmids require. *S. cerevisiae* adhesin, such as Flo11p. To test whether these plasmids formed them into the wild-type *S. cerevisiae* FLO11 pYE-6.

**Figure 1.** Fraction of cells remaining attached to polystyrene after successive rounds of selection. *S. cerevisiae* strain SPY308 (flo8Δ) transformed with the genomic library of *C. albicans* strain SC5314 under control of the *S. cerevisiae* GAL1 promoter was grown in synthetic minimal medium containing galactose and incubated in the parallel plate flow chamber for 3 h. Nonadherent cells were removed by flowing sodium phosphate buffer (0.1 M pH 6.0) through the chamber at a shear stress of 2.5 dyn/cm². The fraction of attached cells was quantified by image analysis of three fields before and after flow.

Effects of *FLO8 and FLO11* Deletion on Adhesion of *S. cerevisiae* Transformed with pYE-1 to pYE-6. FLO11 expression is required for adhesion of wild-type *S. cerevisiae* to polystyrene (20, 26), and FLO8 is required for FLO11 expression (25). Therefore, at least two explanations exist for the increased adherence of the flo8Δ strains containing pYE-1 to pYE-6 to polystyrene. First, the *C. albicans* genes contained in these plasmids could encode *C. albicans* cell surface proteins that directly mediate *S. cerevisiae* adhesion to the substrate in the absence of FLO11. Alternatively, the *C. albicans* genes contained in these plasmids could encode proteins that enhance activity or expression of an endogenous *S. cerevisiae* adhesin, such as Flo11p. To test whether these plasmids require FLO11 to increase adhesion, we transformed them into the *S. cerevisiae* haploid flo11ΔA strain and measured adhesion as a function of shear stress in the parallel plate flow chamber. After growth in minimal medium containing galactose, *S. cerevisiae* haploid flo11ΔA cells harboring pYE-1 to pYE-6 exhibited enhanced adhesion to polystyrene to virtually the same extent as flo8Δ cells carrying corresponding plasmids (Figure 3), suggesting that the *C. albicans* genes in these plasmids encode either adhesion molecules or regulators capable of inducing the transcription of genes encoding other *S. cerevisiae* adhesins, such as FLO10. *S. cerevisiae* haploid flo8ΔA cells harboring pYE-2, pYE-3, and pYE-5 exhibited significantly higher adhesion to polystyrene than did haploid flo11ΔA cells carrying corresponding plasmids, suggesting that the increased adhesion to polystyrene by pYE-2, pYE-3, and pYE-5 is dependent on FLO11 (Figure 3). The haploid flo8Δ strain expressing pYE-4 was slightly more adherent than the flo11ΔA strain expressing pYE-4 (Figure 3), suggesting significant Flo11p and Flo11p-independent contributions to adhesion in this strain.

**Sequence Analysis of the Adhesion-Promoting Genes.** An oligonucleotide corresponding to a region in
the GAL1 promoter was used as the primer to obtain the sequence of the DNA inserts adjacent to the GAL1 promoter in pYE-1 through pYE-6 (Table 1) (22). CaAAF1 and CaTEC1, expressed in pYE-5 and pYE-6, respectively, are genes whose involvement in *C. albicans* morphogenesis and/or adhesion has been previously identified. CaAAF1 was discovered via a strategy to complement an adhesion defect, similar to the present study, using tissue-culture-treated polystyrene or endothelial cells as attachment substrates, thus validating our approach (27, 28). The protein encoded by CaAAF1 localizes to the cytoplasm and nucleus but not the cell wall or plasma membrane (27). CaTEC1 is a member of the TEA/ATTS family of transcription factors that regulate *C. albicans* virulence (29). It encodes a functional homologue of *S. cerevisiae* TEC1p that interacts with the terminal transcription factor Ste12p of the MAP kinase cascade to activate the transcription of *FLO11* (13). The ORF contained in pYE-3 encodes a protein that is homologous to Swi1p in *S. cerevisiae*. Swi1p is part of the SWI-SNF chromatin remodeling complex that controls the transcription of MATa-specific genes in *S. cerevisiae* (30). The ORF contained in pYE-4 encodes a protein that is homologous to Msb1p in *S. cerevisiae*. *S. cerevisiae* MSB1 was identified as a multicopy suppressor of temperature-sensitive *cdc24* and *cdc24* mutations in *S. cerevisiae* (31). CDC24 and CDC42 are required for the establishment of cell polarity and for bud formation (32). The ORF contained in pYE-2 is capable of encoding a 785-residue polypeptide whose N-terminal region was found to be conserved among fission yeast *S. pombe* Gti1p and Pac2p as well as two hypothetical proteins encoded by the *S. cerevisiae* ORFs YEL007W and YHR177W, respectively (33). A sequence motif (KRWTDG) found within this region resembles a protein kinase A (PKA1) consensus phosphorylation site (34). This gene has not been annotated so we named it CaEAP2 (enhanced adhesion to polystyrene). Sequence analysis predicted that the ORF contained in pYE-1 encodes a glycosylphosphatidylinositol-dependent glucan-cross-linked cell wall protein (GPI-CWP), with an N-terminal signal sequence and a C-terminal GPI addition signal (6, 20, 35). We named this gene CaEAP1 (20). CaEap1p possesses homology to certain cell wall proteins of *S. cerevisiae*, such as Flo11p and Aga1p, and contains the consensus motif YTTWCPL present in many additional yeast cell wall proteins, including CaHwp1p, the *C. albicans* chitinase CaCht2p, the *S. cerevisiae* flocculation protein Flo1p, the α-agglutinin subunit Aga1p, the pheromone-regulated protein Fig2p, and the cell wall protein Sed1p (20, 36).

**Effects of Expressing *C. albicans* Adhesion-Promoting Genes on FLO11 Transcription.** All of the selected *C. albicans* adhesion-promoting genes identified in this study, except *EAP1*, are homologous to *S. cerevisiae* regulatory factors involved in *FLO11* transcription and/or pseudohyphal growth. *FLO8* is required for the transcription of *FLO11* in wild-type diploid *S. cerevisiae* strains and the corresponding filamentous growth (25, 37, 38). No *FLO11* transcripts could be detected in a haploid *flo8A* strain (Figure 4). Expression of all of the *C. albicans* adhesion-promoting genes identified in this screen, except CaEAP1, was able to suppress the *FLO11-*transcription defect in the *flo8A* strain (Figure 4). These results, together with the reduced adhesion of *flo8A* and *flo11A* strains expressing these plasmids (Figure 3), suggest that these five *C. albicans* genes induce *S. cerevisiae* adhesion via FLO8-independent transcriptional activation of endogenous adhesins.

**Figure 4.** Northern blot analysis of *FLO11* expression in a *S. cerevisiae* *flo8A* strain expressing *C. albicans* adhesion-promoting genes. Total RNA was isolated from strains *S. cerevisiae* SPY308 (*flo8A*) transformed with vector pYESR (a), pYE-1 CaEAP1 (b), pYE-3 CaSWI1 (c), pYE-2 CaEAP2 (d), pYE-5 CaAAF1 (e), pYE-4 CaMSB1 (f), and pYE-6 CaTEC1 (g) grown in synthetic minimal medium containing galactose for 2 h. A total of 20 μg of RNA was applied and transcripts were detected using probes specific for *FLO11* and ACT1 (loading control).
Figure 5. Adhesion of S. cerevisiae flo8Δ strain expressing C. albicans adhesion-promoting genes to HER 293 cells. S. cerevisiae strain SPY308 (flo8Δ) transformed with vector pYesR (a), pYE-1 CaEAP1 (b), pYE-2 CaEAP2 (c), pYE-3 CaSWI1 (d), pYE-4 CaMSB1 (e), pYE-5 CaAAF1 (f), and pYE-6 CaTEC1 (g) were grown in minimal medium containing galactose and incubated on a confluent 293 cell monolayer for 1 h at 37 °C. The monolayer was gently rinsed via pipetting PBS containing Ca2+ and Mg2+ over the cells to remove nonadherent yeast. The 293 cell monolayer and associated yeast were detached by trypsinization and added to YPD plates. Adhesion is quantified as the number of yeast colonies formed on the YPD plate divided by the number of yeast initially added to the 293 monolayer. Error bars represent the standard deviation of three separate experiments.

Figure 6. Invasive growth of haploid S. cerevisiae strains expressing C. albicans adhesion-promoting genes. (A) S. cerevisiae strain SPY311 (flo8Δ) transformed with vector pYesR (a), pYE-1 CaEAP1 (b), pYE-2 CaEAP2 (c), pYE-3 CaSWI1 (d), pYE-5 CaAAF1 (e), pYE-4 CaMSB1 (f), and pYE-6 CaTEC1 (g) were streaked onto synthetic low-ammonia medium containing galactose. Colonies were allowed to grow at 30 °C for 2 days and then were photographed. (B) S. cerevisiae strain SKY2021 (flo8Δ) transformed with vector pYesR (a), pYE-1 CaEAP1 (b), pYE-2 CaEAP2 (c), pYE-3 CaSWI1 (d), pYE-4 CaMSB1 (e), pYE-5 CaAAF1 (f), and pYE-6 CaTEC1 (g) were streaked onto synthetic low-ammonia medium containing galactose. Colonies were allowed to grow at 30 °C for 2 days and then were photographed. The scale bar represents 100 μm.

Figure 7. Pseudohyphal formation in diploid S. cerevisiae strains expressing C. albicans adhesion-promoting genes. (A) S. cerevisiae strain SPY311 (flo8Δ/flo8Δ) transformed with vector pYesR (a), pYE-1 CaEAP1 (b), pYE-2 CaEAP2 (c), pYE-3 CaSWI1 (d), pYE-4 CaMSB1 (e), pYE-5 CaAAF1 (f), and pYE-6 CaTEC1 (g) were streaked onto synthetic low-ammonia medium containing galactose. Colonies were allowed to grow at 30 °C for 2 days and then were photographed. (B) S. cerevisiae strain SPY309 (flo8Δ) transformed with vector pYesR (a), pYE-1 CaEAP1 (b), pYE-2 CaEAP2 (c), pYE-3 CaSWI1 (d), pYE-4 CaMSB1 (e), pYE-5 CaAAF1 (f), and pYE-6 CaTEC1 (g) were streaked onto synthetic low-ammonia medium containing galactose. Colonies were allowed to grow at 30 °C for 2 days and then were photographed. The scale bar represents 100 μm.

haploid flo8Δ strain to invade agar and a diploid flo8Δ/ flo8 strain to form pseudohyphae, presumably by bypassing the FLO8-mediated transcriptional activation of FLO11. However, none were able to restore the ability of a diploid flo8Δ/flo8Δ strain to form pseudohyphae except CaEAP1, suggesting that FLO11 expression is required for these genes to promote pseudohyphal growth in S. cerevisiae (Figure 7). Expression of the ORFs contained in pYE-2 through pYE-4 in the haploid flo8Δ strain was not able to complement its invasive growth defect (Figure 6). CaAAF1 and CaTEC1 are putative transcriptional regulators of other S. cerevisiae flocculins, such as FLO10, perhaps resulting in their ability to complement the invasive growth defect in the absence of FLO11 (Figure 6).

Increasing adhesion alone appears to be sufficient to restore invasion and filament formation. Expression of S. cerevisiae FIG2 or FLO10, which encode GPI-anchored proteins mediating adhesion, can bypass the requirement for FLO11 for both filamentation and invasion (24). The expression of CaEAP1 was able to restore the haploid invasive growth to both the flo8Δ and flo11Δ strains (Figure 6) and diploid pseudohyphal formation to both the diploid flo8Δ/flo8Δ and flo11Δ/flo11Δ strains (Figure 7). CaEAP1 is very likely to encode a cell adhesin that is functionally similar to Flo11p during invasive and filamentous growth.

Discussion

To identify potential C. albicans adhesion factors, we used a parallel plate shear flow assay to screen a C. albicans genomic library expressed in S. cerevisiae. This screen isolated genes already known to be involved in C. albicans adhesion and morphogenesis as well as genes not previously connected to these phenotypes. All of the selected adhesion-promoting genes except CaEAP1 were able to activate the transcription of FLO11 when expressed in the flo8Δ S. cerevisiae strain (Figure 4). CaEAP2, CaSWI1, and CaTEC1 were shown to induce expression of a FLO11-lacZ reporter in S. cerevisiae (40),

![Graph showing fraction of adherence](image-url)

![Image of adhesion experiments](image-url)
 validating our approach. However, the genes identified in this study were able to activate the transcription of FLO11 in the absence of FLO8. These C. albicans regulatory factors may have target genes in addition to FLO11 in S. cerevisiae. Expression of CaTEC1 enhanced the adhesion of S. cerevisiae to polystyrene (Figure 2) and the invasive growth (Figure 6) in the absence of FLO11, suggesting that it was able to activate the transcription of other S. cerevisiae adhesins. CaTEC1 also complemented the pseudohyphal growth defect of the diploid S. cerevisiae tec1/tec1 mutant strain (29), suggesting that it is a functional homologue of S. cerevisiae TEC1. In S. cerevisiae, Tec1p binds Ste12p downstream of the MAPK pathway to drive the transcription of genes that promote filamentation (41). In C. albicans, however, CaTEC1 is one of the downstream effectors of Efg1p, a key regulator of hyphal growth in the CAMP-PKA pathway (42).

The ORF contained in pYE-3 encodes a protein that is homologous to S. cerevisiae Swi1p. The Swi/Snf complex regulates gene expression by translocating histone octamers and forming nucleosome-free regions to which transcriptional activators can bind (43). This complex is also involved in the regulation of FLO1 expression and remodels the FLO1 promoter (44). It is informative to note that the amino termini of both C. albicans CaAaf1p and CaSwi1p are rich in glutamine, which is characteristic of multiple regulatory factors and similar to the amino terminus of Mss11p in S. cerevisiae (45). Mss11p in S. cerevisiae plays an essential role in the transcriptional regulation of FLO11 and functions at the convergence of at least two signaling cascades, the filamentous growth MAPK cascade and the cAMP-PKA pathway (45). Flo8p interacts physically and functionally with Mss11p and Mss11p is absolutely required for the activation of FLO11 expression by most proteins that affect FLO11 expression, including Flo8p (46). Expression of CaAaf1 in S. cerevisiae caused flocculation (27). Our experiments also revealed that CaAaf1p was able to enhance haploid S. cerevisiae invasive growth in the absence of FLO11, whereas CaSwi1p was not (Figure 6), indicating that they regulate different target genes in S. cerevisiae.

The ORF contained in pYE-3 encodes a protein homologous to Msb1p in S. cerevisiae. CaMSB1 was identified as a multicopy suppressor of temperature sensitive cdc24 and cdc42 alleles in S. cerevisiae (31). Cdc24p is a guanine nucleotide exchange factor that activates Cdc42p by facilitating the formation of its GTP-bound form. Activated Cdc42p interacts with the protein kinase Ste20p to activate the MAP kinase cascade (47), suggesting that the C. albicans gene MSB1 may regulate the transcription of FLO11 through the MAPK pathway. It was also reported that MSB1 is one of the filamentation-specific target genes of Ste12p, suggesting its role in the pseudohyphal growth in S. cerevisiae (48).

Genomics studies have indicated that CaEAP2 is expressed in a and α white cells but repressed by a1-a2 in a/a white cells, and may be involved in C. albicans mating (49). In haploid S. cerevisiae Σ1278b strains, the MAPK cascade regulates both mating and filamentous growth and expression of CaEAP2 activates the transcription of FLO11 (Figure 4). Therefore, CaEAP2 may also regulate the transcription of FLO11 through the MAPK pathway in S. cerevisiae and activate the expression of adhesins involved in C. albicans mating.

CaEAP1 very likely encodes a cell adhesin, which belongs to the GPI-CWP family (6, 20, 35). CaEAP1 might increase the adherence of yeast cells to polystyrene by increasing the surface hydrophobicity. It has been reported that cells that express FLO11 were much more hydrophobic than flo11Δ mutant cells (20, 26). Adhesion of C. albicans to materials and mammalian tissues plays a very important role in pathogenesis (5). Therefore, understanding the nature of CaEap1p-mediated adhesion, as well as the contribution of other adhesins, to different materials will presumably increase our ability to design strategies to prevent adhesion.

A parallel plate flow chamber permits quantitative, reproducible measurements of cell detachment from surfaces by applying a known, regulatable shear stress under conditions of laminar flow. The shear stress at the surface of the chamber is felt as a shear force, which can detach the cells into the bulk medium. Shear forces of different magnitudes can be obtained by varying the volumetric flow rate, solution viscosity, and/or the dimensions of the flow path. Results obtained from this assay were quantitatively consistent, in contrast with many qualitative adhesion assays that rely on operator technique (e.g., shaking or pipetting). High resolution of this system enabled us to isolate and quantify subtle differences in adhesion between different strains. Our system allows visualization of cell adhesion events under well-defined wall shear stress by real-time image acquisition and subsequent image processing. Therefore, this assay may also be used to study the behavior and biochemical responses of yeast cells adhering to host epithelial or endothelial cells by mimicking the physiological flow conditions and the surface characteristics in hosts. The second approach we used to measure yeast adhesion to a substrate is the centrifugation cell adhesion assay. Unlike the parallel plate flow chamber assay that applies shear forces parallel to the surface to peel cells off the surface gradually, the centrifugation assay simultaneously fractures all interactions between a cell and the surface. This assay cannot readily provide as wide a range of forces and the process of detachment cannot be visualized continuously. However, the advantage of this assay is that many samples can be tested simultaneously in multiwell plates, resulting in a much higher throughput.

In summary, these results demonstrate that the parallel plate flow chamber assay provides an efficient way to study yeast cell adhesion and it can be used as an approach to select for yeast strains with an enhanced affinity for a surface. Novel genes encoding both C. albicans adhesins and regulatory factors that activate the expression of endogenous S. cerevisiae adhesins have been identified in our study.

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References and Notes


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