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Cloning and expression of a gene encoding an integrin-like protein in *Candida albicans*

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ABSTRACT **The existence of integrin-like proteins in *Candida albicans* has been postulated because monoclonal antibodies to the leukocyte integrins αM and αX bind to blastospores and germ tubes, recognize a candidal surface protein of ≈185 kDa, and inhibit candidal adhesion to human epithelium. The gene *αINT1* was isolated from a library of *C. albicans* genomic DNA by screening with a cDNA probe from the transmembrane domain of human αM. The predicted polypeptide (αInt1p) of 188 kDa contains several motifs common to αM and αX: a putative I domain, two EF-hand divalent cation-binding sites, a transmembrane domain, and a cytoplasmic tail with a single tyrosine residue. An internal RGD tripeptide is also present. Binding of anti-peptide antibodies raised to potential extracellular domains of αInt1p confirms surface localization in *C. albicans* blastospores. By Southern blotting, *αINT1* is unique to *C. albicans*. Expression of *αINT1* under control of a galactose-inducible promoter led to the production of germ tubes in haploid *Saccharomyces cerevisiae* and in the corresponding *ste12* mutant. Germ tubes were not observed in haploid yeast transformed with vector alone, in transformants expressing a galactose-inducible gene from *Chlamydomonas*, or in transformants grown in the presence of glucose or raffinose. Transformants producing αInt1p bound an anti-αM monoclonal antibody and exhibited enhanced aggregation. Studies of αInt1p reveal novel roles for primitive integrin-like proteins in adhesion and in *STE12*-independent morphogenesis.**

The opportunistic pathogen *Candida albicans* is the leading cause of invasive fungal disease in neonates, diabetics, and immunocompromised patients and carries a high mortality despite prompt and appropriate anti-fungal therapy (1–3). Three important events in the pathogenesis of invasive candidal infection include adhesion to epithelium, penetration of epithelial barriers, and hematogenous dissemination. Complicating this cascade is the yeast's ability to transform from blastospores at the epithelial surface to elongated structures (germ tubes, pseudohyphae, and mycelia) that invade underlying tissues.

Several investigators have reported the existence of surface proteins in *C. albicans* that are antigenically, structurally, and functionally related to the α-subunits of the leukocyte integrins αM/β2 (Mac-1; CD11b/CD18) and αX/β2 (p150,95; CD11c/CD18) (4–11). Many monoclonal antibodies (mAbs) recognizing epitopes of αM or αX bind to blastospores or germ tubes of *C. albicans* (4–10). iC3b-coated sheep erythrocytes rosette with germ tubes of *C. albicans* (9), and the affinity constants for the binding of purified human iC3b to *C. albicans* or to leukocyte αM/β2 are virtually identical (5, 12). Environmental conditions such as increased temperature or glucose concentrations ≥10 mM augment not only the surface expression of this integrin-like protein (5, 11) but also the binding of iC3b (12). Recognition of ligands containing the tripeptide sequence arginine-glycine-aspartic acid (RGD) facilitates the adhesion of *C. albicans* to endothelial and epithelial cells (6, 11).

Among the leukocyte integrins, αM and αX share ≈70% sequence homology and considerable functional identity. These two α-subunits, together with αL, α1, and α2, contain an inserted or I domain of ≈200 amino acids that is involved in ligand binding (13–15). Located just C-terminal to the I domain in αM/αX are three divalent cation-binding sites; at the C terminus are a membrane-spanning region and a cytoplasmic tail, the latter containing a single tyrosine residue in αM and αX (13).

This manuscript reports the isolation of a *C. albicans* gene encoding a protein that shares these integrin motifs.§ Moreover, expression of the gene product in haploid *Saccharomyces cerevisiae* is associated with the production of germ tubes independently of Ste12p, a yeast transcription factor required for morphologic change in response to mating pheromones and nutrient limitations in *S. cerevisiae* (16). These results open the way for the discovery of other integrin-like proteins in primitive eukaryotes, for their study as precursors of vertebrate integrins, and for more detailed investigation of their roles in signal transduction and morphogenesis.

MATERIALS AND METHODS

Yeast Strains, Plasmids, and Reagents for Cloning. *C. albicans* 10261 (B311, serotype A) was purchased as a lyophilate (American Type Culture Collection). *Candida tropicalis* 7555 was isolated from the blood of a fungemic patient by the University of Minnesota Clinical Microbiology Laboratory. *S. cerevisiae* YPH500 (*MATα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*) is a galactose-utilizing strain obtained from the Yeast Genetic Stock Center (Berkeley, CA) (17). pBM272, an ARS/CEN-based yeast shuttle vector containing the *URA3* gene and the *S. cerevisiae GAL1-10* promoter (18), pGG201 containing a 990-bp open reading frame encoding a DNA-binding protein from *Chlamydomonas reinhardtii* (19), a 750-bp *Cla* I/*Hin*dIII fragment of the *C. albicans* actin gene, and *S. cerevisiae* strain M12B-T2 were gifts from James Bodley, Judith Berman, Paul Magee, and Beatrice Magee (all of the University of Minnesota), respectively. pSUL16, a gift from Judith Berman, contains the *S. cerevisiae STE12* gene interrupted with the yeast *LEU2* gene (20). *Escherichia coli* JM101, LE392, XL1-Blue-MRF′, and pBluescript II SK(+) were obtained from Stratagene.

Cloning of *αINT1*. DNA from spheroplasts of *C. albicans* 10261 was isolated according to standard procedures (21), digested with *Sau*3AI, and packaged in λEMBL3 (Stratagene).

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Abbreviations: MM, minimal medium; mAb, monoclonal antibody.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U35070).

Preliminary studies confirmed that a 3.5-kbp *Eco*RI fragment of *C. albicans* DNA hybridized with a 314-bp *Eco*RI/*Sma* I cDNA fragment derived from the transmembrane domain of human αM (kind gift of Dennis Hickstein, Veterans' Administration Medical Center, Seattle). A library enriched for 3.0- to 3.8-kbp *Eco*RI fragments was constructed by digestion of genomic DNA with *Eco*RI and ligation to pBluescript II SK(+). Primers for amplification of the *Eco*RI/*Sma* I αM cDNA fragment were as follows: upstream primer, 5′-GAATTCAATGCTACCCTCAA; downstream primer, 5′-CCCGGGGGACCCCCTTCACT. Plasmid minipreparations from a total of 200 colonies were screened by the sib selection technique for hybridization at 50°C with ^{32}P-labeled PCR product after confirmation of its nucleotide sequence (13). Five clones were isolated from three successive screenings. Two of the five clones gave reproducible signals after hybridization with a degenerate oligonucleotide encoding a conserved sequence (KVGFFK) in the cytoplasmic domain of αX (22): 5′-AA(AG) GT(CT) GG(AT) TT(CT) TT(CT) AA(AG)-3′. Both clones contained a 3.5-kbp *Eco*RI insert and failed to hybridize with a degenerate oligonucleotide from the *S. cerevisiae* gene *USO1* (23): 5′-GAA AT(ACT) GA(CT) GA(CT) TT(AG) ATG-3′. One of these clones (probe 2, Fig. 1*A*) was chosen for further analysis. A 500-bp *Hin*dIII subfragment (probe 3, Fig. 1*A*) was used to screen 20,000 clones from a library of *C. albicans* 10261 genomic DNA by the plaque hybridization technique (24). The largest hybridizing insert, a 10.5-kbp *Sal* I fragment (Fig. 1*A*), was isolated by agarose gel electrophoresis, cloned, and sequenced.

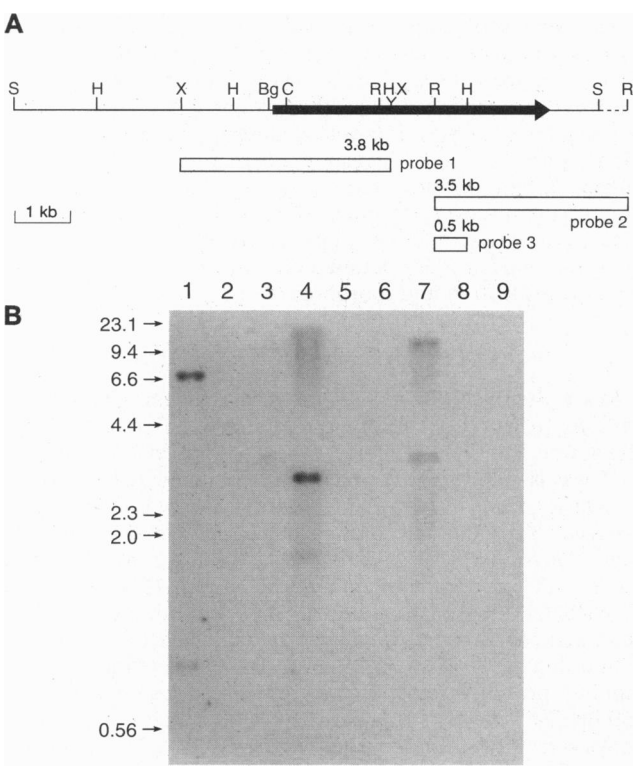

FIG. 1. (*A*) Restriction map of the 10.5-kbp *Sal* I genomic DNA fragment isolated from *C. albicans* 10261, with the open reading frame indicated by the bold arrow. Probe 1, 3.8-kbp *Xba* I fragment used for Southern and Northern blotting. S, *Sal* I; H, *Hin*dIII; X, *Xba* I; Bg, *Bgl* II; C, *Cla* I; R, *Eco*RI. (*B*) Southern blot of genomic DNA from *C. albicans* 10261 (lanes 1, 4, and 7), *C. tropicalis* 7555 (lanes 2, 5, and 8), and *S. cerevisiae* YPH500 (lanes 3, 6, and 9) digested with *Eco*RI (lanes 1–3), *Hin*dIII (lanes 4–6), and *Xba* I (lanes 7–9) and hybridized at high stringency with [α-^{32}P]dGTP-labeled probe 1 (hybridization at 65°C, final wash in 0.2× SSC/0.1% SDS at 65°C). The high molecular weight band (>12 kbp in lane 7) most likely represents incompletely digested DNA. Positions of *Hin*dIII-digested λDNA fragments are indicated on the far left. *Eco*RI and *Hin*dIII digests of four additional *S. cerevisiae* isolates from clinical and laboratory sources, as well as isolates of *Candida glabrata* and *Candida parapsilosis*, also failed to hybridize with probe 1.

Sequence Analysis. Both strands of the 10.5-kbp *Sal* I fragment were sequenced by the method of gene walking on an Applied Biosystems model 373 automated sequencer in the University of Minnesota Microchemical Facility. Nucleotide and protein sequence analyses were performed with the Genetics Computer Group (University of Wisconsin, Madison) Sequence Analysis Software Package, version 7.0 (25).

Yeast Transformation and Gene Expression. The entire open reading frame of *αINT1* (*Bgl* II/*Sal* I fragment) was subcloned into pBM272 after digestion with *Bam*HI and *Sal* I, in order to place the *GAL1-10* promoter upstream of the *αINT1* start codon (pCG01). *S. cerevisiae* YPH500 was transformed with pBM272 or pCG01 by the lithium acetate procedure (26). Transformants were selected on agar-based minimal medium (MM = 0.17% yeast nitrogen base/0.5% ammonium sulfate) with 2% glucose, in the absence of uracil. Induction of *αINT1* was achieved by growing transformants containing pCG01 to mid-exponential phase in noninducing, nonrepressing medium (MM without uracil with 2% raffinose) at 30°C, then harvesting, washing, and resuspending them in inducing medium (MM without uracil with 2% galactose) at 30°C. YPH500 and YPH500 transformed with vector alone (pBM272) were grown under the identical conditions.

Southern and Northern Blotting. Genomic DNA and total RNA were isolated and electrophoresed by standard methods (27–30) and transferred to Hybond N+ nylon membranes (Amersham) by traditional capillary blotting.

Flow Cytometry. Anti-peptide antibodies were prepared in rabbits (Cocalico Biologicals, Reamstown, PA) to a 23-mer peptide encompassing the second divalent cation-binding site [amino acid (aa) 596–618] and to a 17-mer peptide spanning the RGD site and flanking residues (aa 1142–1158) in *αINT1*. The IgG fractions of preimmune and immune rabbit sera were isolated on protein A-Sepharose (Pharmacia). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates) was used as the secondary antibody. For experiments with *S. cerevisiae* transformants, antibodies included OKM1 (anti-αM IgG2b) or MY9 as isotype control (Coulter) and FITC-conjugated goat anti-mouse IgM/IgG (Biosource International, Camarillo, CA) (7, 11).

Insertional Inactivation of *STE12* in *S. cerevisiae*. YPH500 was transformed with pSUL16 by standard techniques (26) and chromosomal integrants of the disrupted *STE12* gene were selected on leucine-deficient MM. After confirmation of sterility, *ste12* mutants were transformed with pCG01 as described above.

Aggregation Assay. The degree of aggregation of *C. albicans* and *S. cerevisiae* transformants was determined according to published methods (31).

RESULTS

Restriction Map and Southern Blotting. The restriction map of *αINT1* with its 5′ and 3′ flanking sequences is displayed in Fig. 1*A*. Fig. 1*B* shows that a 3.8-kbp *Xba* I probe from *αINT1* hybridized with *Eco*RI, *Hin*dIII, and *Xba* I fragments from *C. albicans* (lanes 1, 4, and 7) but not from *C. tropicalis* 7555 or *S. cerevisiae* YPH500. Among the yeast strains tested, this DNA fragment is unique to *C. albicans*.

Sequence Analysis of *αINT1*. Analysis of the nucleotide sequence revealed an open reading frame sufficient to encode a 1664-residue polypeptide with a theoretical molecular mass of 187,989 Da and no extensive homologies with other proteins. Fig. 2 compares the derived aa sequence of αInt1p with the characteristic motifs of several integrin α-subunits. BESTFIT

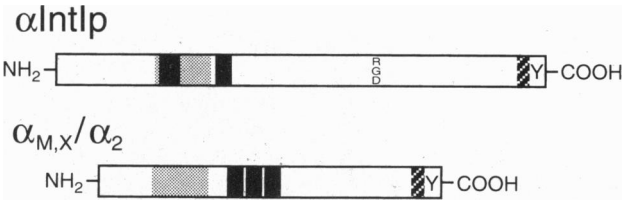

FIG. 2. Schematic diagram comparing the structures of αM, αX, and α2 with that of αIntlp. Gray regions represent the ligand-binding or I domain, the EF-hand divalent cation-binding motifs are indicated in black, and the transmembrane regions are hatched. RGD indicates the approximate location of this sequence in αIntlp (aa 1149–1151). The α-subunit schematic is modified from the sequence reported by Corbi *et al.* (13).

analysis (32) located a putative I domain at aa 230–470, with ≈18% identity to the I domain of human αM. Within this I-domain-like region are three potential partial MIDAS motifs (DXSX) for the coordination of divalent cations (33). This same region (aa 230–470) also displayed 25% identity to the nonrepeat region of the fibrinogen-binding protein of *Staphylococcus aureus* (34). Chou–Fasman analysis (35) indicated multiple α-helices, two of them bracketing the second of two possible EF-hand divalent cation-binding motifs (aa 283–295 and aa 601–613). Fig. 3 shows that the amino acid sequence of the second divalent cation-binding site from αIntlp differs from the EF-hand consensus sequence (36) at only one residue, a non-cation coordinating site. A hydrophobic sequence is located at aa 1592–1617 as determined by Kyte–Doolittle hydrophobicity plotting (37). Just C-terminal to this putative membrane-spanning region in αIntlp is a unique tyrosine residue, also present in the cytoplasmic tails of αM and αX (13, 22).

In the upstream sequence, a putative TATA box is located at −34 from the start codon. The coding sequence also displays 24 N-glycosylation sites, 6 cysteine residues, and the tripeptide sequence arginine-glycine-aspartic acid (RGD) (aa 1149–1151), a feature of many integrin ligands but not of integrins themselves.

Localization of αIntlp in *C. albicans* and *S. cerevisiae*. Polyclonal antibodies prepared against the second divalent cation-binding site and the RGD sequence and flanking residues in αIntlp recognized 64–82% of *C. albicans* blastospores, while preimmune IgG bound to only 0.5–1% of yeast cells ($P < 0.0001$) (Table 1). These results confirm that αIntlp is a surface protein in *C. albicans* and that the second cation-binding site and the RGD site are in the extracellular region of the polypeptide. In *S. cerevisiae*, the binding of the anti-αM mAb OKM1 was significantly greater in transformants expressing *αINT1* vs. transformants containing vector alone for percent yeasts fluorescing (19.0% vs. 6.2%; $P \leq$ 0.004) and for mean channel fluorescence (181.8 vs. 65.7; $P \leq$ 0.013). These results confirm that αIntlp is surface-borne in *S. cerevisiae* transformants and is recognized by an anti-integrin mAb.

A

1	2	3	4	5	6	7
(D)	X	(DNS)	{ILVFYW}	(DENSTG)	(DNQGHKR)	{GP}

8	9	10	11	12	13
(ILVMC)	(DENQTSGCA)	X	X	(DE)	(ILVMFYW)

B

	1	2	3	4	5	6	7	8	9	10	11	12	13
αIntlp $I_{283-295}$	N	-	N	-	N	-	S	-	K	-	V	-	S
αIntlp $II_{601-613}$	D	-	S	-	N	-	D	-	G	-	D	-	R

αIntlp $I_{283-295}$	N	-	N	-	N	-	S	-	K	-	V	-	S
αIntlp $II_{601-613}$	D	-	S	-	N	-	D	-	G	-	D	-	R

	1	2	3	4	5	6	7	8	9	10	11	12	13
αIntlp $I_{283-295}$	N	-	N	-	N	-	S	-	K	-	V	-	S
αIntlp $II_{601-613}$	D	-	S	-	N	-	D	-	G	-	D	-	R

	1	2	3	4	5	6	7	8	9	10	11	12	13
αIntlp $I_{283-295}$	N	-	N	-	N	-	S	-	K	-	V	-	S
αIntlp $II_{601-613}$	D	-	S	-	N	-	D	-	G	-	D	-	R

FIG. 3. Comparison of divalent cation-binding motifs. (*A*) Consensus sequence for the 13-residue EF-hand divalent cation-binding motif (36). (*B*) The N-terminal (I) and more distal cation-binding site (II) in αIntlp. The standard single letter code for aa residues is used. (. . .), Acceptable amino acids; {. . .}, unacceptable amino acids; X, any amino acid. Cation coordinating sites are indicated in boldface type.

Table 1. Percent yeasts fluorescing and mean channel fluorescence of *C. albicans* blastospores after incubation with anti-peptide antibodies

Antibody source	% yeasts fluorescing	Mean channel fluorescence
Control 12	1.0 ± 0.5	67.4 ± 24.6
UMN12	82.4 ± 8.6*	317.0 ± 24.7*
Control 13	0.40 ± 0.36	36.4 ± 9.2
UMN13	64.1 ± 2.3*	266.7 ± 9.2*

Values represent the mean ± SD of three experiments done on different days using different aliquots of *C. albicans* 10261. UMN12 is the antibody to the second divalent cation-binding motif and UMN13 is the antibody to the RGD region of αIntlp. Control 12 and 13 are preimmune IgGs from rabbits prior to immunization with UMN12 and UMN13, respectively. A one-tailed Student's *t* test was used for statistical calculations.
*$P < 0.0001$ vs. control in all comparisons.

Expression of *αINT1* in *C. albicans* and *S. cerevisiae*. Hybridization of probe 1 with total RNA isolated from *C. albicans* blastospores detected message of ≈5.5 kb (Fig. 4*A*). In *S. cerevisiae*, *αINT1* message was detected in pCG01 transformants 6 hr after induction with 2% galactose and continued to be expressed for at least 24 hr (Fig. 4*B*, lanes 1 and 3). As expected, message was not detected in pCG01 transformants grown under conditions of repression (Fig. 4*B*, lanes 2 and 4) or in pBM272 transformants (Fig. 4*B*, lanes 5 and 6).

Coincident with the detection of *αINT1* message, the majority of the pCG01 transformants formed elongated cell projections reminiscent of germ tubes (Fig. 5*A*). These structures continued to be present for 24 hr and could be detected at galactose concentrations ≥ 0.05%. pCG01 transformants exhibited polar budding, typical of haploid organisms, rather

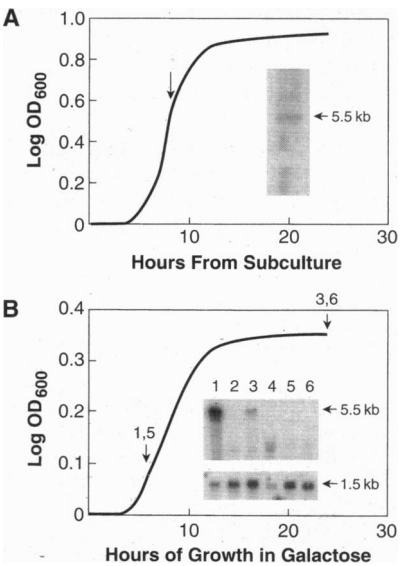

FIG. 4. (*A*) Northern blot of *C. albicans* 10261 total RNA isolated from blastospores in mid-exponential growth (arrow) in MM with 2% glucose and hybridized with probe 1 (see Fig. 1*A*). (*B*) Northern blot of total RNA from *S. cerevisiae* transformants: pCG01 transformants grown in galactose (lanes 1 and 3), pBM272 transformants grown in galactose (lanes 5 and 6), and pCG01 transformants grown to mid-exponential phase (lane 2) and to late exponential phase (lane 4) under conditions of repression (2% glucose). Probe 1 was used for hybridization. The diffuse signal at 2 kbp in lanes 2 and 4 represents nonspecific binding of the probe to the 18S ribosomal RNA band. The signal at 1.5 kbp represents actin transcript.

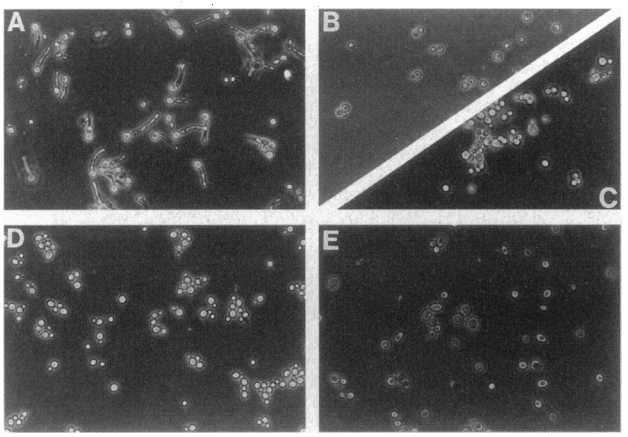

FIG. 5. Phase-contrast photomicrographs of *S. cerevisiae* transformants. pCG01 transformants (*A*) were grown to exponential phase in raffinose and then induced with 2% galactose for 6–24 hr. pBM272 transformants (vector without gene) (*B*), the parent strain YPH500 (*C*), *C. albicans* 10261 (*D*), and pGG201 transformants (galactose-inducible *C. reinhardtii* gene) (*E*) were grown identically. All yeast cells were photographed with a Leitz Wetzlar Laborlux 12 microscope equipped with a WILD MP551 Camera (Heerbrugg, Switzerland). (×500.)

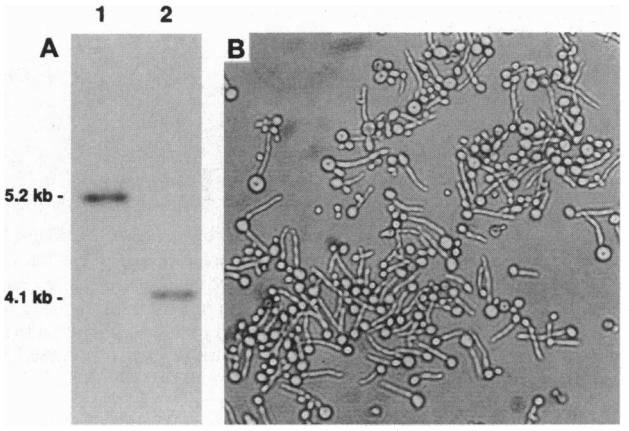

FIG. 6. (*A*) *Cla* I digests of genomic DNA from wild-type YPH500 (lane 1) and *ste12* mutant (lane 2). The blot was probed at high stringency with a 600-bp *Sph* I/*Cla* I fragment from pSUL16. (*B*) Phase-contrast photomicrograph of YPH500 *ste12* mutants transformed with pCG01 and grown in galactose for induction of *αINT1*.

than apical budding, which is typical of diploid organisms. pCG01 transformants (*MATα*) mated to a *MAT***a** yeast strain were able to form diploid organisms (data not shown).

pBM272 transformants, YPH500, and *C. albicans* 10261 did not form germ tubes when grown under the identical conditions (Fig. 5 *B–D*). pCG01 transformants did not exhibit germ tubes when grown in 2% raffinose, 2% glucose, or noninducing concentrations of galactose (0.02%) or when cured of the plasmid (data not shown). In addition, no germ tubes were observed with the galactose-induced expression of an ≈300-residue DNA-binding protein from *C. reinhardtii* (Fig. 5*E*). pCG01 transformants exhibited germ tubes during growth in liquid and on solid medium (MM with 2% galactose). Germ tubes were also observed in yeast strain M12B-T2 transformed with pCG01. Thus, the induction of germ tubes in haploid *S. cerevisiae* is specific to expression of *αINT1* from the plasmid pCG01.

Ability of Yeast Transformants to Aggregate. The aggregation index of pCG01 transformants equaled that of *C. albicans* germ tubes and significantly exceeded the aggregation index of *C. albicans* blastospores and *S. cerevisiae* pBM272 transformants (Table 2). This finding suggests that *S. cerevisiae* germ tubes synthesizing αInt1p are functionally similar to germ tubes in *C. albicans*.

Table 2. Percent aggregation of *C. albicans* and *S. cerevisiae* transformants

Yeast	% aggregation*
C. albicans	
Blastospores	62 ± 1
Hyphae	89 ± 4†
S. cerevisiae	
pBM272	65 ± 4
pCG01	80 ± 2‡

Values represent the mean ± SEM of four experiments, each done in triplicate. *C. albicans* blastospores were grown to mid-exponential phase in YPD medium (1% yeast extract/2% peptone/2% glucose) at 30°C. *C. albicans* hyphae were prepared by growth at 37°C in RPMI 1640 medium (GIBCO/BRL). *S. cerevisiae* pBM272 and pCG01 were grown in galactose-containing medium (see text).

*% aggregation = 100 × (OD_{540} final − OD_{540} initial)/OD_{540} final. A two-tailed Student's *t* test was used to determine statistical significance.

†$P = 0.0013$ vs. *C. albicans* blastospores.

‡$P = 0.026$ vs. *S. cerevisiae* pBM272.

Induction of Germ Tubes in *ste12* Mutants of *S. cerevisiae*. Insertional inactivation of *STE12* in YPH500 shifted the *Cla* I digestion fragment from 5.2 ± 0.1 kbp in the parent to 4.1 ± 0.1 kbp in the *ste12* mutant (Fig. 6*A*). The *Eco*RI fragment shifted from 10.5 ± 0.7 kbp (parent) to 5.0 ± 0.2 kbp (mutant). *ste12* mutants were unable to mate. After transformation of *ste12* mutants with pCG01 and induction of *αINT1* expression by growth in galactose, the mutants formed germ tubes (Fig. 6*B*). Therefore, the observed morphological change is independent of *STE12*.

DISCUSSION

We have isolated a gene encoding a putative integrin-like protein in *C. albicans* by screening a genomic library with conserved sequences from the transmembrane and cytoplasmic domains of human αM. αInt1p exhibits several motifs common to α-integrin subunits, including two EF-hand motifs and three partial MIDAS motifs within a putative I domain, a membrane-spanning domain, and a cytoplasmic tail with a conserved tyrosine residue at the C terminus. Because αM and αX recognize iC3b and fibrinogen as ligands (13), a 25% identity with the fibrinogen-binding protein of *S. aureus* (34) provides additional evidence for relationship.

Divalent cation-binding sites in the amino acid sequence of αM provided initial evidence of the leukocyte integrins' relationship to other vertebrate integrins (13). Both cation-binding motifs in αInt1p conform to the classic EF-hand consensus sequence. In comparison, two of the three cation-binding sites in αM agree at 11 of 13 residues; one of these and the third site require a gap to improve the alignment (13). Chou–Fasman analysis indicates that both divalent cation-binding sites in αInt1p, but not αM, are bracketed by α-helices, a conformation that facilitates cation binding (38). In addition, αInt1p contains three partial MIDAS motifs (DXSX) within the putative I domain. A full or partial MIDAS motif is present in all members of the I domain superfamily (15, 33). Of note, an I-domain-like sequence in *S. cerevisiae* Uso1p binds iC3b and the anti-αM mAb Mn41 (39) but has no divalent cation-binding sites or MIDAS motifs.

The presence of an I domain and an RGD sequence in the extracellular region of αInt1p should contribute to the adhesive capabilities of this protein. For example, an extracellular RGD sequence in the filamentous hemagglutinin of *Bordetella pertussis* facilitates adhesion of the bacterium to eukaryotic

cells (40). Another putative candidal adhesin is encoded by a 3.3-kbp genomic DNA fragment and enables transformed *S. cerevisiae* to adhere to polystyrene or buccal epithelial cells (41). However, its restriction map differs markedly from that of *αINT1*, and the nucleotide sequence has not been published.

In addition to a role as an adhesin, αInt1p leads to the production of germ tubes in haploid *S. cerevisiae* in a process independent of *STE12*. Although the morphological change correlates with expression of the candidal gene product and not with the production of other foreign proteins, we cannot discount the possibilities that αInt1p unnaturally disrupts the cytoskeletal architecture or the growth cycle or that other recognized morphogenic cascades, such as those involving the *CDC* genes (42, 43), may be implicated.

To date, only two genes that participate in morphogenesis in *C. albicans* have been reported. *ACPR*, also called *CPH1*, encodes a protein of 699 aa that is 74% identical to *S. cerevisiae* Ste12p (44, 45). *STE12* is an essential gene in at least two pathways involved in morphogenesis in *S. cerevisiae*: the induction of pseudohyphae in diploid cells on nitrogen-limited medium (46) and the invasive response of haploid cells on rich solid medium (47). Thus, the induction of germ tubes in *S. cerevisiae* transformants expressing *αINT1* after insertional inactivation of *STE12* suggests a novel pathway for integrin-mediated signaling. The second gene, *PHR1*, encodes an ≈580-aa polypeptide essential for pH-dependent morphogenesis in *C. albicans* (48). *ACPR* and *PHR1* encode intracellular regulatory proteins. The isolation of a gene encoding a surface-borne, integrin-like protein in *C. albicans* and its ability to induce morphological variants in haploid *S. cerevisiae* emphasize potential roles for *αINT1* in pathogenesis, signal transduction, and differentiation in *C. albicans* and *S. cerevisiae*.

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