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 flo8Δ 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 flo8Δ and flo11Δ strains as well as filamentous growth to diploid flo8Δflo8 and flo11Δflo11 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, ALAI, and HWP1, have been identified, but their roles in C. albicans pathogenesis remain unclear. ALAI and ALSI 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). Alalp, 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 Alslp 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 floculation 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 CPFI (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 adenyl 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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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.

Yeast strains, media, and genetic methods. The yeast strains used in this study are listed in Table 1. Strains were derived in the low-ammonium (SLAD) medium contained 50 mM ammonium sulfate. Uracil was transformed using lithium acetate transformation (19). 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 centrifuged to remove debris and added 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 3-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.

#### Yeast strains used in this study

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| SKY760 | MATa ura3-52 his3::hisG leu2::hisG | Collection |
| SKY775 | MATa ura3-52 leu2::hisG | Collection |
| SKY2021 | MATa ura3-52 his3::hisG leu2::hisG | Collection |
| SPY308 | MATa ura3-52 his3::hisG leu2::hisG | This study |
| SPY309 | MATa ura3-52 his3::hisG leu2::hisG | This study |
| SPY311 | MATa ura3-52 his3::hisG leu2::hisG | This study |

#### C. albicans strains

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| SCS314 | Clinical isolate | 26 |
| HLC52  | ura3::1 imm3434/ura3::1 imm3434 efg1::hisG::efg1::hisG-URA3-hisG | 37 |
| HLC67  | ura3::1 imm3434/ura3::1 imm3434 efg1::hisG::hisG | 37 |
| HLC74  | ura3::1 imm3434/ura3::1 imm3434 efg1::hisG::efg1::hisG(EFG1) | 37 |
| SPY312 | ura3::1 imm3434/ura3::1 imm3434 efg1::hisG::efg1::hisG EAPI/EAPI-URA3-pACT1-EAPI | This study |

#### 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 centrifuged to remove debris and added to 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 3-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* SCS314 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* GALI promoter. This genomic library was transformed into *S. cerevisiae* SPY308 (MATa ura3-52 his3::hisG leu2::hisG flo8::kan') 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 sonication 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 dynes/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, reprocessed, 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 (Invitro) containing 10% horse serum (Invitro). The cells were washed twice in phosphate-buffered saline (PBS) containing Mg²⁺ and Ca²⁺ (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 adhesion 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.
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 non-adherent cells and the plates were photographed again (50).

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/cm². We also measured adhesion as a function of shear stress in flo11Δ and flo8Δ 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 flo8Δ cells and flo11Δ cells remained attached at shear stresses as low as 3 dyne/cm² (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 srb8Δ (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 flo8Δ 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 flo8Δ 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/cm², 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/cm² 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
is the calculation of shear stress. Less than 1% of cells adhered to 293 cell monolayers (Fig. 5).

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 on the suspension of yeast 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 flo8Δ S. cerevisiae cells carrying empty vector were able to adhere to 293 cells, whereas over 35% of S. cerevisiae haploid flo8Δ 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 S. cerevisiae and certain cell wall proteins of S. cerevisiae, such as Flo11p and Aga1p. Furthermore, the consensus motif YTTCWCPL 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 phenol-regulated protein Fig2p, and the cell wall protein Sed1p (35) (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).

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 insertion 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. The rescued plasmid was transformed back into an S. cerevisiae flo8Δ strain and found to increase adhesion to polystyrene (Fig. 3).

FIG. 3. Eap1p mediates adhesion of S. cerevisiae to polystyrene. S. cerevisiae strains SPY308 (flo8Δ) and SPY309 (flo11Δ) 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.
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). EAPI under the control of the constitutive ACT1 promoter was integrated into the efg1/efg1 strain (efg1/efg1 EAPI/EAPI::pACT1-EAPI1 strain). The resulting strain regained the ability to adhere to 293 cells (Fig. 5).

Expression of EAPI 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 flo8Δ and flo11Δ strains carrying empty vectors were unable to invade agar on synthetic medium lacking uracil (Fig. 6A), and diploid flo8/Δ flo8/Δ 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 EAPI in these strains (Fig. 6). In fact, EAPI expression induced a stronger filamentous phenotype than that observed in the wild-type Σ278b strain on SLAD medium. Thus, EAPI expression rescued several of the flo11Δ defects in S. cerevisiae.

Expression of EAPI 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 EAPI was morphogenically regulated in C. albicans. EAPI 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 EAPI expression was detected by Northern blot analysis in an efg1Δ 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 EAPI was restored in an efg1Δ 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 EAPI expression even though EAPI is not induced during the dimorphic switch in response to serum and 37°C.

**DISCUSSION**

In this study, we identified EAPI, a C. albicans gene that enhances cell adhesion to human epithelial cells as well as to polystyrene when expressed in S. cerevisiae. EAPI expression also complements flo11 mutations in S. cerevisiae, restoring
invasive growth to haploid *flo8Δ* and *flo11Δ* strains as well as filamentous growth to diploid *flo8Δ/flo8Δ* and *flo11Δ/flo11Δ* strains. Transcription of *EAPI* 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 differences 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 *flo11Δ* cells exhibited slightly greater adherence to polystyrene than *flo8Δ* 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 *EAPI* 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 *HW1P1*, perhaps because the adhesins encoded by these genes do not bind to polystyrene as strongly as *EAPI* and these clones were lost in the multiple rounds of selection.

The primary amino acid sequence of *EAPI* shares features of highly glycosylated yeast cell wall proteins with N-terminal signal peptides and C-terminal ω sites mediating GPI anchor addition (24). *HWP1*, *Ala1p*, and *Als1p* from *C. albicans* and *Epa1p* from *Candida glabrata* are members of this class of proteins (62). *EAPI* 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, *EAPI* 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*, *EAPI* exhibits functional similarity to *S. cerevisiae* *FLO11*. *FLO11* encodes a cell wall protein required for both invasion and pseudohyphal 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* *EAPI* complements the deletion of *FLO11* in *S. cerevisiae* to restore both haploid invasive growth and diploid pseudohyphal formation. Increasing adhesion alone appears to be sufficient to restore invasion and filament formation. The hyperfilamentation observed in *S. cerevisiae* cells expressing *EAPI* (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 *flo8Δ* and *flo11Δ* 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 *EAPI* is under the regulation of *EFG1*, it is possible that *EAPI* is also involved in the morphogenesis in *C. albicans*.

*EAPI* 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 *EAPI* 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 *EAPI* induction after 6 h of culture using Northern blot analysis (data not shown). The presence of *EAPI* under both noninducing conditions and hyphae-inducing conditions suggests that *EAPI* might be involved in adhesion of yeast cells.

**FIG. 7.** Northern blot analysis of the expression of *EAPI*. (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 *EAPI* 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 *EAPI* and *ACT1* (loading control).
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 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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