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Expression, Cloning, and Characterization of a *Candida albicans* Gene, *ALA1*, That Confers Adherence Properties upon *Saccharomyces cerevisiae* for Extracellular Matrix Proteins

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Adherence of *Candida albicans* to host tissues is a necessary step for maintenance of its commensal status and is likely a necessary step in the pathogenesis of candidiasis. The extracellular matrix (ECM) proteins are some of the host tissue and plasma proteins to which *C. albicans* adheres through adhesins located on the fungal cell surface. To isolate genes encoding ECM adhesins, an assay was developed based on the ability of yeast cells to adhere to magnetic beads coated with the ECM protein fibronectin, type IV collagen, or laminin. A *C. albicans* genomic library was constructed by cloning *Xba*I-partially-digested and size-selected fragments into pAUR112, an *Escherichia coli*-yeast low-copy-number shuttle vector. The *C. albicans* library was transformed into *Saccharomyces cerevisiae* YPH 499, and clones capable of adherence were selected by using ECM protein-coated magnetic beads. A plasmid containing an ~8-kb insert was isolated from 29 adherent clones. These clones exhibited adherence to all ECM protein-coated magnetic beads and to human buccal epithelial cells. The *ALA1* gene (for agglutinin-like adhesin) was localized by subcloning it into a 5-kb *Xba*I fragment which retained the adherence phenotype in both orientations. The complete DNA sequence of the 5-kb insert was determined, and an open reading frame (ORF) encoding 1,419 amino acid residues was identified. Deletions from the 5′ and 3′ ends extending into the DNA sequence encoding the 1,419-amino-acid ORF product inactivated the adherence phenotype, suggesting that it is the coding region of the *ALA1* gene. A database search identified ALA1 to be similar to the *C. albicans* ALS1 (for agglutinin-like sequence 1) protein and the *S. cerevisiae* agglutinin protein (AGα1), although the homology at the primary amino acid sequence level is limited to the first half of each of these proteins. ALA1 contains a central domain of six tandem repeats of 36 amino acids. We discuss the significance of various predicted ALA1 structural motifs and their relationships to function in the adherence process.

Adherence of *Candida albicans* to a variety of biological surfaces is an important step in the establishment of a commensal relationship with the host (5, 22). It is also important for long-term survival of the fungus and likely to be important for subsequent steps in the pathogenesis of candidiasis (14). Several cell surface adhesins have been identified and characterized biochemically (7, 11, 16). One or several of these surface proteins may play a role in the adherence of *C. albicans* to extracellular matrix (ECM) proteins and host cell surfaces (15). The results of previous studies of *C. albicans* adherence are difficult to interpret with certainty because *C. albicans* probably possesses multiple adhesins interacting with ligands in a variety of ways. This apparent redundancy of adherence molecules in *C. albicans* complicates interpretations of many biochemical results. In recent years genetic and molecular biology approaches have facilitated the investigation by allowing the adhesins to be studied in isolation in heterologous hosts.

Two *C. albicans* adherence genes, *AAF1* and α*INT1*, have been cloned and partially characterized. Barki and coworkers described the first *C. albicans* gene, *AAF1*, which was cloned based upon its ability to confer upon *Saccharomyces cerevisiae* the ability to adhere to polystyrene (4). This gene was also found to be responsible for autoaggregation and adherence to buccal epithelial cells. The AAF1 protein was localized to the *C. albicans* cell surface by immunofluorescence (3). The second *C. albicans* adherence gene, α*INT1*, was cloned by screening with a probe derived from the transmembrane region of a human α-integrin gene (10). Interestingly, expression of α*INT1* from a galactose-inducible promoter in *S. cerevisiae* resulted in the formation of morphological forms similar to *C. albicans* germ tubes. These germ tube-like structures in *S. cerevisiae* showed enhanced aggregation equal to that of *C. albicans* germ tubes. Polyclonal antibodies raised against a specific region of αINT1 recognized 64 to 82% of *C. albicans* yeast cells, indicating a surface localization of the protein (10).

In this report, we describe the cloning of a new *C. albicans* adherence gene, *ALA1*, that transforms *S. cerevisiae* YPH 499 into a strain capable of adhering to various ECM proteins and human buccal epithelial cells. This was accomplished by the development of a screening method that discriminates between *C. albicans* and *S. cerevisiae* YPH 499 with respect to their abilities to adhere to ECM protein-coated magnetic beads. The deduced amino acid sequence of *ALA1* suggests homology to that of the *S. cerevisiae AGα1* gene, which mediates cell-cell interaction between opposite-mating-type yeast cells (20). *ALA1* also has striking homology with the *C. albicans ALS1* gene (12). We discuss the significance of these homologies and possible functional relationships of ALA1 with other eukaryotic cell adhesion proteins.

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MATERIALS AND METHODS

Yeast strains and plasmids. The *C. albicans* isolate was obtained from a human source and has been used extensively in this laboratory, including in studies of the adherence of the fungus to ECM proteins (18). *S. cerevisiae* YPH 499 (*MAT***a** *ura3-52 lys2-801*^{amber} *ade2-101*^{ochre} *trp1-Δ63 his3-Δ200 leu2-Δ1*) was obtained from the American Type Culture Collection. Both isolates were maintained as frozen stocks in 20% glycerol. Yeast cells were cultured in Sabouraud dextrose broth containing neopeptone and dextrose (Difco, Detroit, Mich.), yeast extract-peptone-dextrose (YEPD) (Sigma, St. Louis, Mo.), or Minimal Synthetic Defined Base (SD) (Clontech, Palo Alto, Calif.) with appropriate supplements for 2 to 4 days at 30°C. Germ tubes were induced in *C. albicans* by placing approximately 10^6 yeast cells/ml in Medium 199 (GIBCO, Grand Island, N.Y.) and incubating them at 37°C for 2 h (24). A predominant growth of pseudohyphae was obtained by culturing yeast cells in RPMI (GIBCO) at 30°C for 24 h (1). Plasmid pAUR112 DNA was purchased from PanVera Corporation, Madison, Wis., and was used for construction of the genomic library. The pAUR112 plasmid is an *Escherichia coli*-yeast low-copy-number shuttle vector which selects for uracil and resistance to aureobasidin A in yeast cells.

***C. albicans* genomic library.** Genomic DNA from *C. albicans* was prepared from an actively growing culture in YEPD with the QIAGEN genomic DNA isolation kit (QIAGEN Inc., Chatsworth, Calif.). Lysis of yeast cells was performed with lyticase to which RNase A and proteinase K were added. The genomic DNA was purified with QIAGEN genomic-tip 500/G columns according to the vendor's instructions. The high-molecular-weight DNA (~50 kb) was partially digested with *Xba*I, and DNA fragments of 6 to 10 kb were isolated from low-melting-point agarose gel with the QIAGEN gel extraction kit. DNA fragments were ligated into pAUR112 that had been completely digested with *Xba*I and dephosphorylated. The ligated mixture was then transformed into competent *E. coli* (DH5α) cells, and aliquots were plated on Luria-Bertani plates containing 0.5% yeast extract, 1% Bacto Peptone, and 1% NaCl, to which was added 100 μg of ampicillin per ml. Analysis of the plasmid DNA from these transformants indicated that most of the plasmids (>90%) contained unique inserts. The remaining transformation mixture was inoculated into 50 ml of Luria-Bertani broth containing ampicillin and grown overnight at 37°C. Plasmid DNA was prepared from these cells with the QIAGEN plasmid isolation kit and served as a source for the *C. albicans* genomic DNA library. The quality of the library was tested by transforming DNA into *S. cerevisiae* YPH 499 by the lithium acetate method (13) and scoring colonies capable of growing without uracil or lysine. With the vector containing the *URA3* gene, the number of colonies on plates without uracil indicates the frequency of transformation, whereas the number of colonies on plates without lysine indicates *lys2*-complementing cloned inserts in the plasmid. This analysis indicated that approximately 2.4% of the plasmids contained *lys2*-complementing *C. albicans* inserts in the genomic library.

Magnetic beads. Tosyl-activated magnetic beads (Dynal, Lake Success, N.Y.) were coupled with fibronectin (FN), laminin (LM), and type IV collagen (COL IV) (Collaborative Research, Cambridge, Mass.) according to the manufacturer's instructions. Briefly, about 200 μg of each ECM protein was coupled to ~10^9 magnetic beads by incubation at 37°C for 15 min, followed by the addition of 0.2% bovine serum albumin and incubation for another 16 h. ECM protein-coated beads were washed according to manufacturer's instructions and suspended in phosphate-buffered saline. The beads were stored at a concentration of ~10^8 beads/ml at 4°C until use.

Selection of adherent clones. Following transformation of *S. cerevisiae* YPH 499 with a *C. albicans* genomic library as described above, an aliquot was removed and plated on SD agar without uracil to determine the transformation frequency. The remaining transformants were each transferred to 100 ml of YEPD and cultured overnight at 30°C. The yeast cells were pelleted and washed by centrifugation with Earle's balanced salt solution (EBSS) (GIBCO). A cell suspension was then prepared by adding 4 ml of EBSS to the washed pellet. Into 15-ml glass tubes were added 0.9 ml of the cell suspension and 0.1 ml of ECM protein-coated beads. The glass tubes were incubated at room temperature for 30 min on an end-to-end shaker. The beads were separated by placing the glass tube in a magnet, and the supernatant was removed. The beads were then suspended in 1 ml of EBSS and transferred to a new glass tube. The tubes were incubated on the shaker for another 10 min, and the beads were separated and placed into a new glass tube. This washing procedure was repeated three times. After the last wash the beads were suspended in 0.1 ml of EBSS, plated onto SD agar without uracil, and incubated at 30°C for 2 to 4 days. The CFU on the plate were considered the potentially adherent clones and further analyzed as follows.

Adherence assay. The potentially adherent *S. cerevisiae* YPH 499 clones were screened further in the following manner. Each colony was cultured in 1 ml of YEPD containing 200 μg of aureobasidin A per ml for 18 h at 30°C. Into the wells of a 24-well tissue culture tray were placed 10 μl of yeast culture, 2 μl of ECM protein-coated beads, and sufficient EBSS to make a 200-μl suspension (the yeast cell concentration was ~10^6). The tissue culture tray was then incubated at room temperature for 30 min on a gyratory shaker. Each well was then observed by inverted microscopy for the presence of yeast cells adhering to the ECM-coated beads. *C. albicans* and *S. cerevisiae* were always included as positive and negative controls, respectively. Clones that showed adherence in the above assay were again tested for the ability to adhere to beads in the glass tube assay. Following the washes as described above, an aliquot was placed under the microscope to verify that adherence to beads was occurring. Only at this point were clones said to be positive for adherence. In order to determine the adherence of the clones to buccal cells the following procedure was performed. Human buccal epithelial cells were removed with a tongue depressor, washed three times in EBSS, resuspended with yeast cells (at an approximate ratio of 10 yeast cells to 1 buccal epithelial cell), and incubated at room temperature for 30 min on an end-to-end shaker. An aliquot was removed and observed under the microscope.

DNA sequencing and computer analysis. DNA sequencing of the 5-kb *Xba*I insert carrying the functional *ALA1* gene was performed by fluorescent dye terminator labeling and primer walking methods on an ABI sequence analyzer (ACGT, Inc., Northbrook, Ill.). A double-stranded template was prepared by using the QIAGEN plasmid kit, and sequencing primers were custom synthesized. DNA and protein sequences were analyzed by Vector NTI (InforMax, Gaithersburg, Md.) and GCG (Genetics Computer Group) software. A homology search of GenBank databases at the National Center for Biotechnology Information was performed with the BLAST algorithm.

Construction of plasmid deletions. An *Eco*RI deletion in the 5-kb *ALA1* insert was constructed by complete digestion of pGK102 with *Eco*RI, ligation, and transformation into competent *E. coli* DH5α cells. A *Kpn*I deletion was constructed in a similar way in plasmid pGK103. Both of these deletions inactivate the aureobasidin A gene but retain the functional *URA3* gene. A *Sal*I deletion was constructed by subcloning the *ALA1* 4-kb *Sal*I fragment from pGK100 into *Sal*I-cut pAUR112, resulting in construction of two plasmids (pGK106 and pGK107) carrying the insert in both orientations. pAUR112 contains a unique *Sal*I site adjacent to an *Xba*I cloning site.

Nucleotide sequence accession number. The complete DNA sequence of the *ALA1* gene has been deposited in GenBank under accession no. AF025429.

RESULTS

Adherence assay. To isolate *C. albicans* adherence genes, it was necessary to develop a method that could discriminate between *C. albicans* and *S. cerevisiae* yeast cells. We approached this problem by using ECM protein-coated magnetic beads to which *C. albicans* adhered but *S. cerevisiae* did not. As shown in Fig. 1a, *C. albicans* yeast cells adhere avidly to FN- , LM- , and COL IV-coated magnetic beads, forming large aggregates containing beads and yeast cells. *S. cerevisiae* YPH 499 yeast cells alone or with plasmid pAUR112 do not adhere to any of the ECM protein-coated beads (Fig. 1b). It has been observed previously that *C. albicans* yeast cells aggregate when they adhere to various surfaces (17). In contrast, *S. cerevisiae* YPH 499 autoaggregated but did not adhere to ECM protein-coated magnetic beads (results not shown). Autoaggregation was due, in part, to the increased Ca^{2+} concentration. *C. albicans* germ tubes and pseudohyphae also adhered to ECM protein-coated magnetic beads (Fig. 2a and b).

Cloning of the *ALA1* gene. A *C. albicans* genomic library was prepared in a low-copy-number *E. coli*-yeast shuttle vector, pAUR 112, which contained 2.4% *lys2*-complementing plasmids. This library was transformed into *S. cerevisiae* YPH 499, and transformants were selected for the ability to adhere to ECM protein-coated magnetic beads. After confirming the adherence of the isolated clones, plasmids were recovered by growing them in *E. coli* and retransforming them into *S. cerevisiae* YPH 499, and the adherence phenotype was reconfirmed. The plasmid inserts of the adherent clones were analyzed by digestion with *Xba*I. All 29 clones which were adherent to ECM protein-coated beads contained 5- and 3-kb *Xba*I insert DNA fragments. A plasmid containing this 8-kb insert was designated pGK100. To localize the *ALA1* gene within the 8-kb insert, plasmid pGK100 was digested completely with *Xba*I and, after ligation, transformed into *S. cerevisiae* YPH 499, and the adherence phenotype of each transformant was determined (Fig. 3). Plasmid pGK101, containing a 3-kb *Xba*I fragment, did not confer the adherence phenotype on *S. cerevisiae* YPH 499, whereas plasmid pGK102, containing the 5-kb *Xba*I fragment, transformed *S. cerevisiae* YPH 499 into a strain capable of adhering to ECM-coated magnetic beads in a manner similar to pGK100 (Fig. 3). Plasmid

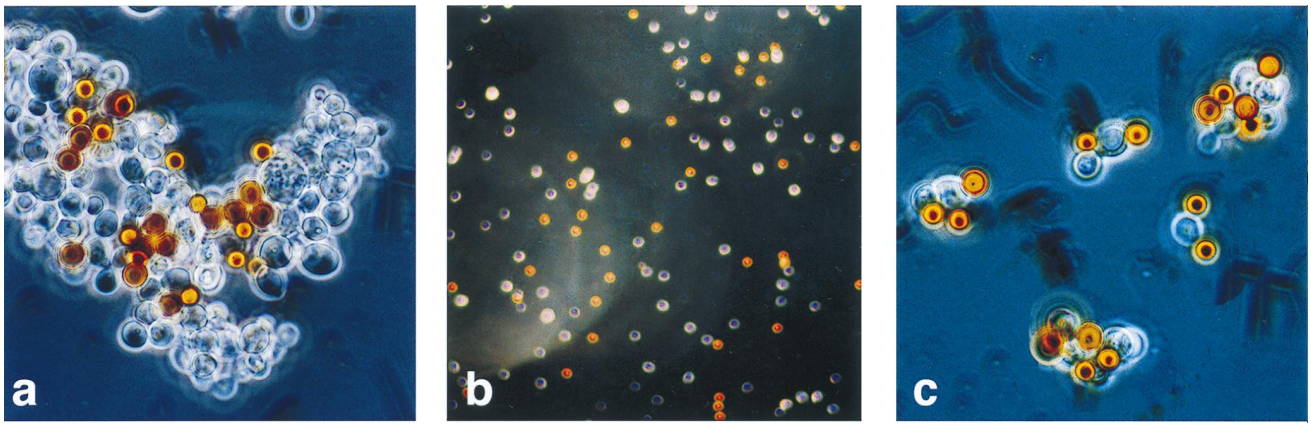

FIG. 1. Adherence of yeast cells to ECM protein-coated magnetic beads. (a) *C. albicans* adhering to and aggregating around COL IV-coated magnetic beads (orange-red spheres). (b) *S. cerevisiae* YPH 499 harboring the pAUR112 vector mixed with COL IV-coated magnetic beads. (c) pGK100 transformant of *S. cerevisiae* YPH 499 adhering to but not aggregating around COL IV-coated magnetic beads.

pGK103, containing the reverse orientation of the 5-kb *Xba*I fragment, was also able to confer an adherence phenotype on *S. cerevisiae* YPH 499, demonstrating that the 5-kb *Xba*I fragment contains the complete *ALA1* gene, including the regulatory sequences required for its expression.

Phenotypic characterization of transformants. Adherence properties of *S. cerevisiae* YPH 499 transformants were compared with those of *C. albicans* to determine similarities and differences. As shown in Fig. 1c, the *S. cerevisiae* YPH 499 transformant carrying plasmid pGK100, which expresses functional *ALA1*, is capable of adhering to COL IV-coated beads. Similar results were obtained with FN- and LM-coated beads. In contrast, the *S. cerevisiae* YPH 499 transformant carrying the vector plasmid, pAUR112, was incapable of adhering to any of the magnetic beads. It is interesting to note that the pGK100 transformant made very few and much smaller aggregates than did *C. albicans* when adhering to ECM protein-coated beads. We have consistently observed examples in the pGK100 transformants in which a single yeast cell was attached to multiple beads or, conversely, one magnetic bead was attached to multiple yeast cells. This observation suggests that *C. albicans* has additional determinants that allow it to make cell-to-cell contacts to form large aggregates after adhering to ECM protein-coated beads. The adherence of yeast cells to ECM protein-coated beads was also observed in Tris-EDTA buffer for both the pGK100 transformant and *C. albicans*. *S. cerevisiae* YPH 499 carrying the pAUR112 vector did not exhibit these properties even when it was induced to autoaggregate in the presence of a high Ca^{2+} concentration.

We next determined the adherence of yeast cells to human buccal epithelial cells. *C. albicans* yeast cells adhered to most buccal cells, mainly in large aggregates (Fig. 4a). The *S. cerevisiae* transformant carrying pGK100 also adhered to buccal cells but never formed aggregates as did *C. albicans* (Fig. 4b). *S. cerevisiae* YPH 499 transformants carrying pAUR112 did not adhere to buccal cells (results not shown).

DNA sequence and analysis of the predicted ALA1 protein. The complete DNA sequence of the 5-kb insert containing the functional *ALA1* gene consisted of 4,723 nucleotides. The translation initiation codon, ATG, was assigned because it was preceded two codons upstream by a termination codon, and two more termination codons were present within 100 nucleotides upstream in the same reading frame. Translation with this initiation codon predicted a 1,419-amino-acid peptide with a calculated molecular mass of 150,000 Da. A BLAST search of databases for homology revealed that the N termini (residues 1 to 657) of the deduced ALA1 protein and the *C. albicans* agglutinin-like sequence (ALS1) protein (12) are highly homologous, with a score of 2785, 78% identity, and 88% similarity in the amino acid sequence and no gap introduced

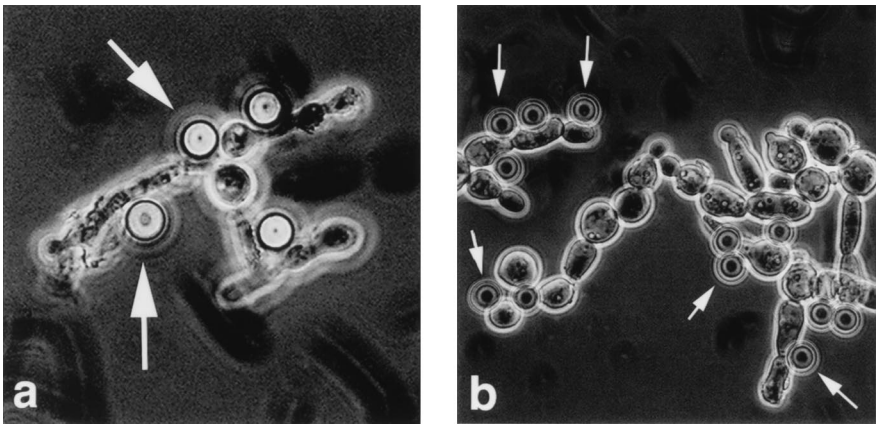

FIG. 2. Adherence of different *C. albicans* morphological forms to COL IV-coated magnetic beads. (a) *C. albicans* germ tubes. (b) *C. albicans* pseudohyphae. The arrows point to adherent magnetic beads.

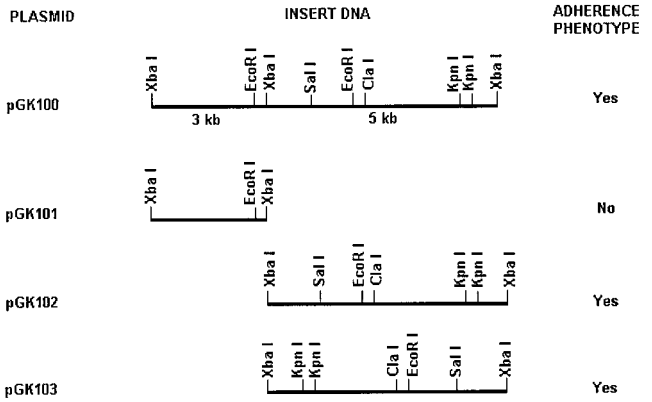

FIG. 3. Restriction map of cloned *ALA1* inserts and localization of *ALA1* to the 5-kb insert. The adherence phenotype of each plasmid in *S. cerevisiae* YPH 499 is shown on the right.

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ALA1:   1 MIQQFTLLFLYLSFATAKAITGIFNSIDSLTWSNAGNYAFKGPGYPTWNAVLGWSLDGTS
          M+QQFTLLFLYLS A+AK ITG+F+S +SLTWSNA NYAFKGPGYPTWNAVLGWSLDGTS
ALS1:   1 MLQQFTLLFLYLSIASAKTITGVFDSFNSLTWSNAANYAFKGPGYPTWNAVLGWSLDGTS

ALA1:  61 ANPGDTFILNMPCVFKFTASQKSVDLTADGVKYATCQFYSGEEFTTFSSLKCTVNNNLRS
          ANPGDTF LNMPCVFK+T SQ SVDLTADGVKYATCQFYSGEEFTTFS+L CTVN+ L+S
ALS1:  61 ANPGDTFTLNMPCVFKYTTSQTSVDLTADGVKYATCQFYSGEEFTTFSTLTCTVNDALKS

ALA1: 121 SIKALGTVTLPIAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGSKKLSIAVNFEKSTVDQ
          SIKA GTVTLPIAFNVGGTGSS DLEDSKCFTAGTNTVTFNDG K +SI V FEKSTVD
ALS1: 121 SIKAFGTVTLPIAFNVGGTGSSTDLEDSKCFTAGTNTVTFNDGDKDISIDVEFEKSTVDP

ALA1: 181 SGYLTTSRFMPSLNKIATLYVAPQCENGYTSGTMGFSTSYGDVAIDCSNVHIGISKGVND
          S YL  SR MPSLNK+ TL+VAPQCENGYTSGTMGFS+S GDVAIDCSN+HIGI+KG+ND
ALS1: 181 SAYLYASRVMPSLNKVTTLFVAPQCENGYTSGTMGFSSSNGDVAIDCSNIHIGITKGLND

ALA1: 241 WNHPVTSESFSYTKSCSSFGISITYQNVPAGYRPFIDAYISPSDNNQYQLSYKNDYTCVD
          WN+PV+SESFSYTK+C+S GI I YQNVPAGYRPFIDAYIS +D NQY L+Y NDYTC
ALS1: 241 WNYPVSSESFSYTKTCTSNGIQIKYQNVPAGYRPFIDAYISATDVNQYTLAYTNDYTCAG

ALA1: 301 DYWQHAPFTLKWTGYKNSDAGSNGIVIVATTRTVTDSTTAVTTLPFNPSVDKTKTIEILQ
             Q  PFTL+WTGYKNSDAGSNGIVIVATTRTVTDSTTAVTTLPFNPSVDKTKTIEILQ
ALS1: 301 SRSQSKPFTLRWTGYKNSDAGSNGIVIVATTRTVTDSTTAVTTLPFNPSVDKTKTIEILQ

ALA1: 361 PIPTTTITTSYVGVTTSYLTKTAPIGETATVIVDVPYHTTTTVTSEWTGTITTTTRTNP
          PIPTTTITTSYVGVTTSY TKTAPIGETATVIVDVPYHTTTTVTSEWTGTITTTTRTNP
ALS1: 361 PIPTTTITTSYVGVTTSYSTKTAPIGETATVIVDVPYHTTTTVTSEWTGTITTTTRTNP

ALA1: 421 TDSIDTVVVQVPLPNPTTTTTQFWSESFTSTTTITNSLKGTDSVIVREPHNPTVTTTEFW
          TDSIDTVVVQVP PNPT +TT++WS+SF +TTT+T    GTD+VI+REP N TVTTTE+W
ALS1: 421 TDSIDTVVVQVPSPNPTVSTTEYWSQSFATTTTVTAPPGGTDTVIIREPPNHTVTTTEYW

ALA1: 481 SESFATTETITSKPEGTDSVIVREPHNPTVTTTEFWSESYATTETITNGPEGTDSVIVRE
          S+SFATT T+T+ P GTDSVI+REP NPTVTTTE+WS+S+ATT T+T  P GTDSVI+RE
ALS1: 481 SQSFATTTTVTAPPGGTDSVIIREPPNPTVTTTEYWSQSFATTTTVTAPPGGTDSVIIRE

ALA1: 541 PHNPTVTTTKFWSESYATTETITNKPEGTDSVIVKEPYNPTVTTTEFWSESYATTETITN
          P NPTVTTT++WS+SYATT T+T  P GTDSVI++EP N TVTTTE+WS+SYATT T+T
ALS1: 541 PPNPTVTTTEYWSQSYATTTTVTAPPGGTDSVIIREPPNHTVTTTEYWSQSYATTTTVTA

ALA1: 601 GPEGTDSVIVREPHNPTVTTTEFWSESYATTETITTGPLGTDSIVIHDPLEELSSTT 657
           P GTD+VI+REP N TVTTTE+WS+S+ATT T+T  P GTD+++I +P     +TT
ALS1: 601 PPGGTDTVIIREPPNHTVTTTEYWSQSFATTTTVTGPPSGTDTVIIREPPNPTVTTT 657
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FIG. 5. BLAST analysis of ALA1 identified the N termini of ALA1 and ALS1 to be homologous. The deduced amino acid sequences of the N termini of both proteins are compared. Identity of the two proteins is indicated by a one-letter amino acid code, and similarity is indicated by + in the middle rows. The positions of the amino acid residues in the proteins are indicated on the left.

for the best alignment (Fig. 5). Like ALS1, the ALA1 N terminus has homology with the *S. cerevisiae* AGα1 protein (12). In the same search, homology at the primary amino acid sequence level did not extend to the C termini of ALA1 with ALS1 or AGα1. Using only the ALA1 C terminus, the search identified homology to proteins containing multiple serine and threonine residues. ALA1 conserves one of the unique features of ALS1, which is the presence of tandem repeats of 36 residues in the middle of the protein (Fig. 6a). ALA1, however, contains 6 tandem repeats, whereas ALS1 has 10 repeats (Fig. 6b). Although the ALA1 and ALS1 tandem repeats are very similar, there are some subtle differences. For example, the 10th residue in all six repeats of ALA1 is phenylalanine, whereas in ALS1 it is tyrosine. Similar differences exist for preferences in amino acids in tandem repeats of these proteins at positions 1, 3, 13, 19, 21, 23, 26, and 33. Another difference is that the residue at position 24 is a conserved proline in ALS1 but is variable in ALA1. Homology searches of databases with ALA1 tandem repeats revealed that they have 30 to 50% identity and 50 to 70% similarity to the *S. cerevisiae* FLO1 protein (Fig. 6c). FLO1 is required for flocculation in yeasts and contains four families of repeated sequences (25). The central region of the ALA1 tandem repeat shows homology to the N terminus of a 45-amino-acid sequence repeated 18 times in FLO1.

Deletion analysis of the *ALA1* gene. To demonstrate that the intact *ALA1* gene is required for the adherence phenotype in *S. cerevisiae* YPH 499, a series of deletions was constructed in plasmids carrying the functional *ALA1* gene (Fig. 7). An *Eco*RI deletion truncating the C terminus of the putative *ALA1* open reading frame (ORF) destroyed the adherence phenotype of plasmid pGK104. Similarly, even a smaller *Sal*I deletion of the C terminus in pGK107 completely destroyed the adherence

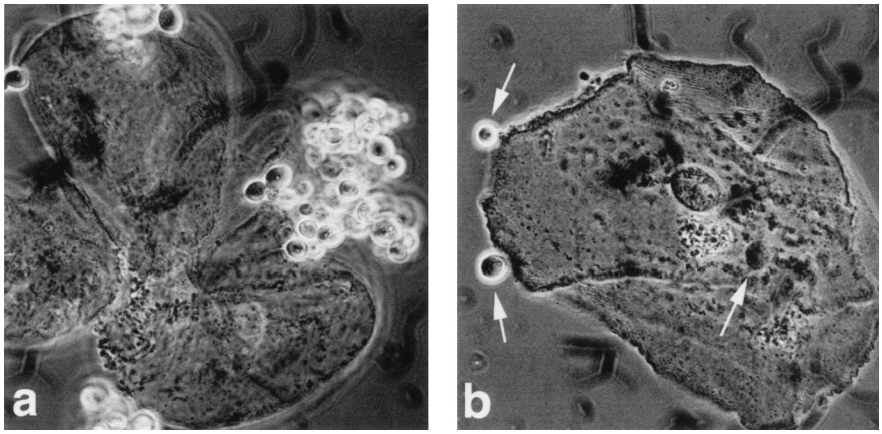

FIG. 4. Adherence to human buccal epithelial cells. (a) *C. albicans* yeast cells adhering to and aggregating on the surface and edges of an epithelial cell. (b) *S. cerevisiae* YPH 499 harboring *ALA1* adhering to but not aggregating on the surface and edges of an epithelial cell (arrows). *S. cerevisiae* YPH 499 with the pAUR112 vector did not adhere to epithelial cells.

(a)

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434  PNP TTTTTQFWSESFTSTTTITNSLKGTDSVIVREP
470  HNPTVTTTEFWSESFATTETITS KPEGTDSVIVREP
506  HNPTVTTTEFWSESYATTETITNGPEGTDSVIVREP
542  HNPTVTTTKFWSESYATTETITNKPEGTDSVIVKEP
578  YNPTVTTTE FWSESYATTETITNGPEGTDSVIVREP
614  HNPTVTTTE FWSESYATTETITTGPLGT DS IVIHDP

ALA1 Consensus
     HNPTVTTTE FWSESYATTETITNgPEGTDSVIVREP
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(b)

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ALS1  PN hTVTTTEYWSQSyATTTTTVTAPPGGTDtVLIREP
        N  TVTTT E+WS+ SYATT  T+T   P  GTD+VI+REP
ALA1  HNPTVTTTE FWSESYATTETITNgPEGTDSVIVREP
      1  3  5  7  9  11  13  15  17  19  21 23  25  27  29 31 33  35
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(c)

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FLO1          TTT ePW ngTFT STST E mTTvTGTNG l PT DETvIVIRTPT tattam
              T TTE  W   + +  +T  T       GT+  +
ALA1  HNPTV TTT EFWSESYATTET I TNgPEGT DSV IVREP
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FIG. 6. (a) The six ALA1 tandem repeats. Each repeat is composed of 36 amino acids. The position of the first amino acid residue in each repeat is shown at the left. The ALA1 consensus sequence derived from the comparison of the six tandem repeats is shown at the bottom. Identical residues are boldface uppercase letters; residues at positions having one mismatch are lightface uppercase letters; residues at positions with two mismatches are uppercase and underlined; and the residue at the position with a lowercase letter indicates that a majority of the tandem repeats possess that residue at that position. (b) Amino acid sequence alignment of the ALS1 (12) and ALA1 consensus sequences. Identity between the two sequences is indicated by a one-letter amino acid code, and similarity is indicated by + in the middle row. The numbers at the bottom denote the positions of amino acid residues in the tandem repeats. (c) Amino acid sequence alignment of the FLO1 (25) and ALA1 consensus sequences. Identity between the two sequences is indicated by a one-letter amino acid code, and similarity is indicated by + in the middle row. Amino acid sequences outside the box show no similarity.

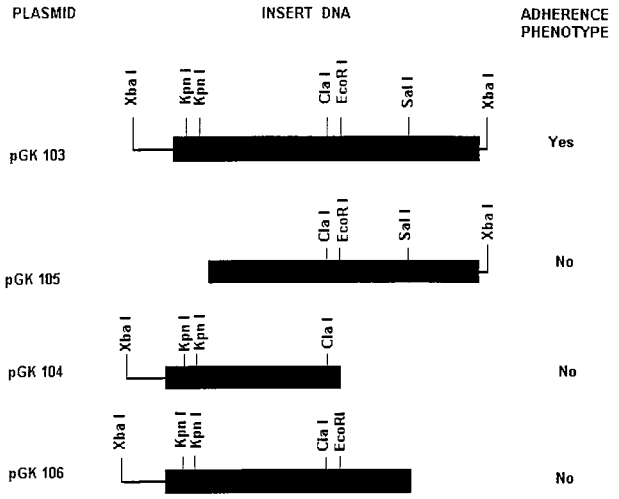

FIG. 7. Deletion derivatives of the 5-kb insert carrying functional *ALA1*. Each filled box indicates the *ALA1* ORF in pGK103 and the deletion derivatives. The adherence phenotype of the plasmid in *S. cerevisiae* YPH 499 is shown on the right.

phenotype. A *Kpn*I deletion removing the putative promoter and the N terminus also destroyed the adherence phenotype of plasmid pGK105. Taken together, these results suggest that the predicted *ALA1* ORF is responsible for conferring on *S. cerevisiae* YPH 499 adherence to various ECM proteins.

DISCUSSION

C. albicans is an opportunistic pathogen that has evolved highly developed mechanisms to adhere to a variety of biological surfaces to initiate and maintain a commensal relationship with the host. Several cell surface proteins which may provide *C. albicans* with this unique ability to adhere have been isolated. We describe in this paper an assay that discriminates between pathogenic *C. albicans* and a nonpathogenic *S. cerevisiae* strain with respect to their adherence properties. Using this assay, we have cloned in *S. cerevisiae* YPH 499 a *C. albicans* gene, *ALA1*, that allows the fungus to adhere to magnetic beads coated with ECM proteins and to human buccal epithelial cells. However, an important difference exists between wild-type *C. albicans* and an *ALA1* transformant of *S. cerevisiae* YPH 499 with respect to their adherence abilities. *C. albicans* yeast cells aggregate upon adherence to ECM protein-coated magnetic beads and human buccal epithelial cells, whereas *S. cerevisiae* YPH 499 *ALA1* transformants adhere but do not aggregate. Although *S. cerevisiae* YPH 499 can be induced to autoaggregate by increasing the Ca^{2+} concentration, it cannot be induced to adhere to ECM protein-coated magnetic beads or human buccal epithelial cells by adding Ca^{2+}. Adherence and aggregation, therefore, are two separate events.

Database searches predicted the structural features shown in Fig. 8. The N terminus (residues 1 to 649) of ALA1 is homologous to the *C. albicans* protein ALS1 and the *S. cerevisiae* α-agglutinin protein AGα1. The function of *ALS1* is not known (12), but AGα1 mediates cell-cell adhesion during mating of haploid yeast cells of opposite mating types by directly interacting with the complementary **a**-agglutinin (20). AGα1 contains two functional regions, a cell surface anchorage region (C terminus) and a ligand-binding region (N terminus). The N terminus is responsible for binding to the complementary protein, **a**-agglutinin, and consists of three V-type immunoglobulin (IgV) domains (6, 8, 27). The presence of an Ig domain is a common feature of cell surface glycoproteins involved in cell adhesion or protein-protein interactions in mammals (26) and nonvertebrate eukaryotes (19). The ALA1 ho-

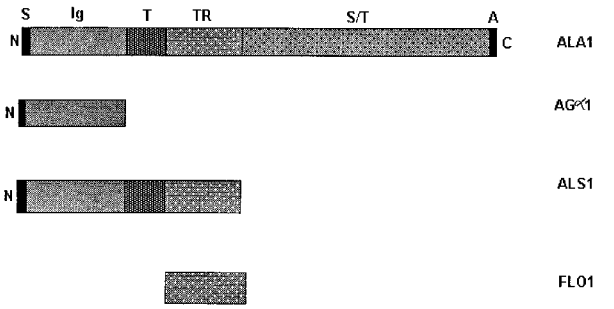

FIG. 8. Proposed structural motifs of ALA1. The N and C termini are indicated. The predicted domains are as follows: S, signal sequence; Ig, region homologous to AGα1 containing an Ig domain; T, region almost identical to ALS1 and rich in threonine residues; TR, region containing six tandem repeats of 36 amino acid residues; S/T, serine- and threonine-rich region showing no significant homology at the primary amino acid sequence level to any protein; A, hydrophobic region similar to the glycosylphosphatidylinositol anchor (9). The homologous regions identified by BLAST searches in AGα1, ALS1, and FLO1 are indicated below the predicted ALA1 structural motif.

mology to AGα1 is in the region containing three IgV domains and therefore may indicate a role of the ALA1 N terminus in recognizing ECM proteins and/or other unknown protein ligands. Chou-Fasman analysis predicted the ALA1 N terminus to be rich in β-strands. This region in ALA1 also contains 8 cysteine residues, of a total of 11 cysteine residues in the entire protein. These features are characteristics of an Ig domain.

Adjacent to the Ig domain in ALA1 and ALS1 is a region highly conserved both at the nucleotide and amino acid levels that is absent in AGα1. As this threonine-rich region is unique to ALA1 and ALS1, it may have a role in *C. albicans* adherence. The presence of tandem repeats in ALA1 and ALS1 but not in AGα1 suggests that they may also have a unique function in *C. albicans*. Although the ALA1 and ALS1 tandem repeats are similar, they are not identical. Both contain consensus N glycosylation sites (N-X-S/T) which may be modified by N glycosylation and several threonine residues which may be modified by O glycosylation. There are 65 proline residues in the protein, most of which are present in the C-terminal half of the molecule. These may add rigidity to the stalk of the protein (27). If the threonine-rich region and the tandem repeats play a role in *C. albicans* adherence, the initial recognition of the target may occur through sugar moieties. In this regard it is significant to note that many pathogens utilize protein repeats for adherence to ECM proteins or cell surfaces (21).

The C-terminal half of ALA1 (residues 650 to 1419) shows no significant homology at the primary amino acid sequence level to any database protein, although proteins rich in serine and threonine are identified. Many of these proteins are heavily glycosylated and localized on the cell surfaces (2). The N-terminal sequence of ALA1, containing residues 1 to 27, is predicted to be hydrophobic, which may serve as a secretory signal for transporting the protein outside the cell membrane (23). The C-terminal sequence, containing residues 1406 to 1419, is also hydrophobic, which is a common feature of eukaryotic cell surface glycoproteins attached to cell surfaces through a phosphatidylinositol anchor (9, 27).

This work is important for two reasons. First, using an assay requiring functional complementation of adherence to ECM proteins in *S. cerevisiae* YPH 499, we have isolated a gene, *ALA1*, encoding a protein whose sequence predicts surface localization and is homologous to a protein (AGα1) with a known adhesion function. Ongoing investigations are directed toward understanding the role of the predicted ALA1 structural motifs in the pathogenesis of candidiasis. Second, during the phenotypic characterization of *ALA1* it has become evident that the adherence of *C. albicans* yeast cells induces yeast cell aggregation, and, as demonstrated in this work, adherence and aggregation are two separate processes.

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