Overexpression of the Candida albicans ALA1 Gene in Saccharomyces cerevisiae Results in Aggregation following Attachment of Yeast Cells to Extracellular Matrix Proteins, Adherence Properties Similar to Those of Candida albicans

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Candida albicans maintains a commensal relationship with human hosts, probably by adhering to mucosal tissue in a variety of physiological conditions. We show that adherence due to the C. albicans gene ALA1 when transformed into Saccharomyces cerevisiae, is comprised of two sequential steps. Initially, C. albicans rapidly attaches to extracellular matrix (ECM) protein-coated magnetic beads in small numbers (the attachment phase). This is followed by a relatively slower step in which cell-to-cell interactions predominate (the aggregation phase). Neither of these phases is observed in S. cerevisiae. However, expression of the C. albicans ALA1 gene from a low-copy vector causes S. cerevisiae transformants to attach to ECM-coated magnetic beads without appreciable aggregation. Expression of ALA1 from a high-copy vector results in both attachment and aggregation. Moreover, transcriptional fusion of ALA1 with the galactose-inducible promoters GAL80, GAL7, and GAL1, allowing for low, moderate, and high levels of inducible transcription, respectively, causes attachment and aggregation that correlates with the strength of the GAL promoter. The adherence of C. albicans and S. cerevisiae overexpressing ALA1 to a number of protein ligands occurs over a broad pH range, is resistant to shear forces generated by vortexing, and is unaffected by the presence of sugars, high salt levels, free ligands, or detergents. Adherence is, however, inhibited by agents that disrupt hydrogen bonds. The similarities in the adherence and aggregation properties of C. albicans and S. cerevisiae overexpressing ALA1 suggest a role in adherence and aggregation for ALA1 and ALA1-like genes in C. albicans.

Candida albicans is capable of maintaining commensal relationships with mammalian hosts, in part, by surviving in small numbers on mucous membranes (3, 26). Since, C. albicans has evolved in the normal host by maintaining a commensal state, it is likely to have developed a mechanism(s) to maintain long-term residence. Adherence of the fungus to host tissue is generally believed to be one such important mechanism, both for initiating and for maintaining the commensal state. Furthermore, the adherence of C. albicans to many substrates is often associated with aggregations of yeast cells. This is a particularly prominent feature of in vitro adherence assays when C. albicans is added in saturating numbers to such targets as buccal cells (25), basement membrane (21), endothelial cells (19), and fibroblasts (23).

Cell surface molecules that may serve as C. albicans adhesins for host tissue include glycoproteins (4), polysaccharides (7), and lipids (10). Genetic approaches have identified and isolated C. albicans genes that may encode potential adhesins. For example, the C. albicans AAF1/CAD1 gene was isolated by expression cloning in Saccharomyces cerevisiae, and transformants exhibited enhanced adherence to polystyrene and to buccal epithelial cells. Transformants also autoaggregated with or without adherence (1). Further work by another group, however, disputed the direct involvement of the AAF1/CAD1 gene product in adherence to endothelial and epithelial cells and demonstrated that the autoaggregation was a form of flocculation inhibited by mannose (3). Another gene, aINT1, was isolated from C. albicans by screening a genomic library with a human α-integrin gene probe (8). Expression of aINT1 in S. cerevisiae caused fungal adherence to epithelial cells, formation of germ tube-like structures, and autoaggregation. However, an observation has been made that a strong similarity exists between the aINT1 gene sequence and the S. cerevisiae gene BUD4, which is involved in yeast cell bud site selection (28). Consequently, the function of this gene may be related more to morphology than to adherence (2). Another novel adhesive mechanism involving C. albicans is the recent description of the covalent interaction of a hypha-specific surface protein and mammalian cell proteins catalyzed by transglutaminases, thus forming a stable yeast cell-mammalian cell bond (29).

We have previously described the isolation of the C. albicans gene ALA1 (agglutinin-like adhesin 1), by expression cloning in S. cerevisiae (9). Expression of ALA1 in nonadherent S. cerevisiae caused transformants to adhere to extracellular matrix (ECM)-coated magnetic beads and to human buccal epithelial cells. The predicted Ala1p sequence shows features consistent with cell surface localization and similarities to the S. cerevisiae agglutinin protein (AGa1) which mediates cell-cell adhesion during the mating of haploid yeast cells. Recently, Fu et al. demonstrated that another C. albicans gene, ALS1 (agglutinin-like sequence 1), causes S. cerevisiae trans-
formants to adhere to human endothelial and epithelial cells when expressed from a galactose-inducible promoter (6).

In the present investigation, we show that adherence of C. albicans and transformed S. cerevisiae to proteins and cells is characterized by two sequential steps: attachment followed by aggregation. This form of adherence of C. albicans and S. cerevisiae expressing ALA1 from different plasmids occurs over a broad pH range to a number of protein ligands and is resistant to shear forces generated by vortexing and competition from free additives. The adherence of both yeasts is inhibited by hydrogen bond-disrupting agents.

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MATERIALS AND METHODS

Media, strains, and transformation. A C. albicans CAI strain (prototroph) isolated from a human source was used in adherence assays in these studies. S. cerevisiae YPH499 (MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1) was obtained from the American Type Culture Collection (ATCC). All fungal strains used in these studies were maintained as frozen stocks in 20% glycerol for the long-term storage. Media used for growing yeast strains were as follows: YPD (1% yeast extract, 2% peptone, 2% glucose), YPGal (1% yeast extract, 2% peptone, 2% galactose), and synthetic defined medium consisting of YPH499, 2% peptone, 2% galactose), and synthetic defined medium consisting of glycerol for the long-term storage. Media used for growing yeast strains were as follows: YPD (1% yeast extract, 2% peptone, 2% glucose), YPGal (1% yeast extract, 2% peptone, 2% galactose), and synthetic defined medium consisting of

FIG. 1. Schematic representation of plasmids used in this study showing plasmid copy numbers and structures of the putative ALA1 promoter and the galactose-inducible promoters. PGK100 is our original plasmid carrying ALA1. It is derived from pAUR112 (PanVera Corp., Madison, Wis.), which is incapable of conferring adherence properties on S. cerevisiae. The ALA1 promoter is unmapped, but deletion of the putative promoter abolishes its expression. Three upstream activation sequences (UAS1, UAS2, and UAS3) required for full promoter induction by galactose are shown by the numbered shaded boxes. The promoter location is shown by a solid box for ALA1 and an open box for the galactose promoter, and each is marked by a P. *, Partial deletion of UAS2.

Plasmid pGK119. Plasmid PGK119 (Fig. 1) was constructed by ligation of the BamHI-XhoI fragment from pGK102 into pRS426 (obtained from the ATCC) that had been restricted with BamHI and XhoI and dephosphorylated. After ligation, DNA was directly transformed into S. cerevisiae YPH499, and the cell pellet was suspended in 5 ml of YPGal and grown at 28°C for 20 h. Cells were collected and washed three times with 5 ml of TE buffer (10 mM Tris-HCl, pH 7.0; 1 mM EDTA) and then suspended in 1 ml of TE buffer. Cells expressing ALA1 were isolated by using fibronectin (FN)-coated magnetic beads as described earlier (9). Two transformants of the six analyzed exhibited adherence with an aggregation phenotype when grown in YPD or YPGal, and one of these was used for the adherence studies.

Plasmids pGK112, pGK116. Plasmid pGK112 and pGK116 carrying galactose-inducible promoters were purchased from the ATCC. Several attempts to clone the ALA1 gene in E. coli by using pG411gal I were not successful. Therefore, we constructed plasmids pGK112 and pGK116 that contain GAL1 and GALS promoters, respectively. In these plasmids, E. coli and S. cerevisiae replicons of pG411gal I and p414GALS were replaced with the replications from pAUR112, in which ALA1 is stable. For construction of pGK112, the SacI-SnaBI fragment from p414GAL1 was gel purified after making blunt ends by using T4 DNA polymerase. This DNA fragment contains the TRP1 marker, the GAL1 promoter, and multiple cloning sites. Similarly, the XhoI-BglII fragment from pAUR112 was blunt ended with T4 DNA polymerase, dephosphorylated, and gel purified. This DNA fragment contains sequences for replication in E. coli and S. cerevisiae and the β-lactamase gene for selection in E. coli. After ligation of these two fragments and transformation into E. coli, a chimeric plasmid (pGK112) was isolated that could be transformed and selected for the TRP marker in S. cerevisiae. By a similar strategy, pGK116 was constructed by ligation of SacI-SnaBI and XhoI-BglII fragments from p414GALS and pAUR112, respectively.

Plasmids pGK114, pGK118, and pGK117. A DNA fragment containing the promoter-less ALA1 gene was cloned into pGK102 and pGK116 to obtain plasmids pGK114 and pGK117, respectively. In contrast to the original plasmids, ALA1 is stable in pGK114 and pGK117. For the construction of pGK114, the EcoRV-XhoI fragment from pGK102 was gel purified after filling in of the XhoI end by using DNA polymerase I (Klenow fragment). This fragment was ligated to pGK112 that had been restricted by SmaI and was then dephosphorylated. After transformation into E. coli, recombinant plasmids were analyzed, and a plasmid containing ALA1 in the right orientation was called pGK114. The plasmid was transformed into S. cerevisiae YPH499, and transformants were analyzed for adherence to FN-coated magnetic beads. All six transformants analyzed exhibited adherence with an aggregation phenotype when grown in YPGal medium but not when grown in YPD medium. For the construction of pGK117, the promoter-less ALA1-containing EcoRV-XhoI fragment from pGK102 was isolated by gel purification. The PGK116 vector DNA was prepared by restricting with SmaI and XhoI and dephosphorylation. After ligation and transformation into S. cerevisiae YPH499, ALA1-expressing cells were isolated as described for the construction of pGK119. Three of six transformants selected for analysis
exhibited adherence with an aggregation phenotype when grown in YPGal but not inYPD. One of these transformants was used for further studies. Plasmid pGK118 was constructed by ligation of the EcoRV-Xhol fragment containing ALAI into p414GALL that had been restricted with Smal and Xhol and dephosphorylated. The ligated DNA was transformed into *S. cerevisiae* YPH499, and ALAI-expressing cells were isolated as described above. In this case, four of six transformants exhibited adherence with an aggregation phenotype when grown in YPGal but not in YPD, and one of these transformants was used in the adherence studies.

**Antibodies to ALAIp.** In order to demonstrate the presence of ALAI on the surface of the *S. cerevisiae* transformants, rabbit polyclonal antibodies to 15-mer peptides predicted by Chou-Fasman analysis to be on the surface of the protein were produced. The antigenic peptides used in this study were obtained (Research Genetics, Huntsville, Ala.). The approximate locations of the 15-mer peptides in ALAIp are shown in Fig. 2.

FIG. 2. Diagrammatic representation of ALAIp and approximate locations of the antigenic peptides used in this study. The N-terminal peptide begins with residue 258 (SFSDDDNYQYLSKYND). The C-terminal peptide begins with residue 1309 (SKTKSHEESIMNPOS). The molecule includes an immunoglobulin G domain (IgG), a threonine-rich domain (T), a tandem repeat domain (TR), and a serine-threonine-rich domain in the carboxy terminus (S/T).

Fungi were cultured as already described, and ~5 × 10^6 cells of each strain were placed in 10 ml of Tris-OL (pH 7.2) with 100 mM phenylmethylsulfonyl fluoride and shaken for 30 min at room temperature. After this treatment the cells are not capable of adhering or aggregating (see Results). The cells were centrifuged, and the supernatant was retained and concentrated (Centrrixprep; Amicon, Beverly, Mass.). All volumes were kept equal during the concentration, and samples were derived from each yeast strain were separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose, incubated with the primary antibody (1:2,500 dilution) overnight, and treated with a secondary alkaline phosphatase-tagged antibody (1:250 dilution).

For fluorescent-antibody studies, the samples were suspended in phosphate-buffered saline containing 3% bovine serum albumin (BSA; pH 7), incubated in a 1:10 dilution of primary antibody for 2 h, and then washed and treated with a secondary, fluorescein-tagged antibody at a dilution of 1:100.

**Preparation of cells for adherence assay.** An isolated *S. cerevisiae* colony from a YPD agar plate was inoculated in 5 ml of YPD and grown for 16 to 20 h at 28°C. The overnight culture was transferred to 45 ml of YPD and grown for 6 to 7 h at 28°C. Cells were harvested by centrifugation, and the cell pellet was washed twice with 25 ml of TE buffer (pH 7.0; 10 mM Tris-HCl, 1 mM EDTA). Cells were suspended in 5 ml of TE buffer and stored at 4°C. *S. cerevisiae* YPH499 cells were prepared by inoculating an isolated colony into 50 ml of YPD and then growing at 28°C for 24 h. As described for *C. albicans*, cultures were harvested, washed, and stored in 5 ml of TE buffer. Similarly, transformed *S. cerevisiae* YPH499 harboring pGK110 and pGK119 was grown in 50 ml of YPD at 28°C for 24 h. *S. cerevisiae* YPH499 harboring pGK114, pGK117, and pGK118 was grown in 50 ml of YPGal for 60 to 70 h at 28°C. Cells were harvested, washed, and stored in TE buffer as described for *C. albicans*.

**Adherence assay.** The adherence assay is based on a method that we had previously developed to isolate the *C. albicans* ALAI gene (9). We have now made further modifications in this method which enables us to perform a quantitative adherence assay measuring adherent cells with or without aggregation. The method utilizes ECM-coated, i.e., FN-laminin (LM)-, and type IV collagen (COL IV)-coated, magnetic beads to selectively isolate *C. albicans* and *S. cerevisiae* expressing ALAI. BSA is used to mask unoccupied sites according to the manufacturer’s directions. Masking the unoccupied sites with ethanolamine produced the same results as did BSA; thus, the interactions were with the ECM proteins. The adherence assay was performed in a 15-ml glass tube to which 940 μl of TE buffer (pH 7.0), 10 μl of ECM-coated magnetic beads (final concentration, ~10^7 beads/ml), and 50 μl of cell suspension (final concentration, ~10^9 cells/ml) were added in that order. *C. albicans* at a concentration of ~10^7 cells/ml was determined to be saturating under these conditions since adherence remained constant at higher cell concentrations (results not shown). After an immediate mixing by vortexing, the glass tube was placed on an end-to-end shaker and incubated at room temperature for 30 min with moderate shaking. The mixture was vortexed for 10 to 15 s; the tube was then immediately placed in a magnetic separator to attract magnetic beads on the side, and the supernatant was carefully removed. The separated magnetic beads were washed with 1 ml of TE by adding it along the wall while keeping the tube in the magnet. The supernatant was carefully removed, and magnetic beads were similarly washed with 1 ml of TE while keeping the tube in the magnetic separator. The separated magnetic beads and adherent cells were washed three times with TE as described above and, after the last wash, the cells and magnetic beads were suspended in an appropriate volume of 0.1 N NaOH (0.1 to 2.0 ml). Suspension in NaOH dissociates adherent cells from the magnetic beads. Cells and magnetic beads in each sample were counted four times with a hemacytometer.

**Statistics.** Experiments involving the quantitation of cells were repeated a minimum of three times for each variable. Standard deviation was used as an expression of variation. Comparisons of the means were made by using the Student’s t test, and a P value of <0.05 was considered significant.

**RESULTS**

**Attachment of yeast cells to ECM-coated magnetic beads.** The method utilizes ECM-coated, i.e., FN-laminin (LM)-, and type IV collagen (COL IV)-coated, magnetic beads to selectively isolate *C. albicans* and *S. cerevisiae* expressing ALAI. (i) *C. albicans*. To gain insight into the relationship between attachment and aggregation, we investigated the kinetics of *C. albicans* adherence. Samples were taken at increasing times of incubation. The curve of *C. albicans* binding to FN-coated magnetic beads is shown in Fig. 3. A rapid increase in adherence was observed for the initial 5 min of incubation. The second and slower phase of adherence occurred from 5 to 20 min of incubation. No significant increase in adherence was observed after 20 min of incubation. Samples taken at different times were also observed microscopically. During the first several minutes, cells rapidly attached to magnetic beads but no aggregates formed (the attachment phase). In the second, slower phase, aggregates formed and became increasingly larger in size (the aggregation phase). No aggregation of cells occurred if FN-coated magnetic beads were not added during incubation. Similarly, no aggregation occurred if uncoated magnetic or polystyrene beads of different sizes were incubated with cells. The kinetics of adherence suggest (Fig. 3) and the microscopic observations confirm that the adherence of *C. albicans* cells to an FN-coated magnetic bead is the sum of two distinct steps: attachment (cell-bead interaction) and aggregation (cell-cell interaction). Incubation of *C. albicans* with FN-coated magnetic beads resulted in an average of four to six cells per bead, and aggregates of different sizes can be found (Fig. 4a).

(ii) *S. cerevisiae* harboring a low- or high-copy ALAI plasmid. *S. cerevisiae* harboring the original vector plasmid without ALAI, i.e., pAUR112, did not attach to or aggregate with ECM-coated magnetic beads in any way (Fig. 4b). As reported in our previous study (9), *S. cerevisiae* harboring pGK100, a low-copy ALAI plasmid, exhibited attachment but no appreciable aggregation (Fig. 4c). We interpreted these results to be...
due to the low level of \textit{ALA1} expression in \textit{S. cerevisiae} compared to \textit{C. albicans}. \textit{pGK100} is a low-copy plasmid in \textit{S. cerevisiae} and may not have enough gene copies to produce sufficient amounts of Ala1p necessary to exhibit the aggregation phenotype. Therefore, we constructed a high-copy plasmid, \textit{pGK119}, by cloning \textit{ALA1} in \textit{pRS426} that uses a high-copy (2 \textit{m}) replicon in \textit{S. cerevisiae} (Fig. 1). In contrast to \textit{pGK100}, \textit{S. cerevisiae} harboring \textit{pGK119} exhibited attachment and aggregation phenotypes (Fig. 4d). Quantitative measurement of adherence indicated that expression of \textit{ALA1} from a high-copy plasmid (\textit{pGK119}) resulted in an approximately threefold increase in adherence compared to the adherence of \textit{S. cerevisiae} carrying the low-copy plasmid, \textit{pGK100} (Fig. 5). However, the increase in adherence was not proportional to the expected increase in plasmid copy number, which is at least 20-fold higher for \textit{pGK119} than for \textit{pGK100} (12). This suggested that transcription from the \textit{ALA1} promoter might be regulated in \textit{S. cerevisiae}.

\textbf{(iii) \textit{S. cerevisiae} expressing \textit{ALA1} from a series of galactose-inducible promoters.} In order to solve the problem described above, we investigated the positive effect of increased Ala1p levels on the aggregation phenotype by replacing the putative \textit{ALA1} promoter with a series of galactose-inducible promoters (GAL1, GALL, and GALS) (Fig. 1). GAL1 is a galactose-inducible promoter that contains three upstream activating sequences (UAS1, UAS2, and UAS3) (24). Deletion of UAS3 yields GALL, and deletion of UAS3 and one-half of UAS2 yields GALS. Therefore, GALS is the weakest promoter, GALL is a moderate promoter, and GAL1 is the strongest promoter.

Adherence to FN-coated magnetic beads of \textit{S. cerevisiae} expressing \textit{ALA1} from a series of galactose-inducible promoters is shown in Fig. 5. As expected, there was no adherence in all three \textit{ALA1} fusions when yeast cells were grown in a medium containing glucose as the sole carbon source. \textit{C. albicans} adherence was similar with either glucose or galactose as the carbon source (results not shown). However, when glucose was replaced with galactose, \textit{S. cerevisiae} harboring the \textit{pGK117}, \textit{pGK118}, and...
pGK114 plasmids exhibited adherence directly correlated with the strength of the GAL promoter (Fig. 5). This finding is corroborated by Fig. 6, demonstrating that the amount of surface-extractable Ala1 from yeast cells is directly correlated with the strength of the GAL promoter. Aggregation after attachment of \textit{S. cerevisiae} harboring pGK114 to FN-coated magnetic beads is shown in Fig. 4e and f. \textit{S. cerevisiae} harboring pGK114 also attached and aggregated on human buccal epithelial cells (data not shown). As shown in Fig. 5, \textit{S. cerevisiae} harboring pGK114 had more adherent cells (attached and aggregated) than did \textit{C. albicans}. This may be due to the greater amount of Ala1p on the surface of the transformant than on \textit{C. albicans}, thus resulting in greater adherence. The relationship of cell-surface Ala1p detected by Western blot assay and cell-surface fluorescence microscopy, demonstrated a direct correlation of the amount of cell surface protein detected with the intensity of the reaction proportional to the strength of the GAL promoter, as shown in Fig. 6. It is interesting that fluorescence did not occur on the cell surface using the antibody to the IgG domain. Lane 1, molecular mass markers at 205 and 118 kDa; lane 2, \textit{S. cerevisiae} with the original vector alone (pAUR112, i.e., there is no ALA1); lanes 3, 4, and 5, supernatants of strains of \textit{S. cerevisiae} harboring pGK117, pGK118, and pGK114 representing low, medium, and strong GAL promoters, respectively. Ala1p is marked by an arrow and is ca. 135 kDa.

**Fig. 6.** Immunoblot of Ala1p antigen extracted from the surface of cells demonstrating a direct correlation of the amount of cell surface protein detected and the strength of the GAL promoter. Nitrocellulose was blotted with the antibody to the peptide from the IgG domain. Lane 1, molecular mass markers at 205 and 118 kDa; lane 2, \textit{S. cerevisiae} with the original vector alone (pAUR112, i.e., there is no ALA1); lanes 3, 4, and 5, supernatants of strains of \textit{S. cerevisiae} harboring pGK117, pGK118, and pGK114 representing low, medium, and strong GAL promoters, respectively. Ala1p is marked by an arrow and is ca. 135 kDa.

**Table 1.** Relationship between the expression of \textit{ALA1} as determined by the amount of adherence and aggregation of \textit{S. cerevisiae} transformants to FN-coated magnetic beads and the detection of surface Ala1p by Western blot assay and cell-surface fluorescence microscopy.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Amt with \textit{S. cerevisiae} plasmid:</th>
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<tr>
<td>Attachment to beads</td>
<td>pAUR112</td>
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<tr>
<td>Aggregation of cells</td>
<td>–</td>
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<tr>
<td>Western blot detection with antibody to IgG domain</td>
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<td>Western blot detection with antibody to S/T domain</td>
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<tr>
<td>Surface fluorescence using antibody to IgG domain</td>
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<td>Surface fluorescence using antibody to S/T domain</td>
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* For the immunoglobulin G (IgG) and serine-threonine (S/T) domains of Ala1p, see Fig. 2. Qualitative estimates of the amount of aggregation range from none (–) to extensive, involving hundreds of yeast cells (++++), and qualitative estimates of the amount of protein range from none (–) to the maximum amount (+++) (see Fig. 6).

The conclusion from these experiments is that the magnitude of adherence is directly correlated with the plasmid copy number and the strength of the galactose-inducible promoter driving the \textit{ALA1} expression.

**Fig. 7.** Effect of pH on adherence and aggregation of \textit{C. albicans} and \textit{S. cerevisiae} overexpressing \textit{ALA1}. Since overexpression of \textit{ALA1} in \textit{S. cerevisiae} causes aggregation of cells after attachment to FN-coated magnetic beads and human buccal epithelial cells morphologically similar to \textit{C. albicans}, we decided to investigate additional adherence properties.

(i) **Resistance to shear forces.** While developing this assay, we observed an unusual stability of \textit{C. albicans} adherence to FN-coated magnetic beads. The adherent cells were resistant to shear forces generated by vortex mixing at full speed. We therefore compared the stability of adherence of \textit{S. cerevisiae} harboring pGK114, pGK117, and pGK100 with that of \textit{C. albicans}. In this experiment, tubes containing cells adherent to FN-coated magnetic beads were continuously vortexed at full speed for different times. \textit{C. albicans} cells remained adherent to the beads after up to 5 min of vortexing. Similarly, \textit{S. cerevisiae} cells expressing \textit{ALA1} from pGK114, pGK117, or pGK100 remained adherent to the beads after up to 5 min of vortexing. Interestingly, resistance to shear forces was similar in \textit{S. cerevisiae} harboring pGK114 and pGK100, which formed the most and the least aggregates, respectively. These results suggest that the stability of adherence is not affected by the level of Ala1p although, as shown, aggregation is proportional to the levels of Ala1p.

(ii) **Effect of pH.** We next compared the adherence properties of \textit{C. albicans} and \textit{S. cerevisiae} harboring pGK114 at different pH values. As shown in Fig. 7, both organisms exhibited adherence to FN-coated magnetic beads over a wide pH range. In both cases, significant adherence activity was detected at neutral and acidic pH values, and the activity rapidly declined at pH 9 or higher. Both fungi exhibited broad pH optima for adherence, i.e., from pH 4 to pH 8.

(iii) **Adherence to various ECM-coated magnetic beads.** We also compared the adherence of \textit{C. albicans} and \textit{S. cerevisiae
C. albicans maintains long-term commensal relationships with a number of hosts presumably, in part, by its ability to adhere to a variety of biological surfaces. It can be cultured from mucosal surfaces with great ranges in pH values. The residence of C. albicans on these mucosal surfaces is resistant to competition from other microorganisms, the presence of a variety of potentially competing molecules in biological fluids, and the shear forces generated by the flow of body fluids (26). In this report we characterize a form of C. albicans adherence consisting of two sequential steps: an attachment phase followed by an aggregation phase. This type of adherence is noteworthy for its occurrence in acidic to neutral pH ranges, its resistance to strong shear forces, and the presence of numerous competing molecules and applies to human cells and proteins, alike. It is possible that this type of adherence endows C. albicans with the ability to maintain a long-term commensal relationship with the host.

Our results imply that the expression of ALA1 and ALA1-like genes is responsible for a type of adherence in C. albicans characterized by attachment and aggregation of yeast cells. The presence of the ALS gene family in C. albicans is inferred by the hybridization of multiple genomic DNA fragments to an ALS family-specific probe (15). The complete sequences of three members of the gene family, ALA1, ALS1, and ALS3, have been reported, but only ALA1 and ALS1 have been functionally characterized (6, 9). We isolated ALA1 by its ability to confer adherence properties on S. cerevisiae transformants for ECM-coated magnetic beads (9), and Fu et al. isolated ALS1 by its ability to cause S. cerevisiae transformants to adhere to endothelial and epithelial cells when the gene was expressed from a galactose-inducible promoter (6). We have found that ALS1 behaves like ALA1 in our assay (unpublished results). This adherence function appears to be reserved for Candida agglutinin-like proteins, since S. cerevisiae expressing α-agglutinin (encoded by JAG1, to which Ala1p shows similarity in the N terminus) does not adhere to ECM-coated magnetic beads (unpublished results).

Adherence of C. albicans to the ECM proteins has been well

**DISCUSSION**

C. albicans maintains long-term commensal relationships with a number of hosts presumably, in part, by its ability to adhere to a variety of biological surfaces. It can be cultured from mucosal surfaces with great ranges in pH values. The residence of C. albicans on these mucosal surfaces is resistant to competition from other microorganisms, the presence of a variety of potentially competing molecules in biological fluids, and the shear forces generated by the flow of body fluids (26). In this report we characterize a form of C. albicans adherence consisting of two sequential steps: an attachment phase followed by an aggregation phase. This type of adherence is noteworthy for its occurrence in acidic to neutral pH ranges, its resistance to strong shear forces, and the presence of numerous competing molecules and applies to human cells and proteins, alike. It is possible that this type of adherence endows C. albicans with the ability to maintain a long-term commensal relationship with the host.

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Adherence of C. albicans to the ECM proteins has been well

**FIG. 8.** Comparison of adherence of C. albicans and S. cerevisiae harboring pGK114 or pGK117 to magnetic beads coupled with the indicated proteins.

**FIG. 9.** Effects of different additives on the attachment and aggregation of S. cerevisiae harboring pGK114. Additives were included in the adherence assay before the addition of yeast cells. The final concentrations of the additives were as follows: 1% each of D-glucose, D-galactose, or D-mannose; 1 M NaCl; 100-μg/ml concentrations of COL IV, LM, and FN; 50% formamide; 6 M urea; 100 mM Tris-HCl (pH 12.0). Data for l-fucose (1%) and Tween 20 at 0.1% (vol/vol) are not shown, but neither caused the inhibition of adherence or aggregation. The error bar equals one standard deviation from the mean. Significant differences (P < 0.05) from the control mean value (None) are marked with asterisks.
described by numerous groups and obviously encompasses the attachment step we describe. However, the aggregation of adherent \textit{C. albicans} is also a well-recognized phenomenon, particularly in in vitro experiments (21, 22). The term aggregation has been used to describe the interaction of \textit{C. albicans} germ tubes with one another (14, 27), and the term coaggregation has been used to describe yeast cells adhering to bacteria (16). Here, the term aggregation is reserved for yeast cell-cell interactions which occur when the microorganisms are already attached to a substrate. Cell-cell interactions that occur in suspension are more appropriately termed flocculation. Aggregation of \textit{C. albicans} has been noted with such adherence targets as ex vivo porcine endothelium (19), human intestinal epithelial cells (22), and human buccal epithelial cells (for a full discussion, see reference 25). Aggregation as it occurs in our assay is the second of two steps in the adherence of cells to a target. First, cells attach to an appropriate target, such as an ECM-coated magnetic bead. The attachment of cells to these beads is a transitional step that, with or without the second step, culminates in what is measured as adherence. For example, \textit{S. cerevisiae} harboring pGK100 apparently does not express sufficient amounts of Ala1p, and thus, cells harboring this low-copy plasmid attach to the beads but do not aggregate. However, when cells express sufficient amounts of Ala1p, both attachment and aggregation occur and the high levels of Ala1p increase the magnitude of adherence by increasing the extent of aggregation. Not all forms of cell adherence lead to aggregation. For example, adherence of \textit{C. albicans} to uncoated plastic beads, cationic beads, or an oil-water interface does not result in aggregation (17, 20). The implication is that the form of adherence that we describe here, involving attachment and aggregation mediated by Ala1p, somehow involves specific recognition of the target surface by the cells.

Aggregation as described here is different from flocculation, a form of yeast cell-cell interaction governed by cell surface lectins and dependent upon the presence of divalent cations (30). The strain of \textit{S. cerevisiae} used in our studies is readily induced to flocc by the addition of Ca$^{2+}$, and this is reversed upon the addition of EDTA. However, even when flocculation is induced in \textit{S. cerevisiae} by the addition of Ca$^{2+}$, the cells do not interact in any way with the ECM-coated magnetic beads. However, in flocculation, due to the sheer mass of the microorganisms forming a floc, cells may become physically entrapped and thus masquerade as adherent microorganisms, although this form of interaction did not occur in our assay.

The attachment step of adherence in our assay is due solely to the presence of Ala1p on the surface of the cell—without this protein \textit{S. cerevisiae} is unable to adhere to the protein-coated beads. The domain of Ala1p involved in attachment is unknown. Similarly, how Ala1p affects aggregation is unknown. Perhaps aggregation is due to Ala1p-Ala1p interactions of separate cells or, alternatively, to the Ala1p-non-Ala1p cell wall interactions of adjacent cells. It is also possible that different domains of Ala1p are involved in adherence, one for attachment and another for aggregation.

The adherence properties of \textit{C. albicans} and \textit{S. cerevisiae} overexpressing ALA1 are very similar. In both cases adherence can be observed over a wide pH range and is resistant to strong shear forces and to exogenously added competing molecules, such as sugars, free ligands, and detergents. It is likely that \textit{C. albicans} on certain occasions utilizes ALA1 or ALA1-like genes to effect adherence to a host surface. The properties of ALA1-mediated adherence suggest that this mechanism is well suited to maintaining long-term residence in the host. The aggregation step may assist in the colonization of the host by recruiting cells to interact with small numbers of cells that have previously attached to tissue (26).

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REFERENCES


