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EAP1, a *Candida albicans* Gene Involved in Binding Human Epithelial Cells

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Candida albicans **adhesion to host tissues contributes to its virulence and adhesion to medical devices permits biofilm formation, but we know relatively little about the molecular mechanisms governing *C. albicans* adhesion to materials or mammalian cells. *Saccharomyces cerevisiae* provides an attractive model system for studying adhesion in yeast because of its well-characterized genetics and gene expression systems and the conservation of signal transduction pathways among the yeasts. In this study, we used a parallel plate flow chamber to screen and characterize attachment of a *flo8*Δ *S. cerevisiae* strain expressing a *C. albicans* genomic library to a polystyrene surface. The gene *EAP1* was isolated as a putative cell wall adhesin. Sequence analysis of *EAP1* shows that it contains a signal peptide, a glycosylphosphatidylinositol anchor site, and possesses homology to many other yeast genes encoding cell wall proteins. In addition to increasing adhesion to polystyrene, heterologous expression of *EAP1* in *S. cerevisiae* and autonomous expression of *EAP1* in a *C. albicans efg1* homozygous null mutant significantly enhanced attachment to HEK293 kidney epithelial cells. *EAP1* expression also restored invasive growth to haploid *flo8*Δ and *flo11*Δ strains as well as filamentous growth to diploid *flo8*/*flo8* and *flo11*/*flo11* strains. Transcription of *EAP1* in *C. albicans* is regulated by the transcription factor Efg1p, suggesting that *EAP1* expression is activated by the cyclic AMP-dependent protein kinase pathway.**

Candida albicans is the most common fungal pathogen of humans (45). Typically, candidiasis manifests as superficial mucosal diseases, but it also frequently results in systemic infections of immunocompromised patients. Approximately 30% mortality results from systemic candidiasis in susceptible individuals, such as diabetics, surgical patients, and hosts with human immunodeficiency virus infection (65).

Among the factors involved in *C. albicans* pathogenesis are adhesion of *C. albicans* to host epithelial and endothelial cells and the dimorphic transition of *C. albicans* between the ellipsoid yeast form and various filamentous forms: germ tubes, pseudohyphae, and hyphae (36, 41, 45, 53). Signals involved in the yeast-hyphae transition, including temperature, pH, and chemical stimuli, often also lead to increases in adhesin expression (6, 7). Two transcription factors, Cph1p and Efg1p, are required for hyphae formation, adhesion to and penetration of multilayers of human epidermal tissue, and virulence in a mouse model (9, 37).

A number of *C. albicans* genes encoding adhesins, including *ALS1*, *ALA1*, and *HWP1*, have been identified, but their roles in *C. albicans* pathogenesis remain unclear. *ALA1* and *ALS1* were identified based on their abilities to confer upon *Saccharomyces cerevisiae* the capacity to adhere to extracellular matrix proteins or human umbilical vein endothelial cells (11, 18). *HWP1* encodes an adhesion receptor that operates through a transglutaminase-mediated mechanism (59). Ala1p, Als1p, and Hwp1p are predicted to be cross-linked to the β-1,6-glucans of the cell wall of *C. albicans* (61). Furthermore, Hwp1p and Als1p function downstream of the transcription factor Efg1p (12, 55). *INT1*, which was initially characterized as a *C. albicans* adhesion receptor, has similarity to *S. cerevisiae BUD4* and contains a conserved transmembrane region found in the human α-integrin gene (14). *INT1* expression in *S. cerevisiae* is sufficient to direct the adhesion of this yeast to HeLa cells (14, 15). Int1p also colocalizes with septins and is involved in axial bud site selection in *C. albicans* (16). Furthermore, disruption of *ALS1*, *HWP1*, or *INT1* in *C. albicans* attenuates virulence in mouse models (12, 15, 59).

Two different signaling pathways, a mitogen-activated protein kinase (MAPK) cascade and a cyclic AMP (cAMP)-dependent pathway, have been identified to regulate the morphogenetic switch from yeast to pseudohyphae in *S. cerevisiae* (17, 42). In *S. cerevisiae*, both pathways converge on Flo11p, a transmembrane protein that mediates cell flocculation and adhesion to plastic and permits filament formation and invasive growth during nitrogen and carbon source starvation (13, 23, 49, 52). Based on the evolutionary conservation of fungal signal transduction pathways, MAPK and cAMP pathways have been identified in *C. albicans* (3). Functional homologues of the components of the *S. cerevisiae* MAPK cascade have been identified in *C. albicans*, such as *CST20*, *HST7*, *CEK1*, and *CPH1* (5). The cAMP-dependent pathway in *C. albicans* includes *EFG1*, the homologue of *S. cerevisiae PHD1*, and is believed to be regulated by cAMP-protein kinase A Tpk2p downstream of Ras and adenylyl cyclase (37, 51, 58, 60). However, important differences exist between *S. cerevisiae* and *C. albicans* adhesion and dimorphic growth. *S. cerevisiae* is not able to grow in hyphal forms, whereas *C. albicans* is more morphologically diverse. The Tup1p transcriptional repressor

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Source or reference
S. cerevisiae strains		
SKY760	*MAT***a** *ura3-52 his3*::*hisG leu2*::*hisG*	Collection
SKY756	*MAT***a**/α *ura3-52*/*ura3-52*	Collection
SKY2021	*MAT***a**/α *ura3-52*/*ura3-52 flo11*::*LEU2*/*flo11*::*LEU2 leu2*::*hisG*/*leu2*::*hisG*	Collection
SPY308	*MAT*α *ura3-52 his3*::*hisG leu2*::*hisG flo8*::*kan*^{r}	Collection
SPY309	*MAT***a** *ura3-52 his3*::*hisG leu2*::*hisG flo11*::*HIS3*	This study
SPY311	*MAT***a**/α *ura3-52*/*ura3-52 his3*::*hisG*/*his3*::*hisG leu2*::*hisG*/*LEU2 flo8*::*kan*^{r}/*flo8*::*HIS3*	This study
C. albicans strains		
SC5314	Clinical isolate	26
HLC52	*ura3*::*1 imm434*/*ura3*::*1 imm434 efg1*::*hisG*/*efg1*::*hisG-URA3-hisG*	37
HLC67	*ura3*::*1 imm434*/*ura3*::*1 imm434 efg1*::*hisG*/*efg1*::*hisG*	37
HLC74	*ura3*::*1 imm434*/*ura3*::*1 imm434 efg1*::*hisG*/*efg1*::*hisG*(*EFG1*)	37
SPY312	*ura3*::*1 imm434*/*ura3*::*1 imm434 efg1*::*hisG*/*efg1*::*hisG EAP1*/*EAP1-URA3-pACT1-EAP1*	This study

is required for normal pseudohyphal growth in *S. cerevisiae*, while Tup1p functions as a repressor of hyphal development in *C. albicans* (4).

In this study, we isolated *EAP1*, a novel *C. albicans* adhesin which can mediate adhesion of *S. cerevisiae* and *C. albicans* cells to polystyrene and epithelial cells. Expression of *EAP1* can also restore haploid invasive growth and diploid pseudohyphal formation to adhesion-deficient *S. cerevisiae*. Finally, we demonstrate that *EAP1* expression in *C. albicans* is under the regulation of the transcription factor Efg1p.

MATERIALS AND METHODS

Yeast strains, media, and genetic methods. The yeast strains used in this study are listed in Table 1. Strains were derived in the Σ1278b genetic background (22, 34) using standard genetic methods. Standard yeast culture media and filamentous growth media were prepared as previously described (1, 31). Synthetic low-ammonium (SLAD) medium contained 50 μM ammonium sulfate. Uracil was added to SLAD medium to a concentration of 0.2 mM to make SLAD plus Ura. Galactose was added to medium to replace glucose in order to express genes within plasmids containing the *S. cerevisiae GAL1* promoter. Yeast cells were transformed using lithium acetate transformation (19).

PCR disruption (39) was used to replace *FLO8* and *FLO11* in both *MAT***a** and *MAT*α Σ1278b cells with the kanMX G418 resistance or *HIS3* marker. *FLO8* positions +1 to +2400, relative to the start site, were deleted and *FLO11* positions −26 to + 4170 were deleted.

To overexpress *EAP1* in *C. albicans*, the *C. albicans URA3* gene was obtained as a *Sac*II-*Xba*I fragment from pDDB57 (66) and inserted into *Sac*II/*Xba*I-digested pBluescript KS(+) (Stratagene) to generate pAU1. A 1.1-kb region upstream of *ACT1* from *C. albicans* SC5314 genomic DNA was amplified by PCR and cloned into the *Kpn*I and *Xho*I sites of pAU1 to yield pAU2 (63). The entire open reading frame (ORF) of *EAP1* flanked by an upstream *Cla*I site and a downstream *Ssp*I site was amplified by PCR and cloned into pAU2 cut with *Cla*I and *Sma*I. The resulting construct (pAU3) was linearized by cutting in the *EAP1* ORF DNA with *Pst*I to direct integration to the native *EAP1* locus. The linearized pAU3 was used to transform a *ura3*/*ura3 efg1*/*efg1 C. albicans* strain. Ura$^+$ clones were selected, and integration of the third copy of *EAP1* was verified by PCR.

Parallel plate flow chamber cell adhesion assay. The parallel plate flow chamber (Glycotech, Rockville, Md.) consists of a flow deck that fits inside a 60-mm-diameter petri dish. A silicone rubber gasket is placed between the flow deck and the 60-mm petri dish to form the flow chamber. A peristaltic pump connected to the inlet of the flow chamber provides relatively constant velocity flow through the chamber. The shear stress generated by the flow at the bottom surface of the flow chamber detaches yeast cells from the surface. The shear stress is defined as $\tau = 3Q\mu/2wh^2$, where Q is the volumetric flow rate, μ 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. After assembly, the flow chamber was placed on a motorized X-Y stage (Prior) of an Olympus IX70 inverted microscope.

Yeast cells were cultured overnight in the appropriate medium, as indicated below in Results. Cells were then pelleted and suspended in 0.1 M sodium phosphate buffer, pH 6.0. After brief sonication to break cell lumps, the cell suspension was pumped into the flow chamber and incubated for 3 h to allow the cells to settle on the surface of the petri dish. 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. For each applied shear stress, three fields were selected under the microscope and images were captured using a digital camera (Nikon Spot) and the Metavue software package. The number of cells remaining attached to the surface was automatically identified and counted by Metavue based on contrast of the cells and cell sizes. The adhesion of cells was quantified as the average of the fraction of cells in each of the selected three fields remaining attached after exposure to an applied shear force for 15 min.

Selection for adherent clones of *S. cerevisiae*. The genomic library of *C. albicans* SC5314 constructed in pYesR was kindly provided by Yue Fu and Scott Filler (11). Expression of the genes within the library is regulated by the *S. cerevisiae GAL1* promoter. This genomic library was transformed into *S. cerevisiae* SPY308 (*MAT*α *ura3-52 his3*::*hisG leu2*::*hisG flo8*::*kan*^{r}). Cells carrying the genomic library were cultured overnight at 30°C in synthetic complete medium lacking uracil (SC-ura) and containing galactose as a carbon source. Cells were pelleted and resuspended in 0.1 M sodium phosphate buffer, pH 6.0. After sonicating 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 a shear stress of 2.5 dyne/cm^2, 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, repooled, 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, and plasmids were isolated from those colonies. Approximately 50 individual colonies were sequenced. 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 (48). 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 confluent monolayer in six-well tissue culture plates in minimum essential medium (Invitrogen) containing 10% horse serum (Invitrogen). The cells were washed twice in phosphate-buffered saline (PBS) containing Mg^{2+} and Ca^{2+} (PBS^{++}) at 37°C. Yeast adhesion to 293 cells was measured essentially as described by Fu et al. (11). Briefly, yeast cells (500 cells/μl) suspended in PBS^{++} were sonicated for 30 s and added to confluent monolayer 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 away from the 293 cells using PBS^{++}. Next, trypsin was added to the wells and the yeast cells were suspended in water and plated on yeast extract-peptone-dextrose (YPD) agar. The number of adherent yeast cells was determined by colony counting, and adherence was expressed as the fraction of cells remaining attached.

Pseudohyphal growth assay. The pseudohyphal growth assay was performed essentially as described by Gimeno et al. (20). Strains to be tested were streaked

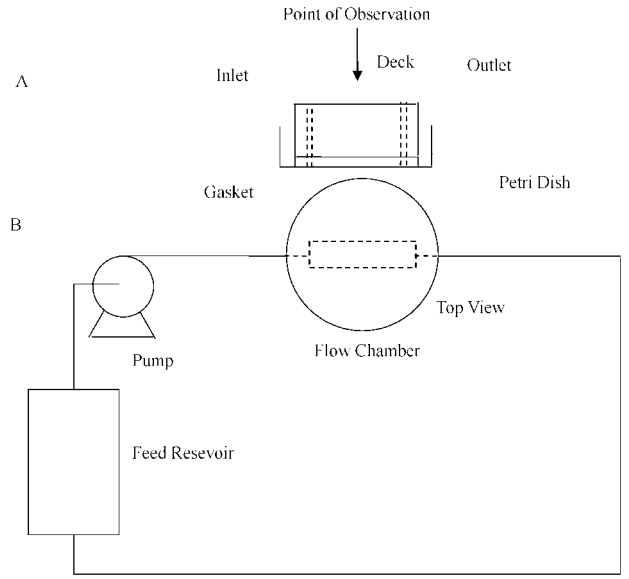

FIG. 1. Schematic diagram of the flow chamber used for yeast detachment measurements. (A) Side view of the chamber apparatus; (B) diagram of flow system.

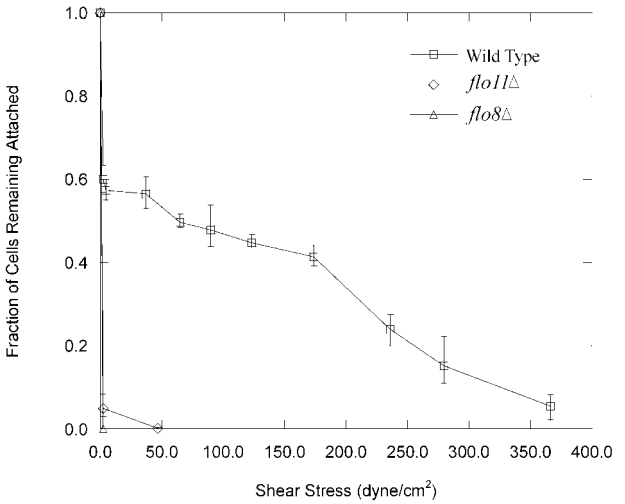

FIG. 2. Effect of applied shear stress on yeast cell detachment from polystyrene. *S. cerevisiae* strains SKY760 (wild type), SPY308 (*flo8*Δ), and SPY309 (*flo11*Δ) were grown in YPD and incubated on the surface of a petri dish in the parallel plate flow chamber for 3 h. Phosphate buffer (0.1 M; pH 6.0) flowed through the chamber for 15 min at a controlled flow rate, and shear stress was calculated. The fraction of cells adhering after flow was determined via image analysis of three fields, containing 600 to 800 cells each, prior to flow. Error bars represent the ranges of three separate experiments.

on SLAD plus Ura plates containing 2% galactose to obtain single cells. Cultures were grown at 30°C for 2 days, and representative colonies were photographed.

Agar invasion assay. Strains to be tested were patched on SC-ura plates containing 2% galactose. Cells were grown at 30°C for 1 day, and the plates were photographed. Next, the plates were rinsed with running water to remove nonadherent cells and the plates were photographed again (50).

Northern blot analysis. *C. albicans* strains were cultured in liquid YPD medium overnight at 30°C. Cultures were diluted 10-fold into fresh YPD medium supplemented with 10 or 20% serum if indicated and incubated for 2 h at 37°C. Total RNA was extracted from cells by phenol-chloroform followed by ethanol precipitation. For each sample, 40 μg of total RNA was separated by electrophoresis on a formaldehyde gel and transferred by capillary action to a nylon membrane. DNA probes (800-bp region of *EAP1* and 1,000-bp region of *ACT1* ORFs) were amplified and radiolabeled by PCR. Hybridization and washes were performed according to the methods of Sambrook et al. (54).

RESULTS

***FLO8* and *FLO11* are required for *S. cerevisiae* adhesion to polystyrene.** A parallel plate flow chamber permits quantitative reproducible adhesion measurements between yeast cells and a surface by applying a known, regulatable shear stress under conditions of laminar flow (40, 64). This shear stress is felt as a shear force, which can detach the cells into the bulk medium or roll them along the surface. A schematic diagram of the flow system is shown in Fig. 1. Shear forces of different magnitudes are obtained by varying flow rate, chamber height, and/or fluid viscosity. Yeast cell adhesion can be quantified as the ratio of the number of attached cells after exposure to an applied shear force to the initial number of attached cells under zero force.

We used a parallel plate flow chamber assay to characterize adhesion of wild-type haploid *S. cerevisiae* strain Σ1278b cells to the surface of an untreated polystyrene petri dish. As expected, increasing shear stress decreased the fraction of adherent cells (Fig. 2). An extremely low shear stress removed over 40% of the cells, which were most likely just resting on the surface (Fig. 2). As shear stress increased, the fraction of cells remaining attached to the surface decreased in a roughly linear manner up to about 350 dyne/cm^2. We also measured adhesion as a function of shear stress in *flo11*Δ and *flo8*Δ strains. *FLO11* encodes a cell surface protein involved in adhesion, invasive growth, and filamentous growth (23, 38), and *FLO8* encodes a transcription factor required for expression of the flocculins *FLO1* and *FLO11* (28, 29). Less than 5% of *flo8*Δ cells and *flo11*Δ cells remained attached at shear stresses as low as 3 dyne/cm^2 (Fig. 2), demonstrating that *FLO8* and *FLO11* are required for *S. cerevisiae* adhesion to polystyrene and suggesting that Flo11p is the protein that mediates this adhesion. *S. cerevisiae* strains that overexpress *FLO11*, such as *srb8*Δ (46), showed increased adhesion to polystyrene compared to the wild-type strain (data not shown).

Identification of *EAP1*, a *C. albicans* gene that increases *S. cerevisiae* adhesion to polystyrene. Based on the observation that an *S. cerevisiae flo8*Δ strain is much less adherent to polystyrene than the wild-type strain, we used the parallel plate flow chamber to apply shear stress to select adherent clones of an *S. cerevisiae* haploid *flo8*Δ strain expressing a *C. albicans* genomic library (11). The *S. cerevisiae GAL1* promoter regulates expression of the *C. albicans* genes within the library. Cells carrying the genomic library were cultured in minimal medium plus galactose overnight at 30°C. 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 a shear stress of 2.5 dyne/cm^2, and the fraction of attached cells was measured. This selection procedure was repeated four times, after which the fraction of cells remaining attached at 2.5 dyne/cm^2 shear stress increased from 1 to 73%. One clone was found to exhibit significantly greater adhesion compared to *S. cerevisiae* cells harboring empty plasmids. The

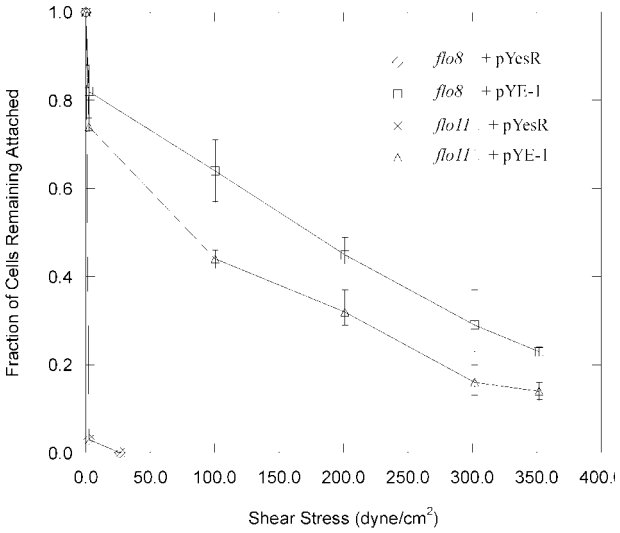

FIG. 3. Eap1p mediates adhesion of *S. cerevisiae* to polystyrene. *S. cerevisiae* strains SPY308 (*flo8*Δ) and SPY309 (*flo11*Δ) containing either pYesR (empty vector) or pYE-1 (containing *EAP1*) were grown in minimal medium containing galactose and incubated on the surface of a petri dish in a parallel plate flow chamber for 3 h. Phosphate buffer (0.1 M; pH 6.0) flowed through the chamber for 15 min at a controlled flow rate, and shear stress was calculated. The fraction of cells adhering after flow was determined via image analysis of three fields, containing 600 to 800 cells each, prior to flow. Error bars represent the ranges of three separate experiments.

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Eap1p_R1    TDTAYTTTVITVTKCDGGSCSHTAVTTGVTIITVTTNDVITEYTTYCPL
Eap1p_R2    TVIVPSTTVITVSSCYEDKCSVSSVTTGV....VTISSEETIYTTYCPI
Hwp1p_R1    TTTEHDTTVVTVTSCSNSVCTESEVTTGV....IVITSKDTIYTTYCPL
Hwp1p_R2    TSTEQSTTVITVTSCSESSCTESEVTTGV....VVVTSEETVYTTFCPL
Cht2p       TVTDVQKTVITITSCSEHKCVATPVTTGV....VVVTDIDTVYTTYCPL
Flo1P_R1    TKTSEQTTLVTVTSCESHVCTESISPAIVSTATVTVSGVTTEYTTWCPI
Flo1p_R2    TETTKQTTVVTISSCESDVCSKTASPAIVSTSTATINGVTTEYTTWCPI
Flo1p_R3    TESRQQTTLVTVTSCESGVCSETASPAIVSTATATVNDVVTVYPTWRPQ
Aga1p_R1    ..................TTTVSPALVSTSTIVQAGTTTLYTTWCPL
Aga1p_R2    ..................STTIPSFSMSTYFTTVSGVTTMYTTWCPY
Sed1p_R1    ........................PSTDYTTDYTVVTEYTTYCPE
Sed1p_R2    .......................PTTTSTTEYTVVTEYTTYCPE
Fig2p_R1    ..................TSTTSPAYVSTATKTVDGVITEYVTWCPL
Fig2p_R2    ..................TSTTSPAYVSTATKTVDGVITEYVTWCPL
Fig2p_R3    ..................TSGMQTLVLSTVTTTVNGAATEYTTWCPA
Fig2p_R4    ..................SSTPSQYSLSTATTTINGIKTVYTTWCPL
Fig2p_R5    ..................SQTSIQYTLSTATTTISGLKTVYTTWCPL
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FIG. 4. Alignment of the *C. albicans* Eap1p sequence with sequences of known adhesin and cell wall proteins containing the conserved YTTWCPL motif. R1, R2, etc. indicate consecutive repeats within the same protein. Residues conserved in at least 50% of the sequences within the region of overlap are boxed.

plasmid contained in this clone was designated as pYE-1. The rescued plasmid was transformed back into an *S. cerevisiae* *flo8*Δ strain and found to increase adhesion to polystyrene (Fig. 3).

There are two possibilities for the increased adherence of the *flo8*Δ strain containing pYE-1. First, the *C. albicans* gene contained in pYE-1 could encode a cell wall protein of *C. albicans* that can directly mediate *S. cerevisiae* adhesion to the substrate. Alternatively, the *C. albicans* gene contained in pYE-1 could encode a protein that enhances activity or expression of an *S. cerevisiae* adhesin, such as Flo11p. To test whether pYE-1 requires *FLO11* to increase adhesion, we transformed pYE-1 into the *S. cerevisiae* haploid *flo11*Δ strain and measured adhesion as a function of shear stress (Fig. 3). *S. cerevisiae* haploid *flo11*Δ cells harboring pYE-1 exhibited enhanced adhesion to polystyrene (Fig. 3), indicating that pYE-1 does not increase adhesion via *FLO11* and likely encodes an adhesion molecule. Both *flo8*Δ and *flo11*Δ strains harboring pYE-1 demonstrated a higher fraction of cells adhering to the surface than wild-type yeast without pYE-1, and those adhering possessed a higher affinity for the surface. Also, the *flo8*Δ strain with pYE-1 had a slightly higher adhesion than the *flo11*Δ strain with pYE-1.

Sequence analysis of the adherence-promoting gene. 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 (48). Sequence analysis revealed a 1,962-bp ORF capable of encoding a 653-residue polypeptide (ORF number 6.5354). This gene was named *EAP1* (enhanced adherence to polystyrene). Sequence analysis showed that Eap1p possesses homology to Hwp1p of *C. albicans* and certain cell wall proteins of *S. cerevisiae*, such as Flo11p and Aga1p. Furthermore, the consensus motif YTT WCPL present in Eap1p is conserved in many additional yeast cell wall proteins, including Hwp1p, the *C. albicans* chitinase Cht2p, the *S. cerevisiae* flocculation protein Flo1p, the α-agglutinin subunit Aga1p, the pheromone-regulated protein Fig2p, and the cell wall protein Sed1p (55) (Fig. 4). All of these proteins are either known or predicted to be glycosylphosphatidylinositol (GPI)-anchored cell surface proteins. Eap1p also contains a serine/threonine-rich sequence that may provide glycosylation sites. Analysis of the deduced amino acid sequence predicts the existence of an N-terminal signal sequence (44). The analysis of the C-terminal sequence suggests a GPI-attached site ω and a valine at the ω-5 site that is important for incorporation into the cell wall (24, 25).

Expressing *EAP1* enhances the adhesion of *S. cerevisiae* cells to 293 cells. Adhesion to host epithelial and endothelial cells is hypothesized to be a critical step involved in the pathogenesis of *C. albicans* (45). To test whether *EAP1* mediates the adhesion of yeast cells to epithelial cells, yeast cell suspensions were added to confluent monolayers of 293 human embryonic kidney cells grown in six-well tissue culture plates and incubated for 1 h at 37°C. Nonadherent yeast cells were dislodged by gentle agitation followed by a rinse. Adherent yeast cells were collected and quantified by trypsinizing and detaching the monolayer from the plate and then transferring the suspension to a YPD plate to count the number of yeast colonies. Adhesion is expressed as the fraction of yeast cells remaining attached to the 293 cell monolayer after rinsing. Shear flow detachment was unsuitable for this assay, since placing the 293 monolayer in the flow chamber often peeled the cell monolayer from the substratum. Also, the rough surface of the cell monolayer disrupted the laminar flow pattern, complicating the calculation of shear stress. Less than 1% of *flo8*Δ *S. cerevisiae* cells carrying empty vector were able to adhere to 293 cells, whereas over 35% of *S. cerevisiae* haploid *flo8*Δ cells expressing *EAP1* adhered to 293 cell monolayers (Fig. 5). Wild-type *C. albicans* also exhibited similar adhesion to 293 cells, as did *S. cerevisiae* expressing *EAP1* (Fig. 5). Therefore, *EAP1* expression is sufficient to mediate yeast attachment to

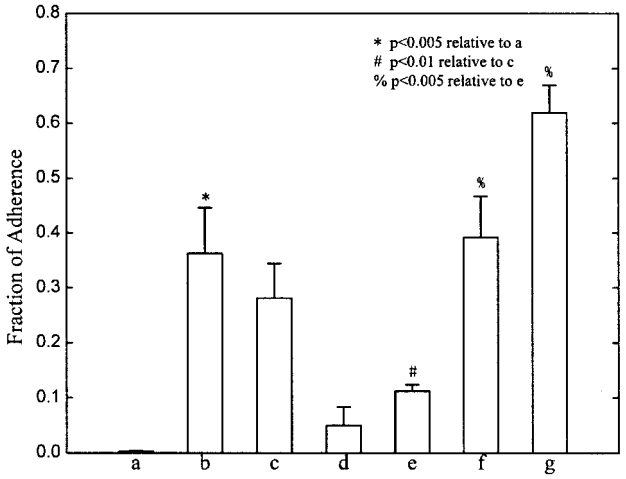

FIG. 5. Eap1p mediates adhesion of *S. cerevisiae* to 293 human embryonic kidney cells. (a) *S. cerevisiae* strain SPY308 (*flo8*Δ) transformed with vector pYesR (empty vector); (b) *S. cerevisiae* strain SPY308 (*flo8*Δ) transformed with pYE-1 (*EAP1*); (c) *C. albicans* SC5315; (d) *S. cerevisiae* strain SKY760 (wild type); (e) *C. albicans* HLC52 (*efg1*/*efg1*::*URA3*); (f) *C. albicans* HLC74 (*efg1*/*efg1*/*EFG1*); (g) *C. albicans* strain SPY312 (*efg1*/*efg1 EAP1*/*EAP1*::*pACT1-EAP1*). Cells were grown in minimal medium containing galactose and incubated on a confluent 293 cell monolayer for 1 h at 37°C. The cells were rinsed with PBS containing Ca^{2+} and Mg^{2+} to remove nonadherent cells. The 293 cell monolayer and associated yeast were detached by trypsinization and added to a YPD plate. Adhesion is quantified as the number of colonies formed on the YPD plate divided by the number of yeast initially added to the 293 monolayer. Error bars represent the standard deviations of three separate experiments. Statistical significance was determined by using Student's *t* test.

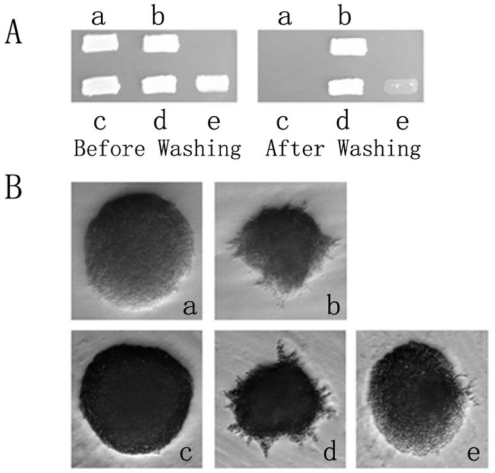

FIG. 6. Expression of *EAP1* restores haploid invasive growth and diploid pseudohyphal formation to adhesion-deficient *S. cerevisiae*. (A) *EAP1* mediates *S. cerevisiae* invasive growth. (a) *S. cerevisiae* strain SPY308 (*flo8*Δ) transformed with vector pYesR (empty vector); (b) *S. cerevisiae* strain SPY308 (*flo8*Δ) containing vector pYE-1 (*EAP1*); (c) *S. cerevisiae* strain SPY309 (*flo11*Δ) containing pYesR; (d) *S. cerevisiae* strain SPY309 (*flo11*Δ) containing vector pYE-1; (e) *S. cerevisiae* strain SKY760 (wild type). Cells were patched on SC-ura medium containing galactose and allowed to grow overnight at 37°C. The plate was photographed and then held under gently running water for several minutes and photographed again. (B) Expression of *EAP1* restores diploid pseudohyphal formation to a *flo11*/*flo11* strain. (a) *S. cerevisiae* strain SPY311 (*flo8*/*flo8*) transformed with vector pYesR;, (b) *S. cerevisiae* strain SPY311 (*flo8*/*flo8*) transformed with pYE-1; (c) *S. cerevisiae* strain SKY2021 (*flo11*/*flo11*) transformed with vector pYesR; (d) *S. cerevisiae* strain SKY2021 (*flo11*/*flo11*) transformed with pYE-1; (e) *S. cerevisiae* strain SKY756 (wild type). Cells were streaked onto synthetic low-ammonia medium containing galactose. Colonies were allowed to grow at 30°C for 2 days and then were photographed.

human epithelial cells. The transcription factor Efg1p is an essential regulator of morphogenesis, cell wall remodeling, and virulence of *C. albicans* (33, 37, 57). Since the Ura status of isogenic mutants affects the adhesion of *C. albicans* (2), we used an *efg1*/*efg1* Ura^+ strain to study its adhesion to 293 cells. The adhesion of *efg1*/*efg1 C. albicans* cells to 293 cells was decreased compared to that of wild-type *C. albicans* cells, but complementing *EFG1* in the *efg1*/*efg1* null mutant restored adhesion to wild-type levels (Fig. 5). *EAP1* under the control of the constitutive *ACT1* promoter was integrated into the *efg1*/*efg1* strain (*efg1*/*efg1 EAP1*/*EAP1*::*pACT1-EAP1* strain). The resulting strain regained the ability to adhere to 293 cells (Fig. 5).

Expression of *EAP1* restores haploid invasive growth and diploid pseudohyphal formation to adhesion-deficient *S. cerevisiae* strains. Flo8p and Flo11p are required for haploid invasive growth and diploid pseudohyphal formation of *S. cerevisiae* (32, 35, 38). *S. cerevisiae* haploid *flo8*Δ and *flo11*Δ strains carrying empty vectors were unable to invade agar on synthetic medium lacking uracil (Fig. 6A), and diploid *flo8*/*flo8* or *flo11*/*flo11* cells failed to form any filaments on SLAD medium (Fig. 6B). However, the ability to penetrate agar and form filaments could be restored by the expression of *EAP1* in these strains (Fig. 6). In fact, *EAP1* expression induced a stronger filamentous phenotype than that observed in the wild-type Σ1278b strain on SLAD medium. Thus, *EAP1* expression rescued several of the *flo11*Δ defects in *S. cerevisiae*.

Expression of *EAP1* in *C. albicans* is under the regulation of *EFG1*. The morphological transformation of *C. albicans* from yeast form to hyphal and pseudohyphal growth can be induced by serum at 37°C. Northern blot analysis was used to determine whether the transcription of *EAP1* was morphogenically regulated in *C. albicans*. *EAP1* was transcribed both in YPD medium at 30°C and YPD medium supplemented with 10% fetal bovine serum at 37°C after 120 min of growth (Fig. 7A).

No *EAP1* expression was detected by Northern blot analysis in an *efg1*/*efg1* mutant in YPD medium supplemented with 10% fetal bovine serum at 37°C or in YPD in the absence of serum at 30°C (Fig. 7A). The transcription of *EAP1* was restored in an *efg1*/*efg1* mutant complemented with *EFG1* in YPD medium supplemented with 20% fetal bovine serum, although at a reduced level (Fig. 7B). Therefore, Efg1p is required for *EAP1* expression even though *EAP1* is not induced during the dimorphic switch in response to serum and 37°C.

DISCUSSION

In this study, we identified *EAP1*, a *C. albicans* gene that enhances cell adhesion to human epithelial cells as well as to polystyrene when expressed in *S. cerevisiae*. *EAP1* expression also complements *flo11* mutations in *S. cerevisiae*, restoring

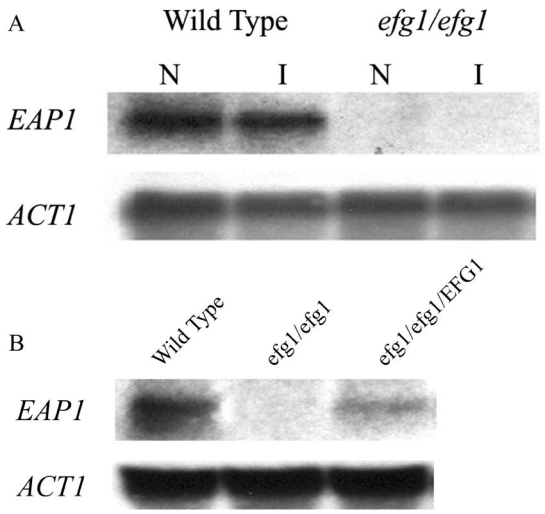

FIG. 7. Northern blot analysis of the expression of *EAP1*. (A) RNA was prepared from *C. albicans* SC5314 (wild type) and HLC52 (*efg1*/*efg1*) grown in YPD at 30°C (N) or in YPD supplemented with 10% fetal bovine serum at 37°C (I). A 40-μg aliquot of total RNA was applied, and transcripts were detected using probes specific for *EAP1* and *ACT1* (loading control). (B) RNA was prepared from *C. albicans* SC5314 (wild type), HLC52 (*efg1*/*efg1*), and HLC74 (*efg1*/*efg1*/*EFG1*) grown in YPD supplemented with 20% fetal bovine serum at 37°C. A 60-μg aliquot of total RNA was applied, and transcripts were detected using probes specific for *EAP1* and *ACT1* (loading control).

invasive growth to haploid *flo8*Δ and *flo11*Δ strains as well as filamentous growth to diploid *flo8*Δ/*flo8*Δ and *flo11*Δ/*flo11*Δ strains. Transcription of *EAP1* in *C. albicans* requires the transcription factor Efg1p, a key regulator of hyphal growth in the cAMP-protein kinase A pathway.

To identify potential *C. albicans* adhesion receptors, we used a parallel plate shear flow assay to screen a genomic library expressed in *S. cerevisiae*. Results obtained from this assay were quantitatively accurate and consistent, in contrast with results from many qualitative adhesion assays that rely on operator technique (e.g., shaking or pipetting). Desired forces can be applied to yeast cells by varying the volumetric flow rates, solution viscosity, or the dimensions of the flow path. The high resolution of this system enables us to isolate and quantify subtle difference in adhesion between different strains. We are also capable of identifying trends of cell adhesion with increasing shear forces. We found that *FLO8* and *FLO11* are required for *S. cerevisiae* adhesion when polystyrene is used as the substratum. This finding agrees with a previous report (49). Haploid *flo11*Δ cells exhibited slightly greater adherence to polystyrene than *flo8*Δ cells (Fig. 2), probably because Flo8p can activate the transcription of other genes encoding adhesins, such as *FLO1*, which can also contribute to the adhesion to polystyrene in a less efficient manner than *FLO11* (29).

Although *EAP1* was initially found by selecting transformants adhering to polystyrene, this gene concomitantly confers upon *S. cerevisiae* the ability to adhere to human kidney epithelial cells. It is feasible to modify the parallel plate flow chamber assay to select adherent clones to more biologically realistic surfaces by functionalizing the polystyrene and reacting with or adsorbing extracellular matrix proteins to the surface. This assay may also prove promising in testing roles of putative adhesins in systemic candidiasis by mimicking the physical forces and the surface characteristics in the host. Glee et al. investigated adhesion interactions of *C. albicans* with endothelial monolayers under simulated physiologic shear stress using capillary tubes and demonstrated that hydrophobic *C. albicans* cells have a higher binding affinity than hydrophilic *C. albicans* cells for endothelial cells and other *C. albicans* cells (21). In our assay we were not able to identify known *C. albicans* adhesins, such as *ALS1*, *ALA1*, and *HWP1*, perhaps because the adhesins encoded by these genes do not bind to polystyrene as strongly as Eap1 and these clones were lost in the multiple rounds of selection.

The primary amino acid sequence of *EAP1* shares features of highly glycosylated yeast cell wall proteins with N-terminal signal peptides and C-terminal ω sites mediating GPI anchor addition (24). Hwp1p, Ala1p, and Als1p from *C. albicans* and Epa1p from *Candida glabrata* are members of this class of proteins (62). Eap1p also contains a valine five residues upstream from the ω site that determines linkage to β (1,6)-glucan of the cell wall (25). According to the above features, *EAP1* has been predicted to be a new *C. albicans* adhesin by searching the *C. albicans* genome for proteins in the GPI cell wall protein class (62).

When expressed in *S. cerevisiae*, *EAP1* exhibits functional similarity to *S. cerevisiae FLO11*. *FLO11* encodes a cell wall protein required for both invasion and pseudohyphae formation by wild-type *S. cerevisiae*, presumably by enabling cell-cell and cell-substrate adhesion (38, 47). Cells of the *S. cerevisiae* strain Σ1278b with deletions of *FLO11* do not form pseudohyphae as diploids nor invade agar as haploids (38). *C. albicans EAP1* complements the deletion of *FLO11* in *S. cerevisiae* to restore both haploid invasive growth and diploid pseudohyphae formation. Increasing adhesion alone appears to be sufficient to restore invasion and filament formation. The hyperfilamentation observed in *S. cerevisiae* cells expressing *EAP1* (Fig. 6) might be due to greater adhesion exhibited by this strain than by the wild-type strain. Expression of *S. cerevisiae FIG2* or *FLO10* encoding GPI-anchored proteins mediating adhesion can bypass the requirement for *FLO11* for both filamentation and invasion (23). Likewise, inhibiting mother-daughter separation following cytokinesis by mutating *CTS1*, *ACE2*, or *EGT2* restores invasive growth in *flo8*Δ and *flo11*Δ strains (10, 27, 30). Since morphogenesis in *S. cerevisiae* and *C. albicans* is governed in part by the same signal transduction pathways, MAPK cascade and cAMP-dependent pathway, and *EAP1* is under the regulation of *EFG1*, it is possible that *EAP1* is also involved in the morphogenesis in *C. albicans*.

EAP1 is transcribed both in cells grown in YPD at 30°C and in YPD supplemented with 10% fetal bovine serum at 37°C. Nantel et al. reported that the transcription of *EAP1* is induced by twofold when yeast cells are cultured in YPD supplemented with 10% serum at 37°C for 6 h by using a DNA microarray of the *C. albicans* genome (43). We found that there was no significant *EAP1* induction after 6 h of culture using Northern blot analysis (data not shown). The presence of *EAP1* under both noninducing conditions and hyphae-inducing conditions suggests that *EAP1* might be involved in adhesion of yeast cells

to mammalian cells before hyphal formation and in the process of forming hyphae.

The cell surface component that Eap1p binds remains unclear. An increase in *C. albicans* hydrophobicity increases binding to fibronectin (56); adhesion to both cell surfaces and polystyrene may be explained by such a mechanism. The sequence of Eap1p shows slight homology to yeast lectins, and certain lectins, such as *C. glabrata* Epa1p, can mediate attachment to mammalian cells, while others, including *S. cerevisiae* flocculins, cannot (8). *C. glabrata* Epa1p binds *N*-acetyl lactosamine glycoconjugates (8), but ligands for other *Candida* spp. adhesins have not been conclusively identified.

In summary, these results demonstrate that *C. albicans EAP1* encodes a protein that permits *S. cerevisiae* cell adhesion to epithelial cells as well as invasive and filamentous growth. In *C. albicans*, *EAP1* functions downstream of Efg1p in the cAMP-PKA pathway.

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