Candida albicans is the leading cause of systemic fungal infections in immunocompromised humans. The ability to form biofilms on surfaces in the host or on implanted medical devices enhances C. albicans virulence, leading to antimicrobial resistance and providing a reservoir for infection. Biofilm formation is a complex multicellular process consisting of cell adhesion, cell growth, morphogenic switching between yeast form and filamentous states, and quorum sensing. Here we describe the role of the C. albicans \textit{EAP1} gene, which encodes a glycosylphosphatidylinositol-anchored, glucan-cross-linked cell wall protein, in adhesion and biofilm formation in vitro and in vivo. Deleting \textit{EAP1} reduced cell adhesion to polystyrene and epithelial cells in a gene dosage-dependent manner. Furthermore, \textit{EAP1} expression was required for \textit{C. albicans} biofilm formation in an in vitro parallel plate flow chamber model and in an in vivo rat central venous catheter model. \textit{EAP1} expression was upregulated in biofilm-associated cells in vitro and in vivo. Our results illustrate an association between \textit{Eap1p}-mediated adhesion and biofilm formation in vitro and in vivo.

\textit{Candida albicans} is the leading cause of candidiasis, most often manifesting as superficial mucosal infections. \textit{Candida} spp. are also major agents of systemic bloodstream infections, causing 8% of all such nosocomial infections in the United States, and are behind only coagulase-negative staphylococci, \textit{Staphylococcus aureus}, and enterococci as causes of bloodstream infections (13, 25, 43). Frequently, candidemia is associated with \textit{Staphylococcus} aureus bloodstream infections (1535-9778/07/$08.00 © 2007, American Society for Microbiology. All Rights Reserved.

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**TABLE 1. C. albicans strains used in this study**

| Strain      | Genotype or description               | Source or reference |
|-------------|--------------------------------------|---------------------|
| SC5314      | Clinical isolate                     | 19                  |
| BWP17       | ura3::XhoI344::ura3::XhoI344 His1::hisG/His1::hisG arg4::hisG/arg4::hisG         | 59                  |
| YJB6284     | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/URa3::arg4::hisG/arg4::hisG | 6                   |
| SPY319      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4/His4::His4 eap1::URA3/EAP1 | This study          |
| SPY314      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 eap1::URA3/EAP1 | This study          |
| SPY315      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 eap1::URA3/EAP1 | This study          |
| SPY316      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 eap1::URA3/EAP1 | This study          |
| SPY317      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 eap1::URA3/EAP1 | This study          |
| SPY387      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 ACT1/ACT1-URA3::pACT1-HAEAP1 | This study          |
| SPY388      | ura3::XhoI344::ura3::XhoI344 HIS1::hisG/His1::hisG ARG4/His4::His4::His4::His4 ACT1/ACT1-URA3::pACT1-HAEAP1 | This study          |

of its ability to mediate adhesion to polystyrene when expressed in a *Saccharomyces cerevisiae* flocculin-deficient strain (32). EAPI expression also restored invasive and filamentous growth to *S. cerevisiae* flo11Δ mutants and enhanced attachment of *S. cerevisiae* to HEK293 kidney epithelial cells (32). In this study, we demonstrate that Eap1p is a glucan-cross-linked cell wall-localized protein. In addition, we found that eap1 mutants exhibited reduced adhesion to plastic surfaces and epithelial cells and that Eap1p was able to mediate adhesion to yeast cells. EAPI expression was also required for biofilm formation under shear flow in vitro and in an in vivo central venous catheter biofilm model. Finally, we show that the expression of EAPI was differentially regulated between planktonic and biofilm-associated cells. Our results suggest that the adhesin Eap1p plays a role in *C. albicans* biofilm formation.

**MATERIALS AND METHODS**

**Strains and media.** The *C. albicans* and *S. cerevisiae* strains used in this study are listed in Tables 1 and 2. Escherichia coli strain DH5α was used for general recombination techniques according to protocols described by Sambrook et al. (48). Yeast strains were routinely cultured in YPD medium (1% yeast extract, 2% peptone, 2% glucose) or minimal defined medium (2% glucose, 0.67% yeast nitrogen base without amino acids) at 30°C. YPD medium was supplemented with 80 μg/ml uridine when Ura− strains were used in this study. Synthetic minimal medium lacking specific nutrients was made prototrophic (strain YJP6284) as described by Bensen et al. (6). Synthetic minimal medium lacking specific nutrients was made prototrophic (strain YJP6284) as described by Bensen et al. (6).

**Plasmid construction.** A partial open reading frame (ORF) encoding the N-terminal 42 amino acids of Eap1p was amplified from *C. albicans* SC5314 genomic DNA with the oligonucleotides EAP1.S and EAP1.HA.Sig3, which contains the sequence encoding the hemagglutinin (HA) epitope of influenza virus. The PCR product was digested with Pael and SpeI and ligated into pHP1Sig.HA.GFP (37) to yield pEElS1gSigHA.GFP.Hwp1GPI. A partial ORF encoding Eap1p without the N-terminal 42 amino acids was amplified from *C. albicans* SC5314 genomic DNA. This PCR product was digested with SpeI and Smal and ligated into pEElS1gSigHA.GOFP.Hwp1GPI to generate pEElS1gSigHA.GFP.Hwp1GPI. The ORF encoding HA-tagged Eap1p was amplified from pSigHA.Eap1.GPI cloned into the Xhol and Xbal sites of pUA3 (32) to yield pHAeAP1.pHAeAP1.NOGPI is essentially identical to pHAeAP1 except that 21 amino acid residues from the C terminus of Eap1p, encoding the GPI anchor signal, were deleted. For all constructs in which fragments were generated by PCR, the final constructs were verified by DNA sequence analysis.

**Expression and detection of HA-tagged Eap1p.** The pHAEAP1 and pHAEAP1.NOGPI constructs were used by cutting in the promoter of CaACT1 with BglII to direct integration to the CaACT1 locus. The linearized pHAEAP1 and pHAEAP1.NOGPI constructs were used to transform *C. albicans* BWP17. Ura− clones were selected, and integration of the third copy of EAPI was verified by PCR. The transformants were cultured in 3 ml SC-Ura medium overnight at 30°C (optical density at 600 nm [OD600] = 1.0). The cells were blocked with 10% goat serum in phosphate-buffered saline (PBS) and then incubated with an anti-HA monoclonal antibody (Covance, Berkeley, CA) for 30 min, followed by incubation with an anti-mouse antibody–fluorescein isothiocyanate–conjugate (Zymed, San Francisco, CA) for 30 min. The cells were examined for green fluorescence by using an inverted epifluorescence Olympus IX70 microscope coupled to a Spot charge-coupled device camera (Diagnostic Instruments, Sterling Heights, MI) and MetaVue image acquisition and analysis software (Universal Imaging Corporation, Downingtown, PA).

**Western analysis.** Yeast cells expressing HA-tagged Eap1p constructs were cultured as described above and harvested by centrifugation. To analyze the HA-tagged Eap1p protein in medium, the supernatant was concentrated with 100-kDa-cutoff Microcon centrifugal filters (Amicon, Billerica, MA). Yeast cell walls were isolated according to the method of Mao et al. (37). Briefly, 3 ml yeast cells (OD600 = 1.5 to 2.0) was harvested and disrupted by vortexing with glass beads. The resulting yeast debris was centrifuged to obtain supernatants and pellets. The pellets were boiled twice in 2% sodium dodecyl sulfate (SDS) for 5 min each and washed five times in 1 M NaCl and five times in water. The washed pellets were treated with lamarinase (β-1,3-glucanase from *Penicillium*; Sigma) (0.25 U/g wet weight) in 100 mM sodium acetate, pH 5.5, or with the buffer alone for 4 h at 37°C. The proteins released into the supernatant by enzymatic digestion or from concentrated cell-free medium were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Western blotting using an ECL Western blot kit (Amersham) and anti-HA monoclonal antibody.

**Construction of eap1 mutant strains.** To construct *C. albicans* disruption strains, we used PCR-mediated gene disruption methods as previously described (20, 58). The marker cassettes used for gene disruption were amplified by primers that provide 100 bp of sequence homology corresponding to the EAPI ORF at its 5′ or 3′ end. An eapi::URA3 PCR product was amplified with pDBR57 (58) as the DNA template and transformed into BWP17 (59) to generate the heterozygous strain SPY316. SPY316 was transformed with an eapi::ARG4 disruption fragment amplified from the DNA template pFA-ARG4 (20) to generate the eapi− null mutant strain SPY317. SPY316 and SPY317 were made prototrophic by transforming them with pGEM-HIS1 (59) linearized with NsiI and/or pRS-Arg4SpeI linearized with ClaI to generate strains SPY313 and SPY314, respectively. BWP17 was made prototrophic (strain YJP6284) as described by Bensen et al. (6).

To complement the eapi−/eapi− mutant with EAPI, HIS1 was amplified by PCR with pGEM-HIS1 as the DNA template and cloned into the SalI and SpeI sites of pH Bluescript KS(+) (Strategene) to generate pHSI1. A full-length EAPI gene, including a 562-bp sequence upstream and a 337-bp sequence downstream of the ORF for Eap1p, was PCR amplified from *C. albicans* BWP17 genomic DNA. This PCR product was cloned into pHSI1 cut with SpeI and SacII to generate

**TABLE 2. S. cerevisiae strains used in this study**

| Strain      | Background and genotype | Source or reference |
|-------------|-------------------------|---------------------|
| SKY760      | MATa ura3−his3::HisG  | Our collection      |
| SPY308      | MATa ura3−his3::HisG  | 32                  |
| SPY415      | MATa ura3−his3::HisG  | This study          |
pHSI-EAPI. The final construct was verified by DNA sequence analysis. This plasmid was linearized by cutting in the EAPI promoter with Sphl and was then integrated into the native EAPI locus of strain SPY317 to generate strain SPY315.

Parallel plate flow chamber cell adhesion assay. Yeast cell adhesion to polystyrene surfaces was quantified using a parallel plate flow chamber (Oxytech, Rockville, MD) as previously described (33). Briefly, yeast cells were cultured in YPD overnight, pelleted, and resuspended in 0.1 M sodium phosphate buffer, pH 6.0. After brief sonication to disperse cellular aggregates, the cell suspension was pumped into the flow chamber and incubated for 15 min. The detachment assay was performed by increasing the flow rate of the sodium phosphate buffer, thus and the shear stress, in a stepwise manner. For each applied shear stress, the number of cells remaining attached to the surface was identified and counted, using an inverted Olympus microscope coupled to a Spot charge-coupled device camera and MetaVue image acquisition and analysis software. The adhesion of cells was quantified as the mean fraction of cells in each of three selected fields remaining attached after exposure to an applied shear force.

*S. cerevisiae* adhesion to *C. albicans* cells. To distinguish *S. cerevisiae* cells bound to a *C. albicans* monolayer, an *S. cerevisiae* floßA strain constitutively expressing green fluorescent protein (GFP) under the control of the ADH1 promoter was constructed. Primers floßADHGFP_F (5′-GTT TAT AGA CAT AAA TAA AGA GGA AAC GCA TCG CTT CGT GGT ATG ATA GAA TAC GAG CCT GTT TAA AC-3′) and floßADHGFP_R (5′-TATA AGA GTT TTT ATT TTT TAT TAT AAT CAA ACT CAC GTA TCT ATA TCC GTG TAT CCC TAT CCC-3′) were designed to amplify the HIS-pADH1-GFP cassette from plasmid pÅta-Has-MxO-pADH1-GFP (54). Plasmid-specific regions of these primers are indicated in bold. The resulting DNA cassette was transformed into SKY1 and then carried out on monoclonal polystyrene plates lacking agar. Replacement of the FLO8 ORF with the HIS-pADH1-GFP construct in the resulting strain (SPY415) was verified by PCR. SPY415 was then transformed with plasmid pYE-1, which contains a fragment of *C. albicans* DNA that carries EAPI under the control of the GAL1 promotor (33).

*C. albicans* BWPI7 was cultured overnight in a petri dish in YPD with mild shaking. Cells were allowed to settle to the dish surface, and the flow chamber apparatus was placed atop them. The chamber was filled with 0.1 M sodium phosphate buffer, pH 6.0, and the flow rate was increased incrementally from 0 to 30.7 dyn/cm² to generate a “monolayer” of cells.

The inoculum was adjusted to 10⁶ CFU/ml and instilled in each catheter in a 500-µl volume (the entire catheter volume) for 4 h. After 24 h of development, the distal 2 cm of catheter was cut perpendicular to the catheter length into 5-mm segments. For scanning electron microscopy, the catheter segments were placed in fixative (1% [vol/vol] glutaraldehyde and 4% [vol/vol] formaldehyde) overnight. The specimens were then dehydrated in a series of ethanol washes and dessicated by critical point drying (Tousimis, Rockville, MA). The specimens were then coated with gold and observed with a scanning electron microscope (Hitachi S-7000) in high-vacuum mode at 10 kV. The images were processed for display by using Adobe Photoshop software (Adobe, Mountain View, CA).

**RESULTS**

**Eap1p is a glucan-cross-linked CWP.** Sequence analysis predicted that Eap1p is a member of the family of GPI-CWPs, with an N-terminal signal sequence and a C-terminal GPI addition signal (11, 32, 53). To verify that Eap1p is a GPI-anchored CWP, the HA epitope of influenza virus was engineered into the EAPI coding sequence C-terminal to the predicted EAPI signal peptide. The HA-tagged Eapi protein (HAEAPI) was expressed in *C. albicans* BWPI7. Transformants expressing HAEGAPI exhibited localized fluorescence at the cell surface (Fig. 1A), consistent with a CWP. We next investigated if HAEAPI was anchored to the cell surface via a GPI anchor by expressing a version of HAEAPI lacking the GPI anchor site (HAEAPI.NOGPI); this protein was not detected at the cell surface (Fig. 1A), indicating that GPI anchoring is essential for localization. We tested the cell-free medium of the HAEAPI.NOGPI transformants for secretion of HA-tagged Eapi protein by Western blotting with an anti-HA monoclonal antibody. The HAEAPI.NOGPI transformants secreted a protein that was detected by the anti-HA antibody, whereas HA-tagged Eapi was not detected in culture supernatants of the HAEAPI transformants (Fig. 1B).

To address whether Eapip anchors to the plasma membrane or is incorporated into the cell wall, purified cell walls from *C. albicans* strains transformed with HA-tagged Eapi constructs were digested with β-glucanase. HA-tagged Eapi was re-
leased from the cell walls of the HAEAP1 transformants and detected with anti-HA antibody (Fig. 1C). Based on these results, we concluded that Eap1p is a GPI-CWP.

**Deletion of EAP1 reduces C. albicans adhesion.** Eap1p localized to the yeast cell wall, as demonstrated in Fig. 1, and heterologous expression of EAP1 in *S. cerevisiae* enhanced adhesion to polystyrene and to HEK293 kidney epithelial cells (32). These findings suggest that EAP1 expression may affect adhesion of *C. albicans* to materials or mammalian cells. We used a parallel plate flow chamber to quantify the shear stresses required to detach wild-type and *eap1* mutant strains from polystyrene surfaces. A dramatic difference existed in the ability of the strains to adhere (*P < 0.001*); adhesion of the eap1/eap1 strain to polystyrene was reduced approximately 50% relative to that of the wild-type strain, up to a shear stress of 200 dyn/cm² (Fig. 2A), as defined by the number of adherent cells at any value of shear stress. In wild-type and eap1/eap1 strains, the number of cells adhered to the surface was relatively independent of shear stress over the range tested. EAP1 expression affected the number of cells that adhered to the surface, but cells that adhered appeared to have a high affinity for the surface, suggesting a very low dissociation rate constant or cooperative binding to the surface. Adhesion was not completely abolished in the *eap1/eap1* strain, as approximately 25 to 30% of cells were able to adhere even at high shear rates, indicating that EAP1-independent mechanisms also play a role in adhesion to polystyrene. Diminished adhesion of the heterozygous strain compared to that of the wild-type strain implies that the gene dosage of *EAP1* affected the extent of adhesion to polystyrene (Fig. 2A). The *eap1/eap1::EAP1* strain adhered to polystyrene to a similar extent as the heterozygous strain (Fig. 2A), indicating that the reduction in adhesion resulted from the absence of *EAP1*.

To confirm that *EAP1* is also involved in *C. albicans* adhesion to mammalian cells, we measured the adherence of strains expressing different gene dosages of *EAP1* to HEK293 kidney epithelial cell monolayers. Compared with that of the wild-type strain, adhesion of the *eap1/eap1* strain, measured as the fraction of cells remaining attached to the 293 monolayer following a rinse in PBS, was reduced 37% (Fig. 2B). Similar to the observations with polystyrene, the adhesion of the null mutant to epithelial cells was not eliminated (Fig. 2B), suggesting that while *EAP1* expression affects *C. albicans* adhesion to epithelial cells, other attachment factors also contribute to this adhesion.

**Eap1p mediates cell-cell adhesion.** During biofilm development, sessile cells proliferate and daughter cells must remain attached to the surface-bound cells or to the polysaccharide matrix in the biofilm. To test whether Eap1p plays a role in cell-cell adhesion in addition to cell surface adhesion, we expressed *EAP1* in an adhesin-deficient *S. cerevisiae* strain and measured the shear stress required to detach these cells from a *C. albicans* monolayer. A cassette encoding GFP regulated by the constitutive ADH1 promoter was integrated into the *FLO8* locus, deleting the *FLO8* ORF, in a haploid *S. cerevisiae* strain. This GFP-expressing strain was transformed with pYE-1, carrying *EAP1* under control of the *GAL1* promoter. A parallel plate flow chamber was assembled atop *C. albicans* BWP17 cells that had been grown overnight in a polystyrene petri dish, and flow was applied to generate a monolayer of cells. This
monolayer remained stably attached to the surface at shear stresses of up to 31 dyn/cm\(^2\) (not shown). *S. cerevisiae* cells expressing GFP and carrying either a plasmid containing *EAPI* or an empty vector were then introduced into the flow chamber and allowed to attach to the *C. albicans* monolayer. The fraction of *S. cerevisiae* cells remaining adherent was quantified as a function of shear stress, using fluorescence microscopy to detect GFP-expressing cells (Fig. 3). Eighty-one percent of *S. cerevisiae* cells expressing *EAPI* were able to adhere to the *C. albicans* monolayer under very low flow (0.6 dyn/cm\(^2\)), while only 40% of cells containing the empty vector were adherent. Likewise, 76% of *S. cerevisiae* cells expressing *EAPI* resisted detachment at 31 dyn/cm\(^2\), while only 3% of cells containing the empty vector remained on the surface. These results suggest that Eap1p mediates adhesion to one or more components of the *C. albicans* cell surface.

**EAPI expression is required for biofilm formation under flow in vitro.** *EAPI* is involved in adhesion to surfaces and adhesion to other *C. albicans* cells. These processes play roles in biofilm formation and development (7). To determine whether *EAPI* expression affects biofilm formation, we tested the ability of *C. albicans eap1* mutant strains to form biofilms under shear flow in a parallel plate flow chamber. In this assay, *C. albicans* cells experience well-controlled shear stresses, and a previous report demonstrated that biofilms formed under flow produce more extracellular matrix than do biofilms formed under static conditions (21). *C. albicans* cells were cultured in YPD medium at 30°C overnight, resuspended in 0.1 M sodium phosphate, pH 6.0, and added to the parallel plate flow chamber. After 30 min of incubation at 37°C, fresh YPD was allowed to flow through the chamber at a shear stress of 6 dyn/cm\(^2\) for 20 h to allow cell growth and biofilm development.

We were able to observe all stages of biofilm formation by capturing phase-contrast images at different times. Wild-type *C. albicans* biofilm formation proceeded in three developmental phases. During the early phase, yeast cells adhered to the uniform surface of the petri dish and began to divide to form microcolonies (Fig. 4). At 8 to 12 h, wild-type cells produced confluent multiple layers of adherent blastospores, and initial differentiation into pseudohyphae and hyphae occurred (Fig. 4). Finally, multilayer biofilms were formed at 20 h, in which *C. albicans* communities were completely encased within extracellular material (Fig. 4). The *eap1* null mutant was able to adhere to the polystyrene surface under this shear stress and formed pseudohyphae and hyphae at 8 h (Fig. 4). However, the biofilm reached the maximum thickness of a few layers of cells at 8 h, and the number of cells retained in the chamber decreased after 8 h. Almost all *eap1* null mutant cells were washed out of the chamber at 20 h, perhaps reflecting the defect in cell-cell and cell-substrate adhesion as colony size increased and the colonies experienced greater shear forces away from the chamber wall (Fig. 4). The heterozygous strain formed a confluent monolayer containing yeast and filamentous cells (Fig. 4) but was not able to form a thick biofilm, even after 48 h (not shown). Thus, it appears that *EAPI* gene dosage, not solely the kinetics of biofilm formation, affects the qualitative ability of *C. albicans* to form biofilms in vitro. The *eap1/eap1::EAPI* strain formed a biofilm to a similar extent as the heterozygous strain (Fig. 4).

**EAPI expression is required for *C. albicans* biofilm formation in vivo.** While an in vitro biofilm assay provides a regulatable environment and permits monitoring of biofilm formation in real time, it lacks certain features that are important for biofilm formation in vivo, such as the host immune response and surface fouling with serum and extracellular matrix com-
ponents. To determine if EAP1 expression affects biofilm formation in vivo, we tested wild-type and eap1 mutant C. albicans strains in a rat model that simulates a central venous catheter biofilm infection (2). Cells were grown in YPD, resuspended in PBS (pH 7.0), and instilled in catheters that had been placed in the internal jugular veins of rats and conditioned for 24 h prior to infection. After 24 h, the catheters were removed from the rats, cut into 5-mm transverse segments, and examined by scanning electron microscopy. Wild-type cells formed a mature biofilm consisting of C. albicans cells embedded in a network of extracellular matrix strands that covered virtually the entire surface of the catheter (Fig. 5). In contrast, the eap1 null mutant was not able to form a biofilm on the catheter surface (Fig. 5). The heterozygous strain formed a thinner biofilm that contained fewer cells and was sparsely distributed over the catheter surface (Fig. 5). The eap1/eap1::EAP1 strain formed a biofilm to a level similar to that of the heterozygous strain (Fig. 5). Together, the observations shown in Fig. 4 and 5 illustrate the necessity of EAP1 expression for biofilm formation and development under flow in vitro and on a catheter surface in vivo.

EAP1 expression is upregulated in biofilms. Total RNA was isolated from C. albicans YJB6284 cells associated with in vivo biofilms developed in catheters or with in vitro biofilms grown in RPMI 1640 in tissue culture flasks undergoing agitation. RNA was also obtained from suspension-cultured cells under identical culture conditions as a reference. No variations in the morphology of planktonic and in vitro sessile cultures were observed at different time points up to 24 h; both yeast and filamentous cells were observed (not shown). The change in abundance of EAP1 mRNA extracted from the biofilm relative to that obtained from planktonic cells, measured by quantita-

tive real-time RT-PCR, is shown in Fig. 6. In both cases, the EAP1 mRNA abundance in the biofilm state was nearly twice that found in the corresponding planktonic cells (P < 0.01). These differential patterns of EAP1 expression in sessile cells compared with their planktonic counterparts further suggest a role for EAP1 in C. albicans biofilm formation. However, it is not clear whether cells with higher levels of EAP1 expression are more likely to adhere to the surface and participate in biofilm formation or whether growth in a sessile state induces EAP1 expression.

DISCUSSION

We report that expression of the C. albicans EAP1 gene affects cell adhesion and biofilm formation in vitro and in vivo. The primary amino acid sequence of Eap1p suggests that it is a member of the family of GPI-CWPs. We demonstrated that the C-terminal sequence of Eap1p was required to target HA-tagged Eap1p to the surfaces of C. albicans cells. In addition, HA-Eap1p was released from the cell wall by glucanase treatment. These results strongly suggest that Eap1p is indeed a GPI-CWP.

The availability of the C. albicans genome provides a strategy for predicting ORFs that potentially encode GPI-CWPs based on their common sequence features. Fifty-four ORFs in assembly 6 of the C. albicans genome have been predicted to encode GPI-CWPs (53), and 104 putative GPI-anchored protein ORFs have been identified in assembly 19 of the C. albicans genome (11). Most of these proteins have not been char-
characterized functionally, although several adhesins have been identified and at least partially characterized, including Eap1p, Hwp1p, and members of the Als family of proteins (24, 55). The existence of multiple putative adhesins in *C. albicans* may account for the diversity of substrates, from human tissues to plastic prostheses, to which *C. albicans* cells are able to attach. Furthermore, genetic recombination and epigenetic regulation have been suggested to provide a reservoir of adhesins with new functions in *S. cerevisiae* (56) and may also have contributed to evolution of adhesion function in *C. albicans*. In this work, we demonstrated that *EAP1* expression confers adhesion of *C. albicans* to polystyrene, human epithelial cells, and *C. albicans* cells, although it is not the sole contributor to adhesion to these surfaces. Understanding the nature of Eap1p-mediated adhesion, as well as the contributions of other adhesins, to different materials will presumably increase our ability to design strategies to prevent such adhesion and resulting infections.

*Many Candida* infections involve the formation of biofilms on implanted devices, such as central venous catheters. These biofilms exhibit resistance to a variety of antifungal agents currently in clinical use, including amphotericin B and fluconazole (1, 4, 23). Adhesion to the surface is the initial step in biofilm formation, and cell-cell interactions may be important in the hierarchical organization of cells within the biofilm, so presumably adhesin expression and activity can regulate the biofilm formation rate and perhaps the biofilm structure. We found that the *EAP1* mRNA abundance in the biofilm state was nearly twice that in the corresponding planktonic cells, and *eap1* null mutants were defective in *C. albicans* biofilm development under shear flow in vitro and in an in vivo central venous catheter biofilm model. This increase in *EAP1* expression may be the result of a selection process in which cells containing higher surface densities of Eap1p are more likely to attach to surfaces and to form biofilms. Alternatively, surface binding may directly induce *EAP1* expression; the mitogen-activated protein kinase Mkc1p is activated by cell surface binding and enhances hyphal growth and biofilm formation (30).

Als3p and Hwp1p have also been implicated, directly and indirectly, in biofilm formation (39, 41, 60). The transcription factor Bcr1p contributes to biofilm formation and induces expression of the adhesins Als3p and Hwp1p, among other CWPs (40). While microarray data did not identify changes in *EAP1* transcription upon *BCR1* deletion (40), alternative transcriptional pathways may affect biofilm formation via *EAP1* expression. Eap1p and Hwp1p both appear to be necessary for biofilm formation in the rat central venous catheter model, while Als3p is not strictly required (39, 41). The functional redundancy of adhesins and mechanisms of their regulation in biofilm formation are not yet clear.

No significant effect of *EAP1* expression on *C. albicans* morphogenesis was observed. Hypha formation by the *eap1/eap1* strain was similar to that of the wild-type strain in liquid cul-

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**FIG. 4.** *EAP1* expression is required for in vitro biofilm formation under flow. *C. albicans* strains YJB6284 (*EAP1/EAP1*), SPY313 (*eap1Δ/EAP1*), SPY314 (*eap1Δ/eap1Δ*), and SPY315 (*eap1Δ/eap1Δ/EAP1*) were cultured on the surface of a parallel plate flow chamber withYPD at 37°C flowing at 6 dyn/cm². Cells were allowed to incubate on the surface in sodium phosphate buffer for 30 min prior to the initiation of flow.
FIG. 5. EAPI expression affects biofilm formation in a rat central venous catheter model. Scanning electron microscopy images of C. albicans strain YJB6284 (EAPI/EAPI), SPY313 (eap1Δ/EAPI), SPY314 (eap1Δ/eap1), and SPY315 (eap1Δ/eap1Δ/EAPI) biofilm cross sections formed in a rat central venous catheter model. Catheters were conditioned for 24 h, 5 × 10⁵ CFU were instilled into the catheters, and biofilms were allowed to develop for 24 h. Catheter segments were fixed overnight and dehydrated by a series of ethanol washes. Samples were observed in the high-vacuum mode at 10 kV. Bar = 30 μm.

FIG. 6. EAPI mRNA abundance for in vivo biofilm-associated or in vitro sessile C. albicans YJB6284 cells compared to that for suspension-grown cells. Five hundred nanograms of RNA harvested from biofilm-associated C. albicans cells isolated from the rat central venous catheter model, from sessile cells cultured in RPMI 1640, or from suspension-grown cells was used in separate quantitative RT-PCRs. ACT1 expression was used as an internal control. Data represent the x-fold changes in EAPI expression in sessile versus planktonic cells. Error bars indicate the standard deviations for two independent experiments.

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