

## *MF $\alpha$ 1*, the Gene Encoding the $\alpha$ Mating Pheromone of *Candida albicans*†

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Received 31 July 2003/Accepted 22 August 2003

*Candida albicans*, the single most frequently isolated human fungal pathogen, was thought to be asexual until the recent discovery of the mating-type-like locus (*MTL*). Homozygous *MTL* strains were constructed and shown to mate. Furthermore, it has been demonstrated that opaque-phase cells are more efficient in mating than white-phase cells. The similarity of the genes involved in the mating pathway in *Saccharomyces cerevisiae* and *C. albicans* includes at least one gene (*KEX2*) that is involved in the processing of the  $\alpha$  mating pheromone in the two yeasts. Taking into account this similarity, we searched the *C. albicans* genome for sequences that would encode the  $\alpha$  pheromone gene. Here we report the isolation and characterization of the gene *MF $\alpha$ 1*, which codes for the precursor of the  $\alpha$  mating pheromone in *C. albicans*. Two active  $\alpha$ -peptides, 13 and 14 amino acids long, would be generated after the precursor molecule is processed in *C. albicans*. To examine the role of this gene in mating, we constructed an *mf $\alpha$ 1* null mutant of *C. albicans*. The *mf $\alpha$ 1* null mutant fails to mate as *MTL $\alpha$* , while *MTL $\alpha$  mf $\alpha$ 1* cells are still mating competent. Experiments performed with the synthetic  $\alpha$ -peptides show that they are capable of inducing growth arrest, as demonstrated by halo tests, and also induce shmooing in *MTL $\alpha$*  cells of *C. albicans*. These peptides are also able to complement the mating defect of an *MTL $\alpha$  kex2* mutant strain when added exogenously, thereby confirming their roles as  $\alpha$  mating pheromones.

*Candida albicans* is the most common opportunistic fungal pathogen of humans and mostly infects immunosuppressed patients (3). It inhabits diverse niches, which include the gastrointestinal tract and the vagina, and causes infection of skin, mucous membranes, and the bloodstream. Since each of these sites of infection presumably requires differences in gene expression, a great deal of effort has been spent in looking at how such adaptation occurs in *Candida*. Much of this effort was predicated on the characterization of *C. albicans* as an asexual obligate diploid.

That this fungus is asexual was disproved after the *C. albicans* genome sequencing project revealed the presence of sequences homologous to the *Saccharomyces cerevisiae* *MAT* (mating-type) loci. The *C. albicans* homologues of the *MAT* loci, the *MTL* (mating-type-like) loci, were found to be heterozygous in common laboratory strains tested (17). Strains homozygous for the *MTL* locus were generated either by a deletion strategy (18) or by loss, induced by growth on sorbose as the sole carbon source (19), of one homologue of chromosome 5, the site of the *MTL* loci (29). Strains thus constructed were able to mate both under laboratory conditions (29) and in the animal host (18). These studies set the stage for dissecting the mating pathway in *C. albicans*.

Mating in fungi has been examined in both yeasts and molds of various sorts. Certain general parts of the process seem to hold across genera and indeed across the kingdom. These include dissimilar regulatory genes (mating-type loci), soluble

pheromones, and signal transduction pathways. There are significant homologies in the structures of the proteins involved in the mating process as well. For example, the *mat A* idiomorph in *Neurospora crassa* encodes a protein similar to the *MAT $\alpha$ 1* gene product in *S. cerevisiae* (9, 12). The general structure of the pheromones is also highly conserved, although the amino acid sequence is usually highly divergent, since this determines the species specificity of the pheromone. Thus, one kind of pheromone (mating pheromone **a**) tends to be farnesylated at the C terminus, while the other (mating pheromone  $\alpha$ ) is synthesized as a precursor which must be cleaved by specific proteinases. Both have been found in fungi as evolutionarily separated as *Schizosaccharomyces pombe* and *Magnaporthe grisea* (7, 37).

Mating in *C. albicans* is relatively unstudied compared to that in other well-characterized fungi, such as *S. cerevisiae*, where the mating pathway has been explored in detail. Haploid cells of *S. cerevisiae* are of two mating types, *MAT $\alpha$*  and *MAT $\alpha$* . Two opposite mating types fuse upon mixing to form a diploid cell. The first step that allows each mating type to sense the presence of the other is the secretion of mating-type-specific diffusible pheromones. *MAT $\alpha$*  cells secrete pheromone **a**, and pheromone  $\alpha$  is secreted from *MAT $\alpha$*  cells. Binding of these pheromones to the opposite cell type triggers a signal transduction pathway which leads to the expression of several genes required for mating in this budding yeast. Cytoplasmic and nuclear fusion occur when the two mating cell types fuse with each other to form a zygote, which gives rise to diploid daughter cells (38).

Recent work on the characterization of the mating pathway in *C. albicans* has revealed that the opaque-phase cells of *C. albicans* are more efficient in mating than the white cell type

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† NRC publication number 46156.

TABLE 1. Strains used in this work

Strain	Genotype	MTL	Reference or source
3752 (RM1000)	<i>his1/his1 ura3/ura3</i>	a/α	34
3842	<i>MFα1/mfα1::HIS1</i>	a/α	This work
3845	<i>mfα1::URA3-dp l200/mfα1::HIS1</i>	a/α	This work
3852	<i>mfα1::dpl200/mfα1::HIS1</i>	a/α	This work
3861 (3852α)	<i>mfα1::dpl200/mfα1::HIS1</i>	α/α	This work
3863 (3852a)	<i>mfα1::dpl200/mfα1::HIS1</i>	a/a	This work
3898 (3861)	<i>mfα1/mfα1 pGEMURA3/MFα1</i>	α/α	This work
3902 (3861)	<i>mfα1/mfα1 pGEMURA3/mfα1Δ</i>	α/α	This work
3896 (CAI4 derivative) <sup>a</sup>	<i>his3/his3::URA3</i>	a/a	Elitra Pharmaceuticals, Montreal, Canada
3897 (CAI4 derivative) <sup>a</sup>	<i>his3/his3::URA3</i>	α/α	Elitra Pharmaceuticals
3142	<i>MTLa/mlα1 mtlα2::hisG ura3/ura3</i>	a/a	18
3716 (3142 derivative) <sup>a,b</sup>	<i>MTLa1 ura3/ura3</i>	a/a	This study
3144 <sup>a</sup>	<i>mla1::URA3/MTLα ade2/ade2</i>	α/α	18
3710	<i>ura3/ura3 his1/his1 arg5,6/arg5,6 (URA3 IMH3-1)<sup>c</sup></i>	a/a	28
3685	<i>ura3/ura3 his1/his1 arg5,6/arg5,6 (URA3 IMH3-1)<sup>c</sup></i>	α/α	28
3301	<i>kex2::hisG kex2::hisG ura3/ura3</i>	α/α	35
3108	<i>kex2::hisG kex2::hisG ura3/ura3</i>	a/a	35
3745(A505 derivative) <sup>a,b</sup>	<i>trp1/trp1 lys2/lys2</i>	a/a	23

<sup>a</sup> The opaque cell type of this strain was used for this work. The strain was streaked on YEPD plates and incubated at room temperature to obtain the opaque cells.

<sup>b</sup> The strain was derived from the indicated parent by growth on minimal medium with sorbose as the sole carbon source.

<sup>c</sup> MPA-resistant strain.

(33). It has also been demonstrated that the genes required in the mitogen-activated protein kinase signal transduction pathway in mating in *S. cerevisiae* are also needed for mating in *C. albicans*, thereby indicating that the mitogen-activated protein kinase pathway is functionally conserved between the two yeasts (6, 28). Furthermore, mating figures or shmoos (cells with elongated, nonseptated projections) have been observed in mixes of *MTLa* and *MTLα* cells (27). Several genes homologous to those that are involved in the processing of the mating pheromones in *S. cerevisiae* are also present in *C. albicans*, including the serine proteinase gene *KEX2* (35). *KEX2* is involved in the generation of the mature active α-peptide in *S. cerevisiae* (22). The *C. albicans kex2* mutant fails to mate if the mating type is *MTLα*, but it mates efficiently if the mating type is *MTLa* (28). Based on these observations, we searched the *C. albicans* genome assembly for potential α pheromone-encoding genes. In this report we describe the identification and detailed characterization of the α pheromone precursor gene *MFα1* (mating factor α1) of *C. albicans*.

#### MATERIALS AND METHODS

**Strains and culture conditions.** Table 1 lists the strains used in these experiments. The *MFα1* null mutant was derived from strain RM1000. Strain 3861 (*MTLα*) was used as the parent for constructing the revertants. The revertant and pseudorevertant strains (3898 [*MTLα*] and 3902 [*MTLα*]) are derived from strain 3861; in these strains the intact *MFα1* gene or a truncated copy has been reinserted by transformation with a plasmid as described below. All of the strains were grown in YEPD (2% Bacto Peptone, 1% yeast extract, and 20% dextrose) or in SD medium (6.7% yeast nitrogen base plus ammonium sulfate, without amino acids, with 2% dextrose, and with 2% Bacto agar for solid media) supplemented with required amino acids and nucleotides at 30°C. Opaque cells were grown in modified Lee's medium (1) at room temperature, and the opaque-phase cell phenotype was confirmed microscopically. Mating was carried out as described previously (29). Revertants were mated against strains 3685 (*MTLα*) and 3710 (*MTLa*), which are mycophenolic acid (MPA)-resistant mating tester strains (28), and the mating products were selected on minimal medium containing 10 μg of MPA per ml.

The *MTL* series of primers listed in Table 2 were used for analysis of the *MTL* alleles in all of the experiments.

**Construction of the *MFα1* null mutant and generation of *MTLa* and *MTLα***

**derivatives from the null mutant.** *MFα1* gene-specific primers (60 bases) *mfαdelF* and *mfαdelR* (Table 2) were used to amplify *HIS1* and the mini-*URA3* blaster cassettes (41) from plasmids pGEMHIS1 and pDDB57, respectively. The amplified fragments were used to transform the strain RM1000 (*his1/his1 ura3/ura3*) by the lithium acetate transformation method as described previously (11). A 432-bp coding region of *MFα1* was deleted by using the *HIS1* marker for the first allele and the *URA3* marker for the second allele, resulting in strains 3842 (*MTLa/α*) and 3845 (*MTLa/α*). Strain 3845 was plated on 5-fluoro-orotic acid (5-FOA) to select for intrachromosomal recombination between the homologous *dpl200* sequences of the mini-*URA3* blaster cassette, resulting in the loss of the *URA3* selectable marker, thereby generating a *ura3* strain, 3852 (*MTLa/α*).

The *MFα1* null mutant, 3852 (*MTLa/α*), was plated on minimal medium with the required supplements and sorbose as the sole carbon source for isolation of strains 3861 (*MTLα*) and 3863 (*MTLa*), which are homozygous for either of the mating types. The plates were incubated at 37°C for 1 week, and the Sou<sup>+</sup> colonies were analyzed by PCR for the *MTL* loci as described previously (29).

Reintroduction of *MFα1* into its original locus was achieved in strain 3861 (*MTLα*) by transformation with plasmid pGEMURA3/*MFα1*. This plasmid consisted of a 2.3-kb DNA fragment containing the 432-bp coding region of *MFα1* preceded by 1.5 kb of the promoter region and followed by a 348-bp region downstream of the open reading frame (ORF). The 2.3-kb fragment was PCR amplified with primers *mfαNotIF* and *mfαMluIR*, which contain the *NotI* and *MluI* restriction enzyme sites (Table 2). The amplified fragment was digested with *NotI* and *MluI* and cloned into *NotI*- and *MluI*-digested pGEMURA3. To rule out the positional effect of the *URA3* gene, a 0.55-kb band containing the truncated ORF of *MFα1* was also cloned into pGEMURA3 by using the same enzyme sites. Both of these clones were linearized with *HindIII*, which is present in the region downstream from the ORF, preceding the *MluI* restriction enzyme site. Integration of these clones into the deleted *MFα1* locus thus occurred at the *HindIII* site.

Proper integration of the two cassettes during the deletion events and the reintroduction of the wild-type gene into the *MFα1* null mutant was verified in each strain and at each step by PCR with primers localized outside the cassettes (primers 1 and 2), a coding-region-specific primer (primer 3), and a *URA3*-specific primer (primer 4) (Table 2; see Fig. 2A to C). All of the fragments were also sequenced for confirmation of their identity. Total genomic DNA was extracted as described previously (14) and used as the template for the PCRs. The PCR mixtures were typically heated to 94°C for 2 min, followed by 29 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. After a final extension at 72°C for 10 min, reaction mixtures were stored at 4°C. The enzyme *Taq* DNA polymerase used for the PCRs was from Sigma-Aldrich, St. Louis, Mo.

**Synthesis and biological activity of the α-peptide.** The 13-amino-acid (SP1, GFRLTNFGYFEPG) and 14-amino-acid (SP2, GFRLTNFGYFEPGK) active peptides were synthesized at the microchemical facility, University of Minnesota, Minneapolis, by solid-phase peptide synthesis with 9-fluorenylmethoxy carbonyl

TABLE 2. PCR primers used in this work

Primer <sup>a</sup>	Nucleotide sequence (5'→3') <sup>b</sup>
mf $\alpha$ delF .....	ATCACCAACAACTACTAATCACTCTATAACATCAACTAATTAATCAACAAAAATAACAGTTTTCCAGT CACGCGTT
mf $\alpha$ delR.....	TTTCATCTAAACAAACATAAAAGTATGATTTCAGTATGCTTTCCATCTTCTTTACTTACTTTGTGGAATTGT GAGCGGATA
5'-detect (1) .....	TTTTTCATTAAGACCATCATC
3'-detect (2) .....	ATAATGGAGATAGAAACTTT
Ura3detect (4) .....	AGACCTATAGTGAGAGAGCA
ORF $\Delta$ detect (3).....	AAGGAAAAAAGCGGCCGCGAAGCTAAGTCTAAAGGTGG
mf $\alpha$ NotIF .....	AAGGAAAAAAGCGGCCGCGGTTTATAGTTTATATTCAAATCAGG
mf $\alpha$ MluIR .....	CGACGCGTAAATATTGCATAACAATAACA
MTLaF .....	TTGAAGCGTGAGAGGCAGGAG
MTLaR .....	GTTTGGGTTCCTTCTTCTCATT
MTLaF .....	TTGAGTACATTCTGGTCCGG
MTLaR .....	TGTAACATCCTCAATTGTACCCGA
KEX2F.....	GTTGAAAGAGGAAAGAAGAGGCGG
KEX2R.....	CGTCATCAGCACGCCTAGTGTCCG

<sup>a</sup> Primers mf $\alpha$ delF and mf $\alpha$ delR were used to amplify both the *HIS1* and the *URA3* cassettes from the plasmids pGEMHIS1 and pDDB57, respectively. The numbers in parentheses are used in the text to describe the deletion strategy.

<sup>b</sup> Restriction endonuclease recognition sites are in bold. *NotI*, 5'-GCGGCCG-3'; *MluI*, 5'-ACGCGT-3'. Sequence homologous to the vectors are underlined.

chemistry (Perkin-Elmer/ABI Pioneer peptide synthesizer). When experiments were performed with both peptides SP1 and SP2, the concentrations were always 10  $\mu$ g of SP1 per ml and 5  $\mu$ g of SP2 per ml to reflect the genomic ratio of 2SP1 to 1SP2. The shmooing experiments with the peptides were performed with the opaque cell type of a given strain. The peptides were dissolved in 2% dimethyl sulfoxide (DMSO) at a stock concentration of 1 mg/ml. Opaque cells from strains 3716 (*MTLa*) and 3144 (*MTL $\alpha$* ) were inoculated into modified Lee's medium and grown for 24 h at room temperature. Approximately  $3 \times 10^6$  cells from each of the two strains were incubated at room temperature with both of the peptides in a final volume of 1.0 ml in a plastic 15-ml Falcon tube. The same experiment was also carried out in the presence of either SP1 (10  $\mu$ g/ml) or SP2 (5  $\mu$ g/ml) separately. The same volume of 2% DMSO was added to control samples. Aliquots of 200  $\mu$ l were taken every 2 h, fixed with 3.7% formaldehyde for 30 min, and stained with Calcofluor at a final concentration of 0.1  $\mu$ g/ $\mu$ l for another 30 min in the dark. The cells were washed twice with water before final resuspension in 200  $\mu$ l of water. Three microliters of cells was examined on an E800 Nikon microscope. Images were recorded with a Nikon Cool camera, and Adobe Photoshop software was used for processing the images.

Experiments with the peptides were also carried out on plates. The two peptides were spotted together or separately (10  $\mu$ g of SP1 per ml or 5  $\mu$ g of SP2 per ml) onto the patched strains 3716 (*MTLa*) and 3144 (*MTL $\alpha$* ). The plates were incubated at room temperature and photographed after 3 days. Cells from the patches were scooped, and phase-contrast images were taken on an E800 Nikon microscope and recorded with a Nikon Cool camera.

**Halo tests with the peptides.** The halo tests were performed with either the SP1 or SP2 peptide at a concentration of 5  $\mu$ g/ml on the opaque cell type of strain 3745 (*MTLa*). Cells from a fresh single opaque colony were resuspended in 200  $\mu$ l of water and spread onto an SD medium plate supplemented with the required amino acids. Five microliters of peptides SP1 and SP2 from a stock solution of 1 mg/ml was spotted onto the plate. The plates were monitored for the appearance of halos after 24 and 48 h.

**Complementation assays.** The complementation assays were performed with the *MTLa* *kek2* (3301) and *MTLa* *mf $\alpha$ 1* (3861) strains. The *MTLa* *kek2* (3108) and *MTLa* *mf $\alpha$ 1* (3863) strains were used as controls for this experiment. Matings were performed as described earlier (29) except that the plates contained the purified peptides SP1 and SP2 added separately or together at an SP1/SP2 ratio of 2:1 to give final concentrations of  $6 \times 10^{-5}$ ,  $6 \times 10^{-6}$ ,  $6 \times 10^{-7}$ , and  $6 \times 10^{-8}$  M. Since the mating experiments performed in our laboratory are nonquantitative assays (28), the number of mating products that appear on plates with SP1 and SP1 plus SP2 for the *MTLa* *kek2* mutant strain varies on the two plates (see Fig. 7A).

Matings in the presence of an *MTL $\alpha$*  helper strain (3897) as a source for the  $\alpha$  pheromone were performed by mixing strain 3301 (*MTLa* *kek2*) with strain 3897 (*MTL $\alpha$* ) in one set and 3861 (*MTLa* *mf $\alpha$ 1*) with 3897 (*MTL $\alpha$* ) in the other set and mating each pair with strain 3896 (*MTLa*). Strains 3896 (*MTLa*) and 3897 (*MTL $\alpha$* ) have the same auxotrophic marker and were chosen for this experiment in order to avoid selecting for the tetraploid products that would arise from mating between these two strains. For the mating experiment, one strip con-

tained cells from strain 3301 (*MTLa* *kek2*) or 3861 (*MTLa* *mf $\alpha$ 1*) costreaked with cells from strain 3897 (*MTL $\alpha$* ) and the second strip had cells from strain 3896 (*MTLa*) on a YEPD plate at 30°C. The rest of the mating experiment was carried as described previously (29). The mating products arising from this experiment were selected on minimal medium (see Fig. 7B). The mating products obtained from the 3301 (*MTLa* *kek2*)  $\times$  3896 (*MTLa*) cross were subjected to PCR analysis of the *MTL* alleles and also to PCR analysis for the *KEX2* gene (for sequences of the primers, see Table 2).

## RESULTS

**Identification and sequence analysis of the gene encoding the  $\alpha$  mating pheromone (*MF $\alpha$ 1*).** In a number of fungi, generation of one of the mating pheromones involves cleavage of the precursor polypeptide specifically at the C-terminal side of a Lys-Arg sequence by a serine proteinase; in *S. cerevisiae*, this proteinase is encoded by *KEX2*. In order to screen for the *C. albicans* *MF $\alpha$ 1*-encoding gene, a genome-wide search for ORFs with *KEX2* proteinase cleavage sites flanking small repeated sequences was carried out. This search led to the identification of a putative gene sequence in *C. albicans* with structural similarities to the  $\alpha$  mating pheromone of *S. cerevisiae* (Orf 6-4306 at <http://www-sequence.Stanford.edu/group/candida/>) (Fig. 1). This gene was designated *MF $\alpha$ 1* for mating factor  $\alpha$ . Another group independently reported this gene sequence as a pheromone candidate but carried out no further analysis (36).

Analysis of the gene sequence revealed a 432-bp ORF that codes for three repeats separated by *KEX2* proteinase cleavage sites, unlike the *S. cerevisiae* *MF $\alpha$ 1* gene, which contains four repeats (26). The three putative  $\alpha$  pheromone peptides are separated by spacer sequences, each containing a pair of Lys-Arg residues, the *KEX2* proteinase cleavage sites. Two of the three  $\alpha$ -peptides (SP1) are identical in sequence; the third contains an extra Lys residue at the C terminus (SP2) (Fig. 1). The N termini of two of the peptides have the -x-A spacer dipeptides, which in *S. cerevisiae* are known to be removed by the action of dipeptidyl peptidase encoded by the *STE13* gene. The presence of a homologue of *STE13* in the *Candida* genome suggests a similar role for this gene. The first repeat has a different N-terminal sequence, KG.

Analysis of the sequence upstream of the ATG start codon

TTATTATTATTTGGGAGAGCATTGAAATAAAATTTGCAACT 44  
 AGAAAAGAAAATCGACACTCAAAAATAAACTTTCGATATTCAATAAAAATACTGAATATACCAATTATTATTTAATTTG 125  
 TTTCTTTTATCTTTTATTTTTTTGAAAAATCATTTATAAACCATTCAATATAAATACTTGATTCACTTTTAAAAGACA 206  
 AAAC***TGAAACA***ATAGTCATCAATTAATCTTACAGCGAAGTAGCCACACTGATCAATATGAATT***TGAAACA***TATGCACCAC 287  
 ATTTTCAATTCATTTAATGTTTATTACATTGTTCTGCAAGTGACATTGTGGCCAATGATTAGCAGGTTAAACTATACAAAA 368  
 GAACATCGTAGAATAAGTTATATATAACATGCATATTCATTGTTTCAAACGCAGTTCATTTATGCACGTCAAGGATAATTT 449  
 CCTCTTTAGTATCGGTAAGGAACCATGTTAAAAACAACCTCTAAAGATAAACTTTTAGTACCACCTGTGCAATGAAAAAAA 530  
 AAAAAAAAAAACCCCTTTAGATCCCCAAAACCTGAAAAACCAAAGCCATACTTTTACCAAGAAGCTGTTTCTTTTGGGTAA 611  
 TAAATTGACTCTTAAAGTAATGATTTCCCCCATTTGAGTATTAGTTTTGTAAAATAAAATAAATAAATAAATAAATAAATAA 692  
 TTACCCAATTTGGCTGCGAAAGGAATTTTGTGTGAACATTAAGTGGTATACTAACCATACACTGGATAGATAGTAATAAG 773  
 TATTTTGATTCAAATTTTTCATTAAGACCATCATCTCTTTCAAATGTAGAAAT***TATAAATA***CATTCTATTTGTTTAAACA 854  
 AATATCATTTAACAAGTTTTTAATTTCAAATCAACAATCACCAACAACCTACTAATCACTCTATAACATCACTAATTTAA 935  
 TCAACAAAAATAACAATGAAATTTTCTTAACTTTATTGACTGCTACTATTGCCACTATTGTTGCTGCTGCTCCTGCTCAA 1016  
           ***M K F S L T L L T A T I A T I V A A A P A Q***  
 TACTGGTCAAGCCATTGACAGTAACCAAGTTGTTGAAATCCCAGAATCTGCCGTTGAAGCCTACTTCCCAATAGATGAT 1097  
   Y T G Q A I D S N Q V V E I P E S A V E A Y F P I D D  
 GAATTAACCTCTGCTTTGGAGAAATGATAACAACCAGTTATTTAATTGTTAATGGTACTACTCTTACCTCTGGTGCT 1178  
   E L T P V F G E I D N K P V I L I V N G T T L T S G A  
 AACAAATGAAAAAGAGAAGCTAAGTCTAAAGGTGGTTTCAGACTCACCAATTTGGGATACTTTGAACCTGGTAAAAGAGAT 1259  
   N N E ***K R*** E A K S K G ***G F R L T N F G Y F E P G K R*** D  
 GCTAACGCCGATGCTGGATTTAGACTTACTAACTTTGGTTATTTTGAACCAGGTAAAAGAGATGCCAATGCTGAAGCCGGT 1340  
   A N A D A ***G F R L T N F G Y F E P G K R*** D A N A E A ***G***  
 TTTAGACTTACCAATTTTGGTTATTTTGAACCTGGTAAATAAGTAAGTAAAGAAGATGGAAAGCATACTGAAATCATACTT 1421  
   ***F R L T N F G Y F E P G K \****  
 TTATGTTGTTTATAGATGAATTGGTATGTCTTTTGGTATTTACTTAAGCTTATTGAAATCCGTTTTCTAAAAGAAATT 1500

FIG. 1. Nucleotide sequence of the *MF $\alpha$ 1* gene and deduced amino acid sequence of its product. The sequence of a 1.5-kb *C. albicans* genomic fragment carrying the *MF $\alpha$ 1* gene is shown. One-letter codes for amino acids are shown beneath the nucleotide sequence. Regions encoding the mature  $\alpha$  mating pheromones are in boldface and underlined. Possible cleavage signals (Lys-Arg) recognized by the *KEX2* proteinase are highlighted in grey. A stretch of 19 amino acids which could serve as the possible signal sequence for translocation of the precursor polypeptide across the membrane of the endoplasmic reticulum is in boldface and italic. A putative TATA box in the region upstream of the ORF is in boldface. Putative PREs are in boldface and italic.

revealed the presence of a putative TATA box and sequences that are important for regulating the expression of this gene in *S. cerevisiae*. An example is the presence of pheromone response elements (PREs) in the upstream region of *MF $\alpha$ 1* (10, 20). The hydrophobicity profile of the 143-amino-acid precursor peptide revealed the presence of a unique hydrophobic region (19 amino acid residues) at the N terminus; this region may serve as a signal sequence during translocation of the precursor across the membrane of the endoplasmic reticulum (Fig. 1). Thus, the overall structural similarity of the *MF $\alpha$ 1* gene of *C. albicans* to the *S. cerevisiae* *MF $\alpha$ 1* gene indicates that the mechanism for maturation of the *C. albicans* *MF $\alpha$ 1* gene product may be similar to that of the *S. cerevisiae* pheromone (26, 38).

**The *MF $\alpha$ 1* null mutant fails to mate as *MTL $\alpha$* .** In order to ascertain the role of *MF $\alpha$ 1* in mating in *C. albicans*, both copies of this gene were deleted in the strain RM1000 (3752). The *MF $\alpha$ 1* null mutant was generated by a complete deletion of the 432-bp ORF as described in Materials and Methods (Fig. 2A, D, E, and F). The null mutant (3852) was then grown on

sorbose-containing medium to obtain *MTL $\alpha$*  (3863) and *MTL $\alpha$*  (3861) strains and tested for mating (Table 1). The *MTL $\alpha$  mfa1<sup>-/-</sup>* cells were unable to mate, but the *MTL $\alpha$  mfa1<sup>-/-</sup>* cells were as fertile as the parent (Fig. 3A). Reintroduction of the *MF $\alpha$ 1* gene in the null mutant restored mating in the *MTL $\alpha$*  cells (3898) (Fig. 2B to F and 3A), indicating that the *MF $\alpha$ 1* gene is cell type specific. Reintroduction of a construct containing the truncated *mfa1* gene in the null mutant (3902) did not restore mating, indicating that restoration of the mating phenotype in the revertant strain (3898) was solely due to the *MF $\alpha$ 1* gene and was not due to problems with the expression of the *URA3* gene (Fig. 2C to F and 3A). PCR analysis of the mating products confirmed the mating phenotype by demonstrating the presence of both the *MTL $\alpha$*  and *MTL $\alpha$*  alleles (Fig. 3B).

**Biological activity of the two  $\alpha$  pheromone peptides and their effect on *MTL $\alpha$*  and *MTL $\alpha$*  cell types.** In *S. cerevisiae* the  $\alpha$ -factor is known to induce morphological changes in *MAT $\alpha$*  cells that are characteristic of the mating process in this budding yeast. The appearance of morphologically abnormal cells

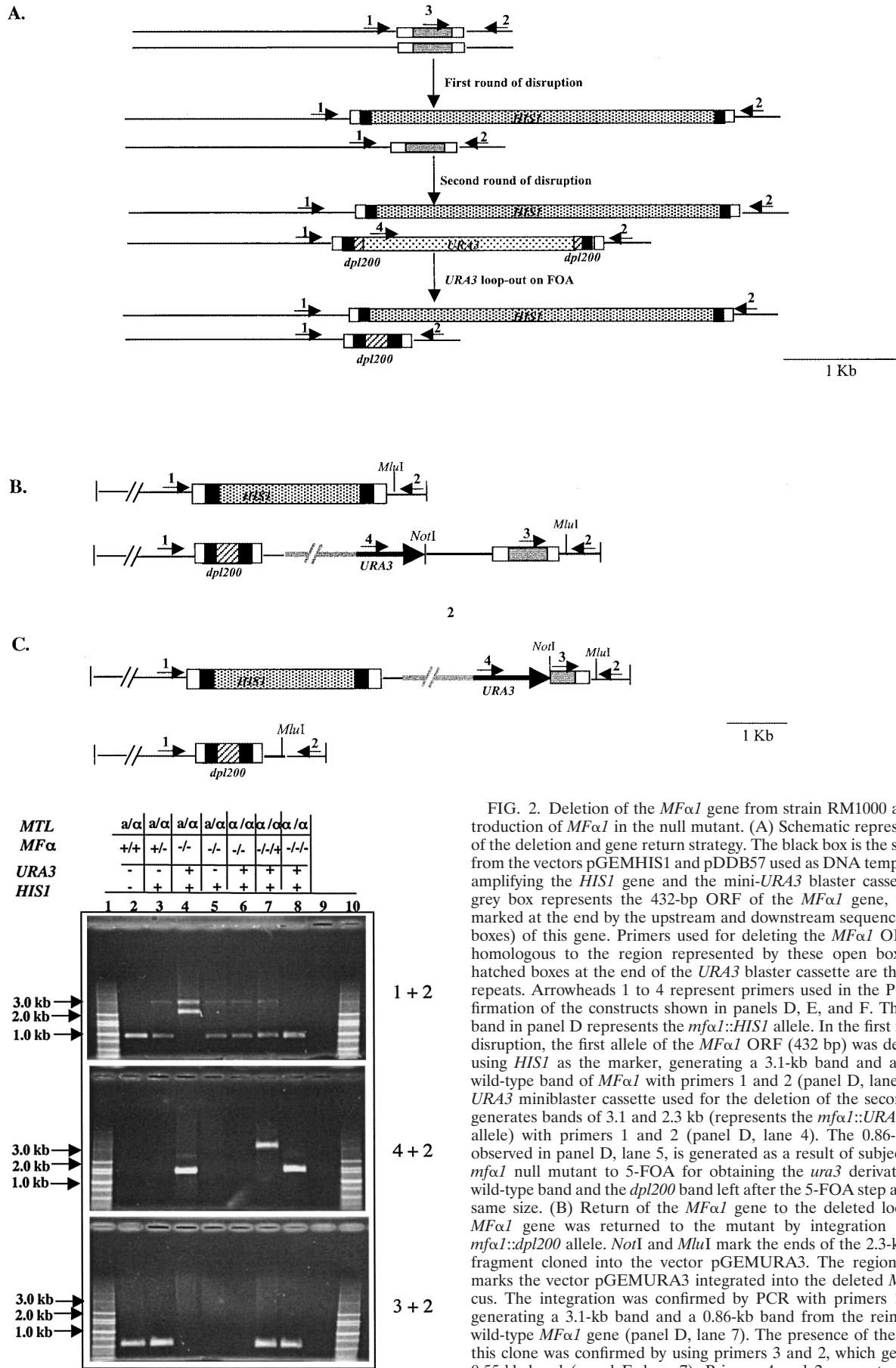


FIG. 2. Deletion of the *MFα1* gene from strain RM1000 and reintroduction of *MFα1* in the null mutant. (A) Schematic representation of the deletion and gene return strategy. The black box is the sequence from the vectors pGEMHIS1 and pDDB57 used as DNA templates for amplifying the *HIS1* gene and the mini-*URA3* blaster cassette. The grey box represents the 432-bp ORF of the *MFα1* gene, which is marked at the end by the upstream and downstream sequences (open boxes) of this gene. The hatched boxes at the end of the *URA3* blaster cassette are the *dpl200* repeats. Arrowheads 1 to 4 represent primers used in the PCR confirmation of the constructs shown in panels D, E, and F. The 3.1-kb band in panel D represents the *mfα1::HIS1* allele. In the first round of disruption, the first allele of the *MFα1* ORF (432 bp) was deleted by using *HIS1* as the marker, generating a 3.1-kb band and a 0.86-kb wild-type band of *MFα1* with primers 1 and 2 (panel D, lane 3). The *URA3* miniblaster cassette used for the deletion of the second allele generates bands of 3.1 and 2.3 kb (represents the *mfα1::URA3-dpl200* allele) with primers 1 and 2 (panel D, lane 4). The 0.86-kb band observed in panel D, lane 5, is generated as a result of subjecting the *mfα1* null mutant to 5-FOA for obtaining the *ura3* derivative. The wild-type band and the *dpl200* band left after the 5-FOA step are of the same size. (B) Return of the *MFα1* gene to the deleted locus. The *MFα1* gene was returned to the mutant by integration into the *mfα1::dpl200* allele. *NotI* and *MluI* mark the ends of the 2.3-kb *MFα1* fragment cloned into the vector pGEMURA3. The region in grey marks the vector pGEMURA3 integrated into the deleted *MFα1* locus. The integration was confirmed by PCR with primers 1 and 2, generating a 3.1-kb band and a 0.86-kb band from the reintegrated wild-type *MFα1* gene (panel D, lane 7). The presence of the ORF in this clone was confirmed by using primers 3 and 2, which generate a 0.55-kb band (panel F, lane 7). Primers 4 and 2 generate a 3.2-kb

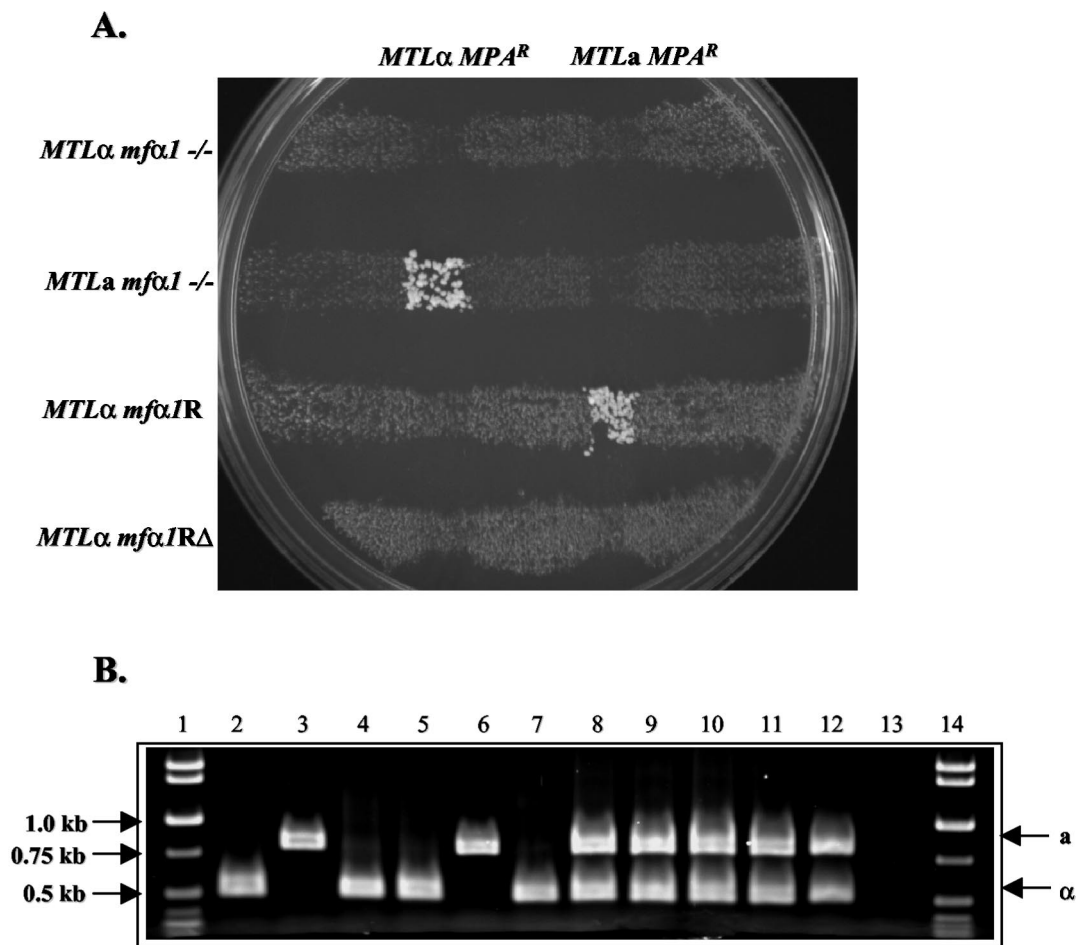


FIG. 3. Mating of the *mfa1* null strains and the revertants. (A) Strains were grown and cross streaked, and mating products were selected on minimal MPA plates as described in Materials and Methods. The strains used were 3861 (*MTL $\alpha$  mfa1<sup>-/-</sup>*), 3863 (*MTLa mfa1<sup>-/-</sup>*), 3898 (*MTL $\alpha$  mfa1* pGEMURA3/*MF $\alpha$ 1*), 3902 (*MTL $\alpha$  mfa1* pGEMURA3/*mfa1 $\Delta$* ), 3685 (*MTL $\alpha$  MPA<sup>R</sup> his<sup>-</sup> arg<sup>-</sup>*), and 3710 (*MTLa MPA<sup>R</sup> his<sup>-</sup> arg<sup>-</sup>*). *MTL $\alpha$  mfa1<sup>IR</sup>* and *MTL $\alpha$  mfa1<sup>IR</sup> $\Delta$*  are the same as strains 3898 and 3902, respectively. (B) PCR analysis of the *MTL* alleles in the parents and the mating products. Mating products were picked from the mating plates and restreaked on a minimal MPA (10  $\mu$ g/ml) plate. Genomic DNAs from strains 3861 (lane 2), 3863 (lane 3), 3898 (lane 4), 3902 (lane 5), 3710 (lane 6), 3685 (lane 7), the 3863  $\times$  3685 mating product (lanes 8 and 9), the 3898  $\times$  3710 mating product (lanes 10 and 11), and SC5314 (lane 12) were used as templates for performing PCR with the *MTLa*- and *MTL $\alpha$* -specific primers. Lane 13, no-DNA control for the PCR. Lanes 1 and 14, DNA molecular size markers. The positions of the *MTLa*-specific and *MTL $\alpha$* -specific bands are marked by the arrows on the right.

fragment (panel E, lane 7) for this integration event. (C) Return of a truncated *MF $\alpha$ 1* gene to the deleted locus. A clone containing the truncated *MF $\alpha$ 1* ORF was integrated into the *mfa1::HIS1* allele. *NotI* and *MluI* mark the ends of the 0.55-kb fragment carrying the truncated *MF $\alpha$ 1* ORF (0.29 kb), cloned into the vector pGEMURA3. Primers 1 and 2 generate the 0.86-kb *dpl200* band only (panel D, lane 8). The presence of *URA3* (1.6 kb) and the truncated *MF $\alpha$ 1* gene (0.55 kb) is confirmed by using primers 4 and 2 and primers 3 and 2, respectively (panels E and F, lanes 8). The details of the deletion and replacement strategies are described in Materials and Methods. (D, E, and F) PCR results confirm the integration of the two cassettes in the deletion construct. Each set of reactions shows the results of PCR with different primer pairs (primers 1 and 2, 4 and 2, and 3 and 2, respectively) carried out on genomic DNA from the wild type (3752) (lanes 2), 3842 (lanes 3), 3845 (lanes 4), 3852 (lanes 5), 3861 (lanes 6), 3898 (lanes 7), and 3902 (lanes 8). Lanes 9, no-DNA controls for the PCR. Lanes 1 and 10, DNA molecular size markers.

allows a sensitive method for the assay of  $\alpha$ -factor biological activity (reviewed in reference 8). Similar morphological changes have been observed in mixtures of *C. albicans MTLa* and *MTL $\alpha$*  cells (27).

When the  $\alpha$ -peptides SP1 and SP2 were spotted onto patches of *MTLa* (3716) and *MTL $\alpha$*  (3144) cells as described in Materials and Methods, only the *MTLa* patch became wrinkled in response to the SP1 peptide after 3 days of incubation at 25°C, while there was no change observed in either the *MTL $\alpha$*  cells (Fig. 4A and B) or the *MTLa* white cells (data not shown) of the same strain. Since Miller and Johnson showed that opaque-phase cells are several orders of magnitude more efficient at mating (33), it is not surprising that they are more sensitive to the pheromone than are the cells in the white phase. When cells were sampled from the wrinkled streak, a high number of cells with evaginations or shmooing were observed (Fig. 4E and G). Every single cell seemed to filament in

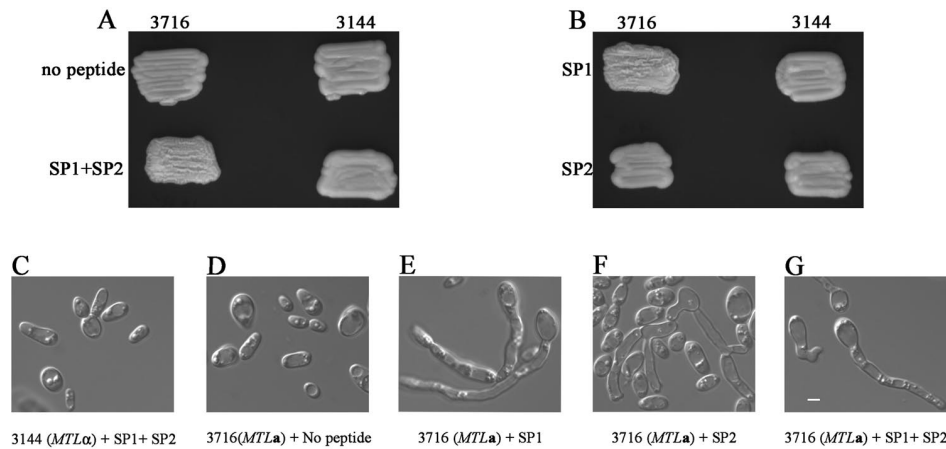


FIG. 4. Colony phenotypes induced by  $\alpha$ -peptides. Strains were patched, and peptides SP1 and SP2 were spotted onto them as described in Materials and Methods. (A) *MTLa* (3716) and *MTLa* (3144) opaque-phase cells spotted with 2% DMSO (upper panel) and with 10  $\mu$ g of SP1 per ml plus 5  $\mu$ g of SP2 per ml (lower panel). (B) Cells spotted with 10  $\mu$ g of SP1 per ml (upper panel) and 10  $\mu$ g of SP2 per ml (lower panel). (C to G) Phase-contrast images of cells scooped from plates A and B. Bar, 5  $\mu$ m.

response to SP1. In contrast to SP1, peptide SP2 did not induce wrinkling in the *MTLa* cells, but cells that were scooped from the patch did show some shmooing (Fig. 4B and F). Fewer cells shmoo in response to SP2 than do so in response to SP1. The

*MTLa* opaque-phase cells from strain 3144 did not respond to either of the two peptides (Fig. 4A to C). Peptide SP2 (GFRLTNFGYFEPGK) differs from SP1 (GFRLTNFGYFEPG) in having an extra lysine residue at the C terminus.

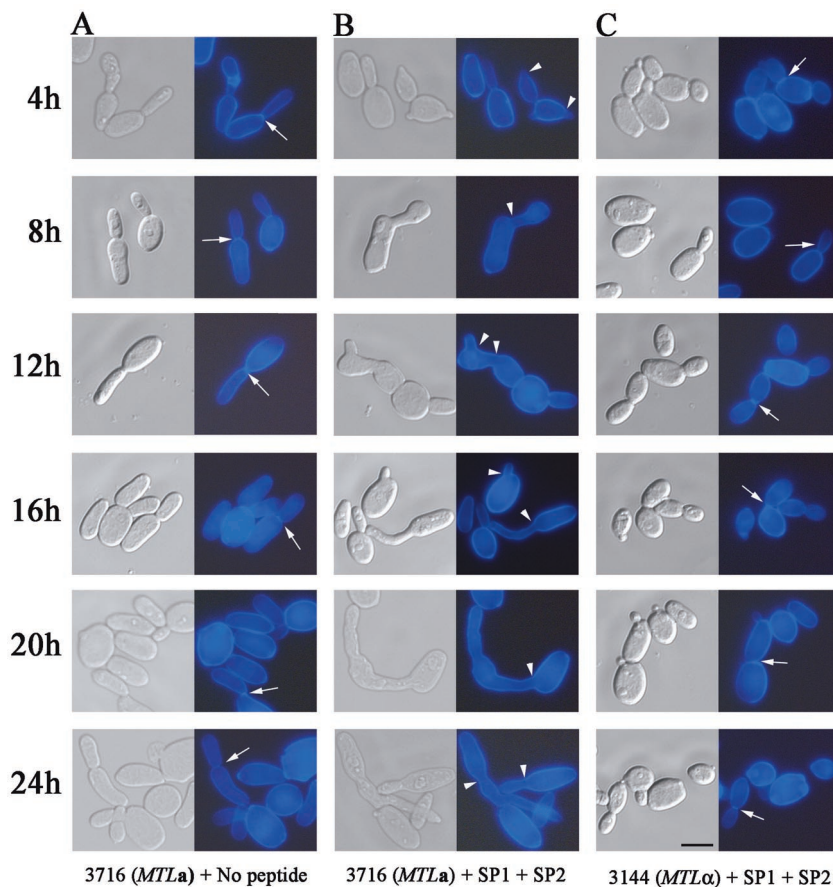


FIG. 5. Shmooing of opaque-phase cells in response to the  $\alpha$ -peptides occurs exclusively in the *MTLa* cell type. The response of the cells in modified Lee's medium is shown. The left and right columns in each panel show phase-contrast and fluorescent images of Calcofluor-stained cells, respectively. (A) 3716 (*MTLa*) opaque-phase cells without peptide; (B) 3716 (*MTLa*) opaque-phase cells with peptides SP1 and SP2 (10 and 5  $\mu$ g/ml, respectively); (C) 3144 (*MTLa*) opaque-phase cells with peptides SP1 and SP2 (10 and 5  $\mu$ g/ml, respectively). The arrows point to the septum (A and C), while the arrowhead points to the region where a septum should have been present (B). Bar, 10  $\mu$ m.

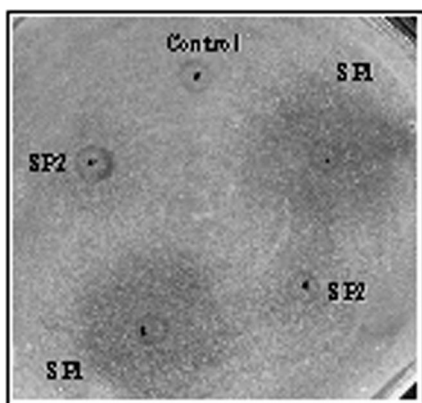


FIG. 6. *MTL $\alpha$*  opaque-phase cells generate a halo in response to the  $\alpha$ -peptides. The dots mark the areas where the peptides were spotted. Methanol (50%) was spotted as the control on a lawn of *MTL $\alpha$*  opaque-phase cells of strain 3745. Five microliters from a 1-mg/ml stock solution of the peptides SP1 and SP2 was spotted onto this lawn of cells in duplicates. The ring seen around the spotting point is an artifact generated due to the solvent. Halos represent the zone of growth inhibition of *MTL $\alpha$*  cells in response to peptides SP1 and SP2, as observed after 48 h.

In order to assay directly for the activity of the *MF $\alpha$ 1*-encoded peptide, opaque-phase cells from homozygous *MTL $\alpha$*  (3716) and *MTL $\alpha$*  (3144) strains of *C. albicans* were incubated with the  $\alpha$ -peptides (SP1 plus SP2) in modified Lee's medium at 25°C and monitored for changes in the morphology at different time points as described in Materials and Methods. The *MTL $\alpha$*  cells started to evaginate 2 h after incubation with the peptides and formed tube-like structures (called shmooing) with time, similar to the mating figures observed in previous studies (27), while the *MTL $\alpha$*  cells without the peptides and *MTL $\alpha$*  cells with peptides did not shmoo even after 24 h of incubation (Fig. 5). Calcofluor, which stains chitin and thus the septa of yeast cells, was used to examine these cells. The shmooing figures formed in response to the peptide were continuous, with no visible septation even after 24 h of incubation, thereby authenticating the absence of hyphae and presence of true shmoos (Fig. 5, right panels). To demonstrate that this effect of the  $\alpha$ -peptide is not strain specific, different strains homozygous at the *MTL $\alpha$*  locus were tested for shmooing (data not shown). In all of the cases shmooing was limited to the *MTL $\alpha$*  cells, while the *MTL $\alpha$*  cells did not shmoo at all.

**SP1 and SP2 generate halos when placed on *MTL $\alpha$*  opaque-phase cells.** The  $\alpha$  and  $\alpha$  pheromones in *S. cerevisiae* are known to act on the opposite mating types by inducing a reversible arrest in the  $G_1$  phase of the cell cycle. This arrest, which occurs prior to shmooing, is important for synchronization of the cell cycles in the two mating types (2, 32, 38). One of the qualitative assays for the mating pheromones is based on their ability to inhibit the growth of cells of the opposite mating type (in this case *MTL $\alpha$*  cells in response to the  $\alpha$ -factor) in the surrounding area, resulting in a clear zone or halo. In order to examine the role of the *MF $\alpha$ 1*-encoded peptides in growth inhibition, we carried out halo tests with peptides SP1 and SP2. We found that peptide SP1 is more capable of inhibiting the growth of the *MTL $\alpha$*  opaque-phase cells, while SP2 has a lesser but observable effect on these cells at the concentrations used

in this experiment (Fig. 6). The halos produced in response to SP1 or SP2 are not as clear as the halos observed with the *S. cerevisiae*  $\alpha$  pheromone (30). This might be due either to some of the cells switching back to the white cell type or to the presence of a barrier activity similar to the endopeptidase observed in *S. cerevisiae* (13). As discussed below, *C. albicans* has candidates for such an activity.

**Exogenously added synthetic  $\alpha$ -peptide renders the *MTL $\alpha$  kex2* mutant mating efficient.** We know from our previous work that the *kex2* mutant does not mate as *MTL $\alpha$* , while the *MTL $\alpha$  kex2* mutant is as fertile as the parent strain (28). If the sole reason for the inability of the *kex2* mutant to mate as *MTL $\alpha$*  is due to the absence of mature  $\alpha$  pheromone, then the addition of the *MF $\alpha$ 1*-encoded peptides, viz., SP1 and SP2, should be able to complement this mating defect. To test this possibility, the *MTL $\alpha$  kex2* mutant was mated against an *MTL $\alpha$*  strain on plates supplemented with various concentrations (as mentioned in Materials and Methods) of peptides SP1 and SP2 added separately and in combination. The *MTL $\alpha$  kex2* strain was able to mate in the presence of SP1 and SP1 plus SP2 at final concentrations of  $6 \times 10^{-5}$  and  $6 \times 10^{-6}$  M (Fig. 7A), but it failed to do so at concentrations of  $6 \times 10^{-7}$  and  $6 \times 10^{-8}$  M. This mutant strain was not able to mate in presence of SP2 alone even at the highest concentration ( $6 \times 10^{-5}$  M), thereby suggesting that although SP2 alone can induce shmooing and a small halo in *a* cells, it is not potent enough to render *kex2* cells mating competent at the concentrations used in this study (Fig. 7A, 5F, and 6). In order to achieve a physiological level of the  $\alpha$  pheromone in the mating reaction, the *MTL $\alpha$  kex2* mutant was mixed with strain 3896 (*MTL $\alpha$* ) in the presence and absence of strain 3897 (*MTL $\alpha$* ). This strain, which is *his3<sup>-</sup>* and cannot complement 3896, was added to serve as a source of  $\alpha$ -factor in this mixture (Table 1). The mating defect of the *MTL $\alpha$  kex2* mutant was also complemented in this experiment (Fig. 7B).

If the colonies arising from these experiments are true mating products, they should harbor a wild-type copy of the *KEX2* gene along with the deleted copy, in addition to containing both of the *MTL* alleles. Therefore, two sets of primers were used for the PCR analysis of these mating products. PCR analysis with the *KEX2*-specific primers indicated the presence of both the wild-type and the deletion copies of this gene (Fig. 7C, lanes 6 to 8). Similarly PCR with the *MTL*-specific primers demonstrated the presence of both of the *MTL* alleles (Fig. 7D, lanes 5 to 7). These experiments confirmed the authenticity of these mating products and demonstrate that the *MF $\alpha$ 1*-encoded peptide SP1 indeed is capable of supplementing the  $\alpha$  pheromone defect in the *MTL $\alpha$  kex2* mutant strains, thus confirming the role of this gene in mating in *C. albicans*. Experiments similar to those with the *kex2* deletion strain were performed with the *MTL $\alpha$  mfa1* mutant strain, but no complementation was observed (Fig. 7A).

## DISCUSSION

Pheromones are ubiquitous in fungal mating systems, and even the general structure is conserved over the fungal kingdom, from *S. pombe* (7) and *N. crassa* (9, 12) to basidiomycetes such as *Cryptococcus neoformans* (5). The discovery of mating in *C. albicans* led to the search for the pheromones involved.



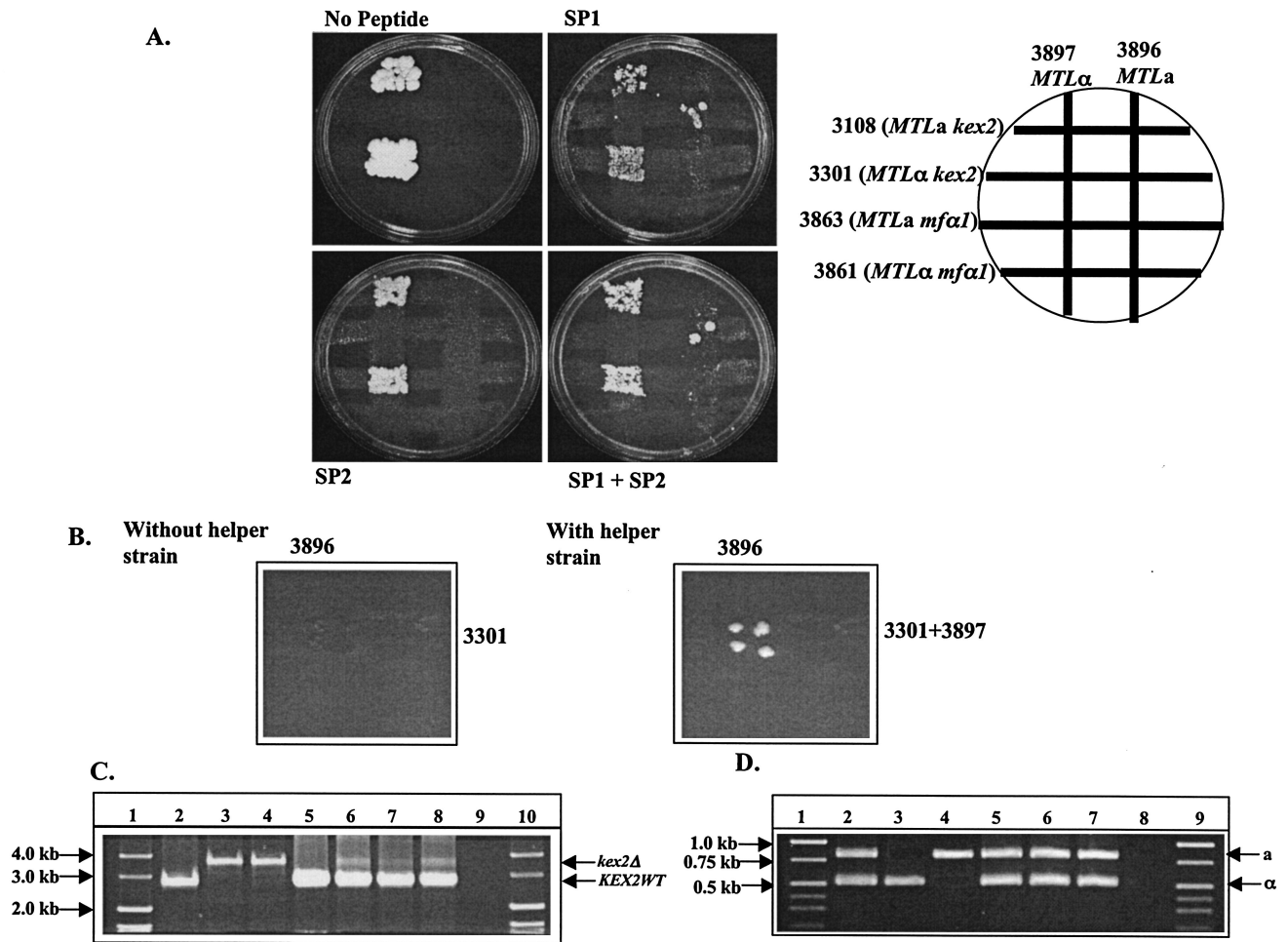


FIG. 7. Mating of the *kex2* and *mfa1* null mutants in the presence of exogenously added  $\alpha$ -peptides. (A) Synthetic peptide can complement the mating defect in an *MTLa kex2* strain but not in an *MTLa mfa1* strain. A diagram of the mating plate is shown on the upper right. The strains used were 3108 (*MTLa kex2*), 3301 (*MTLa kex2*), 3863 (*MTLa mfa1*), 3861 (*MTLa mfa1*), 3896 (*MTLa his3<sup>-</sup>*), and 3897 (*MTLa his3<sup>-</sup>*). SP1 and SP2 concentrations of  $6 \times 10^{-5}$  M of were used in the plates. (B) An *MTLa* helper strain can complement the mating defect in an *MTLa kex2* strain but not in an *MTLa mfa1* strain. The strains used were 3896 (*MTLa his3<sup>-</sup>*), 3301 (*MTLa kex2*), and the  $\alpha$  helper strain 3897 (*MTLa his3<sup>-</sup>*). (C) PCR analysis of the *KEX2* locus in parents and mating products. The position of the wild-type band (*KEX2*WT) and the deletion band (*kex2* $\Delta$ ) of *KEX2* are marked by arrows. Lane 2, SC5314; lane 3, 3301; lane 4, 3108; lane 5, 3896; lane 6, mating product picked from right plate in panel B; lanes 7 and 8, mating products from plates with SP1 and SP1 plus SP2, respectively (A); lane 9, no-DNA control for the PCR; lanes 1 and 10, the DNA molecular size markers. (D) PCR analysis of the *MTL* alleles in parents and mating products. The positions of the *MTLa*- and *MTL $\alpha$* -specific bands are marked by arrows. Lane 2, SC5314; lane 3, 3301; lane 4, 3108; lane 5, mating product picked from right plate in panel B; lanes 6 and 7, mating products from plates with SP1 and SP1 plus SP2, respectively (A); lane 8, no-DNA control for the PCR; lanes 1 and 9, DNA molecular size markers.

The search for the  $\alpha$  pheromone was guided by the similarity of pheromone structure across the fungal kingdom and by the observation that the *KEX2* gene is essential for mating (28). Based on the *KEX2* site specificity, we identified a sequence which encodes a putative protein of 143 amino acids containing a hydrophobic leader sequence, three identical repeats preceded by the sequence KR (the site of cleavage by the *KEX2* proteinase), and sequences similar to the PREs of *S. cerevisiae* in the 5' untranslated region. The pheromone-stimulated transcriptional induction of genes is conferred by the presence of PREs, which are *cis*-acting DNA sequences present in the upstream regions of genes (24, 40). The presence of these sequences suggests that the regulation of the  $\alpha$  pheromone might be similar in the two yeasts. Further confirmation of the

importance of these sequences will come from a detailed mutational analysis of the upstream region. Although there are three identical repeats in the gene, processing by the *KEX2* proteinase would leave one of the three with a C-terminal lysine. We synthesized both the lysine-containing (SP2) and the lysine-free (SP1) peptides to determine their biological effects. Purification of the  $\alpha$ -factor and subsequent determination of its peptide sequence will lead to the identification of the exact cleavage sites in this protein and should also determine whether the peptide containing the extra Lys residue is secreted. A homologue of *KEX1*, whose product cleaves C-terminal lysine and arginine, and a homologue of *STE13*, encoding the dipeptidyl aminopeptidase in *S. cerevisiae* which removes the amino acids at the N terminus, exist in the *Can-*

*did*a genome database (39). However, since the first peptide repeat of *MF $\alpha$ 1* is preceded by a KG sequence, not the DA and EA of the other two repeats, the enzyme from *C. albicans* must have some differences in specificity compared to Ste13p from *S. cerevisiae*, since the *S. cerevisiae* Ste13p is known to cleave at the x-A dipeptides (21). Thus, although the mechanisms of precursor processing in the two yeasts are similar on the surface, some of the details must be different.

The biological significance of the *MF $\alpha$ 1* sequence was demonstrated by the fact that deletion of both alleles of this gene led to sterility in *MTL $\alpha$*  but not *MTL $\alpha$*  strains, and return of the wild-type *MF $\alpha$ 1* gene in the *mfa1* null mutant restored its mating ability. The synthetic peptides (SP1 and SP2) induced morphological changes (shmooing) in *MTL $\alpha$*  but not *MTL $\alpha$*  strains. Furthermore, these peptides could also complement the mating defect of the *kex2* null mutant in the mating reaction. All of these findings suggest that the gene we have isolated is the biologically active *MF $\alpha$ 1* gene and that, unlike *Saccharomyces* but similar to *Neurospora* (11) and *S. pombe* (7), *C. albicans* seems to carry only one copy of this pheromone gene. SP1, the 13-amino-acid (lysine-free) peptide seems to be more active than the 14-amino-acid SP2. In most experiments in which they were tried together and singly, the effect of the two together was not significantly different from that of SP1 alone, and the effect of SP2 was usually much less. SP2 alone also did not complement the mating defect of the *kex2* mutant.

We examined the activity of the *MF $\alpha$ 1*-encoded peptides by taking advantage of the ability of these peptides to diffuse through agar and cause halos in a lawn of *MTL $\alpha$*  opaque-phase cells. The growth inhibition is probably due to cell cycle arrest, but we have not shown this microscopically. Despite the fact that no homologue of the *BARI* (*SST1*) gene has been found in the *Candida* database, the halos were diffuse. Bar1p is an aspartyl proteinase, and *C. albicans* has a large gene family encoding secreted aspartyl proteinases (the *SAP* gene family) with widely differing patterns of expression. Some of these previously identified proteinases may function to degrade the pheromone; for example, the *SAP1* and *SAP3* genes have been shown to be differentially expressed in the opaque (mating) phase (15). Since mutations in each of these genes are available, their role in mating could be easily tested (16).

The ability of the exogenously added  $\alpha$ -peptide (SP1) to alleviate the mating defect of the *MTL $\alpha$  kex2* mutant is consistent with the results obtained with the *MAT $\alpha$  kex2* and the *MAT $\alpha$  ste13* mutant strains of *S. cerevisiae*, wherein the addition of exogenous  $\alpha$ -factor has been shown to partially complement the mating defects of these two strains (4). It has been shown for *S. cerevisiae* that the *MAT $\alpha$  kex2* mutant secretes very small amounts of the *MF $\alpha$*  peptides, which are undetectable by bioassay and just barely detectable by a sensitive radioimmunoassay (21). This might be one reason why the *MF $\alpha$* -encoded peptides were able to restore the mating defect in the *MTL $\alpha$  kex2* mutant strains of both *C. albicans* and *S. cerevisiae*.

On the other hand, neither the addition of exogenous  $\alpha$ -factor at various concentrations nor the presence of a helper *MTL $\alpha$*  strain could complement the mating defect of the *mfa1* null mutant. This result was also obtained for *MAT $\alpha$  mfa1 mfa2* double mutants of *S. cerevisiae* (25). If the precursor peptides have a role in the mating reaction in *MTL $\alpha$*  cells, the failure of the added peptide to complement the *MF $\alpha$ 1* deletion

would be explained, since the *MTL $\alpha$  mfa1* strains of *C. albicans* and the *MAT $\alpha$  mfa1 mfa2* strains of *S. cerevisiae* totally lack the endogenous precursor peptide. One possible role of the precursor peptide could be to trigger a mating signal, distinct from the signals that lead to growth arrest or shmooing, in *MTL $\alpha$*  cells. A second possibility that could account for the failure of the  $\alpha$ -peptides to complement the mating defect of the *MTL $\alpha$  mfa1* null mutant would be the concentration of the peptides used for this experiment. In *S. cerevisiae*, high levels of exogenous  $\alpha$  pheromone can complement the mating defect of the *MAT $\alpha$  mfa1 mfa2* strain in crosses to *MAT $\alpha$*  strains that contain mutations that result in supersensitivity to  $\alpha$ -factor (*sst1-2* and *sst2-1*) (25). The supersensitivity of these mutants is due to a defect in the degradation of  $\alpha$ -factor (30). If *C. albicans* has a highly active degradation process, the pheromones might not have reached the necessary concentration in our experiments.

In keeping with previous observations that the mating reaction in *C. albicans* resembles that in *S. cerevisiae* in a great many ways, the  $\alpha$  pheromone involved is also similar, in that it is a small peptide, it requires processing by the product of the *KEX2* gene, and it causes morphological changes in cells of the *MTL $\alpha$*  mating type. The amino acid sequence of the *C. albicans*  $\alpha$  pheromone, however, is unrelated to that of *S. cerevisiae*, forming a sterility barrier between these two yeasts. The lack of any other *MF $\alpha$ 1* genes in the *C. albicans* genome assembly distinguishes this yeast's pheromone gene organization from that of *S. cerevisiae*, which has two *MF $\alpha$ 1* genes. Although an  $\alpha$  pheromone gene has not yet been reported, it seems very likely that one must exist, and we are presently engaged in an attempt to identify one. This report further supports both the general properties of pheromones in fungal mating and the close homology in the mating processes of two distantly related yeasts, *C. albicans* and *S. cerevisiae*. It is somewhat surprising that the process has been so closely conserved over close to two hundred million years (31); this finding would suggest that mating in *Candida* has an important function, despite the reported absence of meiosis. One possible function of mating, of course, is generation of the diversity required for its unique life style, including both commensality and pathogenesis.

#### ACKNOWLEDGMENTS

We thank Dana Davis and B. B Magee for their comments on the manuscript. We appreciate the assistance of Mark Sanders, Kelly Josephson, and Taryn O'Hall in generating the images on the microscope at the imaging center, University of Minnesota, St. Paul. We deeply appreciate Maryam-Gerami Nejad for helpful suggestions during the preparation of the manuscript.

This work was supported by grant AI16567 awarded to P.T.M.

#### ADDENDUM IN PROOF

After this article was accepted for publication, two reports describing experiments on *MF $\alpha$ 1* and its products in *C. albicans* were published: S. R. Lockhart et al., *Eukaryot. Cell* 2:847–855, 2003; and R. J. Bennett, *Mol. Cell. Biol.* 23:8189–8201, 2003.

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