MOLECULAR AND CELLULAR BIOLOGY, Apr. 1985, p. 787–796
0270-7306/85/040787-10$02.00/0

Vol. 5, No. 4

# α-Factor Structural Gene Mutations in *Saccharomyces cerevisiae*: Effects on α-Factor Production and Mating

JANET KURJAN

*Department of Biological Sciences, Columbia University, New York, New York 10027*

Received 30 July 1984/Accepted 14 January 1985

**The role of the α-factor structural genes *MFα1* and *MFα2* in α-factor production and mating has been investigated by the construction of *mfα1* and *mfα2* mutations that totally eliminate gene function. An *mfα1* mutant in which the entire coding region is deleted shows a considerable decrease in α-factor production and a 75% decrease in mating. Mutations in *mfα2* have little or no effect on α-factor production or mating. The *mfα1 mfα2* double mutants are completely defective in mating and α-factor production. These results indicate that at least one α-factor structural gene product is required for mating in *MATα* cells, that *MFα1* is responsible for the majority of α-factor production, and that *MFα1* and *MFα2* are the only active α-factor genes.**

Each of the haploid cell types of the yeast *Saccharomyces cerevisiae* secretes an oligopeptide pheromone that produces specific responses in cells of the opposite mating type (for a review, see references 22 and 38). *MATα* cells produce α-factor, an oligopeptide of 13 amino acids (9, 29, 35, 36), and *MAT***a** cells produce **a**-factor, an oligopeptide of 11 amino acids (1). The following lines of evidence suggest that α-factor plays an important role in the mating process. (i) α-Factor arrests *MAT***a** cells at the position in the G1 phase of the cell cycle at which mating between *MAT***a** and *MATα* cells can take place (4, 9, 11). (ii) α-Factor induces cell surface agglutinins on *MAT***a** cells that promote aggregation with *MATα* cells (for a review, see reference 39). (iii) Many mating-defective (sterile) mutants are also defective in α-factor production in *MATα* cells or in α-factor response in *MAT***a** cells (20, 32). (iv) Mutations in *MAT***a** strains that result in resistance to α-factor (i.e., which allow growth of the cells in the presence of α-factor) also result in sterility (12, 23). (v) The mating deficiency of *ste13* or *kex2* strains, which are defective in processing of the α-factor precursor, can be partially alleviated by the addition of exogenous α-factor (5, 13, 14). To determine whether α-factor is absolutely essential for the mating process, it is necessary to analyze the effects of mutations in the α-factor structural gene(s). No α-factor structural gene mutations have been identified by genetic analysis. Instead, the α-factor structural genes have been identified by cloning (18, 31).

The cloning results have indicated that α-factor is encoded within a small multigene family consisting of at least two α-factor structural genes, *MFα1* and *MFα2* (18, 31). The first α-factor gene to be identified, *MFα1*, was isolated by virtue of the ability of *MFα1* to overproduce α-factor when present in yeast cells on a high-copy-number plasmid (18). *MFα1* and a second α-factor gene, *MFα2*, were isolated by utilizing synthetic oligonucleotide probes that were predicted to be homologous to α-factor coding sequences based on the amino acid sequence (31). From their nucleotide sequences, *MFα1* and *MFα2* encode α-factor precursors of 165 and 120 amino acids, respectively (18, 31). The *MFα1* and *MFα2* precursors have similar structures consisting of three regions: a signal sequence, a region of ca. 60 amino acids within which are three glycosylation sequences, and a region containing tandem repeats of α-factor or α-factor-like sequences preceded by spacer sequences of 6 to 8 amino acids. The *MFα1* precursor contains four identical α-factor repeats; the *MFα2* precursor contains two repeats consisting of one α-factor-like repeat (differing from α-factor by two amino acid substitutions) and one α-factor repeat. In vitro translation experiments suggest that *MFα1* is responsible for the majority of α-factor precursor produced (2).

To investigate further the relative roles of *MFα1* and *MFα2* in α-factor production and the role of the α-factor structural genes in α mating, I have undertaken a genetic analysis of *MFα1* and *MFα2*. In this paper, I describe the construction and analysis of mutants containing alterations in *MFα1* and *MFα2* that totally eliminate the function of these genes (null mutants). These experiments show that at least one of the two α-factor genes is required for mating by *MATα* cells, that *MFα1* is responsible for the majority of the α-factor produced, and that *MFα1* and *MFα2* are the only active α-factor structural genes. In addition, the ability of exogenous α-factor to alleviate the mating defect of *MATα* *mfα1 mfα2* double mutants has been investigated.

## MATERIALS AND METHODS

**Strains and plasmids.** Yeast strains are listed in Table 1. *Escherichia coli* strains RRI and GM33 (*dam*$^-$ for isolation of plasmids to be cleaved with *Bcl*I) were used for *E. coli* transformations. Plasmid p69A (18) contains the *MFα1* gene on the high copy number, autonomously replicating yeast vector YEp13 (3). Plasmids pHK2 and pBH3 were provided by I. Herskowitz. pHK2 is pBR328 containing the 1.7-kilobase (kb) yeast *Eco*RI fragment from plasmid p69A that includes the *MFα1* gene. pBH3 was constructed by first eliminating the *Hin*dIII site of pBR328 and then subcloning the 1.7-kb *MFα1* *Eco*RI fragment into this plasmid. The resulting plasmid, pBH3, contains two tandem repeats of the 1.7-kb *MFα1* *Eco*RI fragment. Insertion of the *LEU2* or *URA3* gene into *MFα1* and *MFα2* utilized *LEU2*-containing fragments from YEp13 or *URA3*-containing fragments from the plasmid YIp30 constructed by M. Rose.

**Media.** Media were as described previously (18) with the exception of MAC or MAS plates, which are minimal synthetic media plus 20 μg of adenine per ml buffered to pH 4.8 (with 0.1 M sodium citrate) or to pH 6 (with 0.05 M sodium succinate), respectively.

**α-Factor and mating assays.** α-Factor secretion was as-

TABLE 1. Yeast strains[a]

| Strain | Genotype | Source or reference |
|---|---|---|
| DC5 | *MATa his3 leu2-3, 112 can1 gal2* | J. Hicks |
| DC6 | *MATα his4 leu2-3, 112 can1 gal2* | J. Hicks |
| K37 | DC5 × DC6 | This paper |
| MCY414 | *MATa ura3-52 his4-539 gal2* | M. Carlson |
| MCY406 | *MATα ura3-52 ade2-101 suc2-432 gal2* | M. Carlson |
| K38 | MCY414 × MCY406 | This paper |
| W303-1B | *MATα ade2-1 can1-100 ura3-1 leu2-3,112 trp1-1 his3-11,15* | R. Rothstein |
| AB320 | *HO ade2-1 can1-100 lys2-1 trp5-2 leu1-12 met4-1 (ura3-1/ura1-1)* | K. Nasmyth |
| RC618 | *MATa ade2-1 ura1 his6 met1 can1 cyh2 rme* | R. Chan (6) |
| RC629 | *MATa sst1-2 ade2-1 ura1 his6 met1 can1 cyh2 rme* | R. Chan (6) |
| RC631 | *MATa sst2-1 ade2-1 ura1 his6 met1 can1 cyh2 rme* | R. Chan (6) |
| RC622 | *MATα leu1 met1 his6 trp5 can1 cyh2 rme* | R. Chan (6) |
| XK41-10b | *matα2-4 cry1 HMLa leu2-3,112 (his3/his4/his5) met1 can1* | Kurjan and Herskowitz (18) |
| J12-2B | *MATa ade2-1 lys2-1 trp5-2 leu1-12 met4-1 ura1-1* | Kurjan et al. (17) |
| 227 | *MATa lys1 cry1* | I. Herskowitz |
| 70 | *MATα thr3* | I. Herskowitz |

[a] Genotypes shown in parentheses indicate that it is uncertain which of the markers are present in the strain. AB320 is a *MATa/MATαHO/HO* diploid homozygous for all indicated markers.

sayed by using the halo assay described by Kurjan and Herskowitz (18). Patch tests for mating were done by replicating to synthetic media on which a lawn of *MAT***a** cells in yeast extract-peptone-dextrose medium had been spread. The efficiency-of-mating experiments were done as described by Dutcher and Hartwell (10); matings were allowed to proceed for 6 h on yeast extract-peptone-dextrose before transfer to the dropout media on which mating frequencies were scored.

**Probes and hybridization.** The 63-base-pair (bp) spacer-α-factor probe was made by cleaving pHK2 or p69A with *Hin*dIII (Fig. 1A), followed by 3′ end labeling with [$^{32}$P]αCTP (2,000 to 3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) with *E. coli* DNA polymerase I (Klenow fragment) or T4 DNA polymerase. The labeled DNA was electrophoresed on polyacrylamide gels, and the 63-bp fragments were electroeluted from the gel. Other probes (including the 1.7-kb *MFα1 Eco*RI fragment, the 1.8-kb *MFα2 Hin*dIII fragment, and a 0.6-kb *Pvu*II fragment contained within the *Hin*dIII-*Sal*I fragment 3′ to *MFα2* [see Fig. 2]) were end labeled in a similar manner or were labeled by nick translation. Southern hybridization and colony screening were as described by Kurjan et al. (17), with reduced stringency hybridizations done at 50 to 55°C.

**Cloning of *MFα2*.** Total yeast DNA from strain AB320 was cleaved with *Hin*dIII and electrophoresed on a 5% polyacrylamide gel. Fragments in the size range of 1.5 to 2.0 kb were electroeluted and ligated with *Hin*dIII-cleaved pBR322. The clone bank was screened with the spacer-α-factor probe by colony hybridization to isolate pBR-*MFα2*. The 1.8-kb *MFα2 Hin*dIII fragment was subcloned into YEp13 (YEp13-*MFα2A*), and clones containing a more extensive region of *MFα2* DNA (YEp13-*MFα2B*) were isolated from a yeast clone bank in YEp13 (27) by hybridization to the labeled 1.8-kb *Hin*dIII fragment.

**DNA sequencing.** Various restriction fragments from pBR-*MFα2* were subcloned into the M13 bacteriophages mp10 or mp11 (24) and sequenced by the dideoxy terminator method (30).

**Construction of mutant genes.** Several mutant *mfα1* and *mfα2* genes were constructed by subcloning of yeast DNA fragments containing either *LEU2* or *URA3* into *MFα1*- or *MFα2*-containing plasmids. The individual mutant constructions are described in more detail below and diagrammed in Fig. 2. *E. coli* RRI was transformed, and ampicillin-resistant transformants were selected. For the cases involving *LEU2* inserts, transformants were screened for the Leu$^+$ phenotype. Transformants were further screened by quick plasmid DNA preparations and restriction analysis for the presence of the desired insertion.

For the construction of *mfα1*::*LEU2A* and *mfα1*::*LEU2B*, the *LEU2*-containing *Pst*I fragment from YEp13 was subcloned into pHK2 that had been partially cleaved with *Pst*I. For the construction of *mfα1*::*LEU2C*, the *LEU2 Pst*I-*Sal*I fragment of YEp13 was subcloned into pHK2 that had been partially cleaved with *Pst*I and *Sal*I. An ampicillin-resistant, tetracycline-resistant Leu$^+$ transformant, in which the 0.8-kb *Pst*I fragment and the 0.5-kb *Pst*I-*Sal*I fragment of pHK2 had been replaced with the *LEU2* fragment, was isolated. For the construction of *mfα1*::*URA3*, the *URA3 Hin*dIII fragment from YIp30 was subcloned into *Hin*dIII-cleaved pBH3. For the construction of *mfα2*::*LEU2A* and *mfα2*::*LEU2C*, the *LEU2 Bgl*II fragment of YEp13 was subcloned into partially *Bcl*I-cleaved pBR-*MFα2*. *mfα2*::*URA3* was constructed in two steps. First, YEp13-*MFα2B* was cleaved with *Bgl*II and *Sal*I. The ligation of this mixture resulted in a plasmid (pJK2) that contains a YEp13 *Bgl*II-*Sal*I fragment (which consists mostly of pBR322 sequences) and a *Bgl*II-*Sal*I *MFα2* fragment. The *Bgl*II-*Sal*I *MFα2* fragment is ca. 3.5 kb, the central portion of which consists of the 1.8-kb *MFα2 Hin*dIII fragment. The *mfα2*::*URA3* mutation was constructed by replacing the *MFα2 Hin*dIII fragment of pJK2 with the *URA3 Hin*dIII fragment from YIp30.

**Gene replacements.** The plasmids described above were digested with restriction enzymes to release linear fragments containing the mutant gene with flanking *MFα1* or *MFα2* DNA sequences. The enzymes used in the individual cases are described below. Yeast strains containing *leu2* (DC6, K37, W303-1B) or *ura3* (MCY406, K38, W303-1B) mutations or both were transformed with these fragments, and Leu$^+$ or Ura$^+$ transformants were selected. Quick yeast DNA preparations from Leu$^+$ and Ura$^+$ transformants were screened by Southern analysis to identify those transformants in which the desired gene replacement had occurred. For the testing of *mfα1*::*LEU2A*, *mfα1*::*LEU2B*, and *mfα1*::*LEU2C*, the plasmids were partially cleaved with *Eco*RI (due to the *Eco*RI site within the *LEU2* gene). For the testing of *mfα1*::*URA3*, the plasmid was cleaved with *Eco*RI. For the testing of *mfα2*::*LEU2A* and *mfα2*::*LEU2C*, the plasmids were cleaved with *Hin*dIII. For the testing of *mfα2*::*URA3*, the plasmid was cleaved with *Bgl*II and *Sal*I.

**α-Factor addition experiments.** α-Factor (Sigma Chemical Co., St. Louis, Mo.) was spread on MAC and MAS plates to give concentrations ranging from $5 \times 10^{-11}$ to $5 \times 10^{-7}$ M. Mating patch tests were done as described above with the tester lawns RC618 (*MAT***a** *SST1 SST2*), RC629 (*MAT***a** *sst1-2 SST2*) and RC631 (*MAT***a** *SST1 sst2-1*). α-Factor activity was assayed by microscopic observation of *MAT***a** cells from the mating lawns. Elongated cells (shmoos) were observed at the concentrations of α-factor that have been

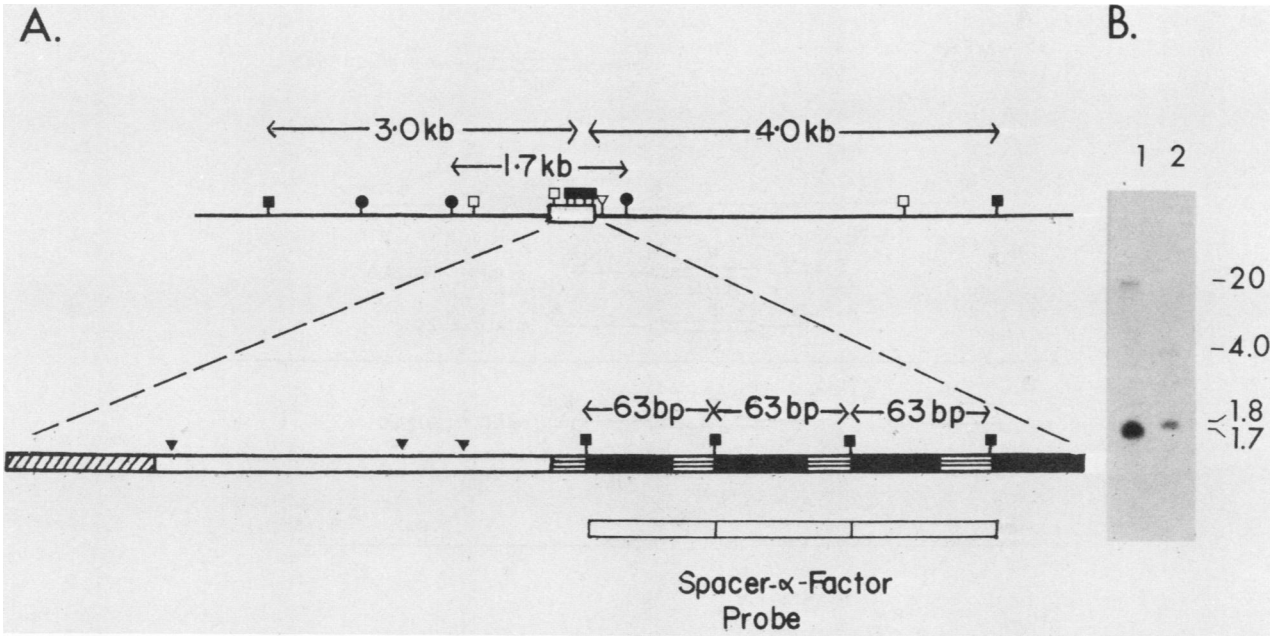


FIG. 1. Homology of genomic sequences to α-factor gene probes. (A) The restriction map of the *MFα1* region is shown. The coding region of *MFα1* is indicated by an open rectangle and is expanded below the line. The signal sequence is indicated by diagonal lines, glycosylation sites by upside-down triangles, spacer sequences by horizontal lines, and α-factor sequences by filled-in rectangles. The region comprising the spacer-α-factor probe is shown. Restriction endonuclease sites are designated by symbols as follows: ▼, *Eco*RI; ▼, *Hin*dIII; ▽, *Sal*I; and ▽, *Pst*I. (B) *Eco*RI-cleaved yeast DNA (lane 1) and *Hin*dIII-cleaved yeast DNA (lane 2). Yeast DNA from strain AB320 (Table 1) was digested, fractionated on a 0.7% agarose gel, transferred to a nitrocellulose filter, and hybridized to the spacer-α-factor probe. The probe hybridizes faintly to the 4.0-kb *Hin*dIII fragment and very faintly or not at all to the 3.0-kb *Hin*dIII fragment of *MFα1*, due to the presence of only a single α-factor or spacer sequence, respectively, on these fragments. The 63-bp spacer-α-factor *Hin*dIII fragments are too small to be detected on these gels.

observed previously (25) with the *MAT***a** *SST1 SST2* strain and at lower concentrations with the *MAT***a** *sst1-2 SST2* and *MAT***a** *SST1 sst2-1* strains.

The effects of addition of a *MAT***a** "helper" strain were done by mixing approximately equal cell numbers of RC622 (*MATα*) with the above *MAT***a** strains before spreading the tester lawn. Mating between the helper *MATα* strain and the *MAT***a** mating tester strains is not detected on the MAC and MAS plates due to noncomplementation of markers. Quantitative tests for the effects of addition of the *MATα* helper strain were done as described below.

## RESULTS

**Cloning and sequencing of *MFα2*.** Our previous results indicated that there are additional sequences in the yeast genome with homology to *MFα1*, suggesting that there might be multiple α-factor structural genes (18). In particular, Southern hybridization experiments with total yeast DNA had revealed a single *Hin*dIII fragment in addition to *MFα1* fragments that hybridized strongly to a probe consisting only of α-factor and spacer sequences. Figure 1B shows an example of a Southern blot of total yeast DNA digested with *Eco*RI (lane 1) or *Hin*dIII (lane 2). In addition to the *MFα1* fragments, significant hybridization is seen to a large *Eco*RI fragment and a 1.8-kb *Hin*dIII fragment. (Several additional faint bands are sometimes detected that have not been further characterized).

Clones containing the 1.8-kb *Hin*dIII fragment were isolated by virtue of *MFα1* homology as described above. To determine whether the 1.8-kb *Hin*dIII fragment contained another gene encoding α-factor, clones containing this fragment (YEp13-*MFα2A*) or a larger genomic fragment (YEp13-*MFα2B*) on a high-copy-number yeast plasmid were isolated (see above) and tested to determine whether they show properties similar to the properties of p69A (YEp13-*MFα1*) (18). Plasmids YEp13-*MFα2A* and YEp13-*MFα2B* showed one of the properties exhibited by p69A: the production of a low level of α-factor in a *matα2* strain which, in the absence of the plasmid, is unable to produce α-factor due to a regulatory mutation in the mating type locus. Unlike p69A, however, YEp13-*MFα2A* and YEp13-*MFα2B* resulted in at most a slight increase in α-factor production in a *MATα* strain.

The behavior of the 1.8-kb *Hin*dIII fragment on a high-copy-number plasmid suggested that this fragment contains an α-factor structural gene but also that this gene shows a lower level of activity than does *MFα1*. The similarity in size of the 1.8-kb *Hin*dIII fragment to the size of the *MFα2*-containing fragment reported by Singh et al. (31) suggested that the 1.8-kb *Hin*dIII fragment might contain *MFα2*. The behavior of the YEp13-*MFα2* plasmids was somewhat different, however, from the behavior of the *MFα2* plasmid of Singh et al. (31), which resulted in a significant increase in α-factor production in a *MATα* strain. A portion of the 1.8-kb *Hin*dIII fragment was sequenced to determine whether it encoded *MFα2* or a third α-factor structural gene. Within the region sequenced (extending from 240 bp upstream of the ATG start codon to 30 bp downstream of the stop codon), the sequence is identical to the *MFα2* sequence determined by Singh et al. (31).

**Construction of *mfα1* and *mfα2* mutants.** The one-step gene disruption technique allows the replacement of a wild-type yeast gene with a null mutant gene in which a yeast DNA fragment containing a selectable marker has been

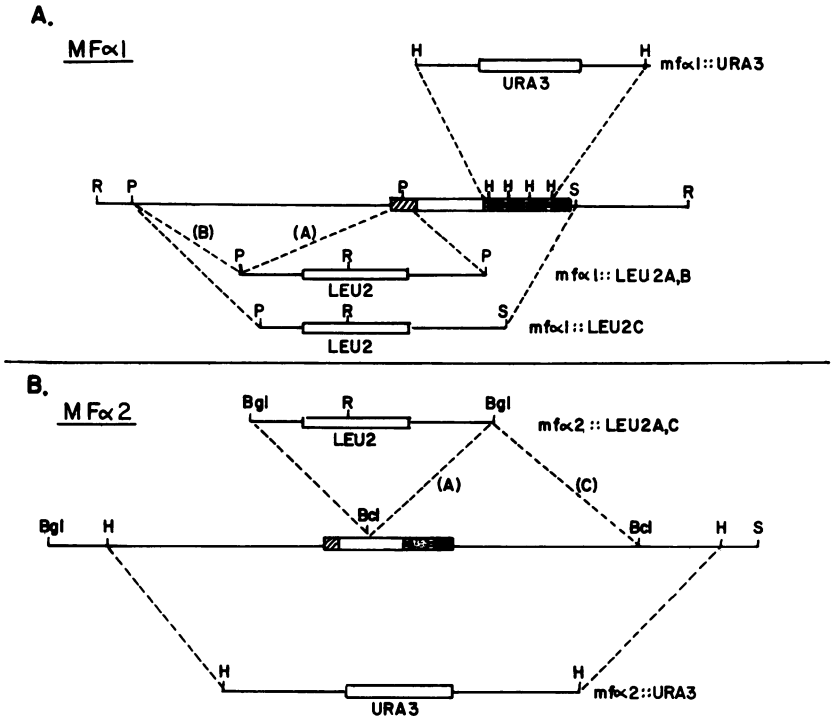


FIG. 2. Construction of *MFα1* and *MFα2* mutations. A partial restriction map of the *MFα1* and *MFα2* regions is shown. *MFα1* and *MFα2* coding regions are diagrammed as described in the legend to Fig. 1, with the *MFα2* α-factor-like sequence indicated by dots and *LEU2* and *URA3* coding regions indicated by open rectangles. (A) *mfα1::URA3* was constructed by inserting a *URA3* fragment into the *Hin*dIII sites in the region of the spacer-α-factor repeats in *MFα1*. *mfα1::LEU2A* and *mfα1::LEU2B* were constructed by inserting a *LEU2 Pst*I fragment into the *Pst*I sites in or near *MFα1*, resulting in an insertion into the signal sequence of *MFα1* (*mfα1::LEU2A*) or a replacement of the 0.8-kb *Pst*I fragment containing the first few amino acids of the *MFα1* precursor and 5′ upstream sequences (*mfα1::LEU2B*). The *LEU2 Pst*I fragment is inserted in opposite orientations in *mfα1::LEU2A* and *mfα1::LEU2B*. *mfα1::LEU2C* was constructed by replacing the 0.8-kb *Pst*I and 0.5-kb *Pst*I-*Sal*I fragments of *MFα1* with a *Pst*I-*Sal*I *LEU2* fragment. (B) *mfα2::LEU2A* and *mfα2::LEU2C* were constructed by inserting a *Bgl*II *LEU2* fragment into the *Bcl*I sites of *MFα2*, resulting in an insertion into the middle of the *MFα2* coding region (*mfα2::LEU2A*) or a replacement of the *Bcl*I fragment containing the carboxy terminus of the *MFα2* coding sequences and 3′ downstream sequences (*mfα2::LEU2C*). *mfα2::URA3* was constructed by replacing the 1.8-kb *MFα2 Hin*dIII fragment with a *URA3* fragment. Gene replacements were done as described in the text.

inserted (28). Mutations in *MFα1* and *MFα2* were constructed by the insertion of fragments containing either the yeast *URA3* or *LEU2* gene into plasmids containing *MFα1* or *MFα2*. Several constructions were made for each of the genes (Fig. 2; see above). Yeast strains mutant for the inserted selectable marker were transformed with the mutant *mfα1* and *mfα2* constructions as described above. In the first set of experiments, both *MAT***a**/*MAT*α diploid strains and *MAT*α haploid strains were transformed in case the *mfα1 or mfα2* mutations had an effect on viability in haploids. Because no effect on viability was seen, further gene replacements have been done in *MAT*α haploids.

To be certain that the correct gene replacement had occurred, the Leu$^+$ and Ura$^+$ transformants were analyzed further. A likely phenotype in *MAT*α strains containing an α-factor mutation is a defect in α-factor production. Many of the transformants obtained by using the mutant *mfα1* constructions showed a significant decrease in α-factor production. Several transformants showing an effect on α-factor production were tested by Southern blot analysis and found to contain a replacement of the wild-type *MFα1* 1.7-kb *Eco*RI fragment by fragment(s) of the size expected for the mutant construction used in the transformation (Fig. 3). None of the transformants obtained by using the mutant *mfα2* constructions showed a difference in phenotype from the parental strains. Several transformants were tested by Southern blot analysis, and most were shown to contain a replacement of *MFα2* by the mutant gene (Fig. 3). Transformants containing *MFα1* or *MFα2* gene replacements were analyzed further.

The *mfα1 mfα2* double mutants were constructed in two ways. One set of constructions involved first replacing the *MFα1* gene with a mutant gene containing either a *LEU2* or a *URA3* insertion, followed by replacing *MFα2* with a mutant gene containing the alternate selectable marker. Southern blots containing an example of the genomic DNA change associated with each of the single and double mutants are shown in Fig. 3.

In a second set of experiments, the double mutants were constructed by genetic crosses. The segregation of the *mfα1* and *mfα2* mutations was followed by using the inserted marker phenotype. First, each of the mutants was mated to a *MAT***a** *MFα1 MFα2 leu2 ura3* strain. An *MFα1 mfα2* segregant from these crosses was mated on an *mfα1 MFα2* segregant of the opposite mating type containing the alternate selectable marker, and the diploid was sporulated and dissected to obtain the *MAT*α *mfα1 mfα2* double mutants. In all cases, the mutations segregated 2+:2− and have no effect on mating or **a**-factor production in *MAT***a** cells (data not shown). The *mfα1* and *mfα2* mutations segregated independ-

ently of one another, indicating that *MFα1* and *MFα2* are unlinked. An isogenic set of mutants constructed in strain W303-1B were analyzed further.

**Effects of *mfα1* and *mfα2* mutations on α-factor production.** α-Factor production was assayed by using the halo assay as described above. Cells to be tested for α-factor production were replica plated to sparse lawns of *MAT***a** cells that contain a mutation (*sst1-2* or *sst2-1*) that results in supersensitivity to α-factor (6). Cells that secrete α-factor inhibit the growth of the surrounding *MAT***a** cells, resulting in a clear halo in which the cells of the *MAT***a** lawn do not grow (Table 2). In addition, an attempt was made to obtain a more quantitative value for the level of α-factor produced by the mutants. Thick suspensions of wild-type and mutant cells were adjusted to equivalent cell concentrations. A dilution series was made, and 2.5 μl of each dilution was spotted on halo tester lawns. The resulting halos were examined to determine at what dilution the wild-type strain gives a halo of equivalent size to the halo generated by the undiluted mutant.

The *mfα1* mutants show a considerable decrease in α-factor production in comparison to the parental strain. With the replica plating technique, the *mfα1* mutants produce only a very small halo on the most supersensitive of the halo lawns (*sst2-1*). The dilution assay indicates that the *mfα1* halos are equivalent in size to a 500-fold dilution of the wild-type strain. These results indicate that *MFα1* is responsible for the majority of the α-factor produced. Both assays show that the *mfα2* mutations have no effect on halo size, indicating that *MFα2* is responsible for, at most, a very small proportion of the α-factor produced. The *mfα1 mfα2* double mutants do not produce a halo on any of the tester lawns, indicating that *MFα2* is responsible for the low level of α-factor production seen in the *mfα1* mutants and that *MFα1* and *MFα2* are the only active α-factor genes.

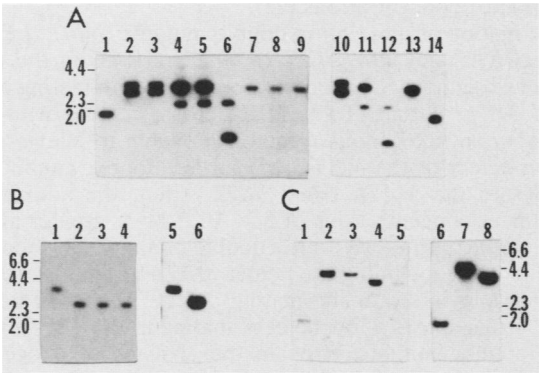


FIG. 3. Genomic changes associated with gene replacements. The genomic change associated with one example of each of the mutations is shown (panel A, lanes 1 to 9; panel B, lanes 1 to 4; panel C, lanes 1 to 5). The plasmids containing the wild-type genes and the mutant genes used to make the gene replacements are also shown (panel A, lanes 10 to 14; panel B, lanes 5 and 6; panel C, lanes 6 to 8). The gene replacements shown were constructed in the *MATα MFα1 MFα2* strain W303-1B. Genomic yeast DNA or plasmid DNA was cleaved as described below, run on 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized to the probe indicated. (A) The probe is the 1.7-kb *MFα1 Eco*RI fragment. The DNAs were cleaved with *Eco*RI. Strains: *MFα1 MFα2* (lane 1); *mfα1*::*LEU2A MFα2* (lane 2); *mfα1*::*LEU2A mfα2*::*URA3* (lane 3); *mfα1*::*LEU2B MFα2* (lane 4); *mfα1*::*LEU2B mfα2*::*URA3* (lane 5); *mfα1*::*LEU2C MFα2* (lane 6); *mfα1*::*URA3 MFα2* (lane 7); *mfα1*::*URA3 mfα2*::*LEU2A* (lane 8); and *mfα1*::*URA3 mfα2*::*LEU2C* (lane 9). Plasmids: p*mfα1*::*LEU2A* (lane 10); p*mfα1*::*LEU2B* (lane 11); p*mfα1*::*LEU2C* (lane 12); p*mfα1*::*URA3* (lane 13); and pHK2 (*MFα1*) (lane 14). (B) The probe is the *Pvu*II fragment 3′ to *MFα2*. The DNAs were cleaved with *Bgl*II and *Sal*I. Strains: *MFα1 MFα2* (lane 1); *MFα1 mfα2*::*URA3* (lane 2); *mfα1*::*LEU2A mfα2*::*URA3* (lane 3); and *mfα1*::*LEU2B mfα2*::*URA3* (lane 4). Plasmids: pJK2 (*MFα2*) (lane 5); and p*mfα2*::*URA3* (lane 6). (C) The probe is the *MFα2* 1.8-kb *Hin*dIII fragment. The DNAs were cleaved with *Hin*dIII. Strains: *MFα1 MFα2* (lane 1); *MFα1 mfα2*::*LEU2A* (lane 2); *mfα1*::*URA3 mfα2*::*LEU2A* (lane 3); *MFα1 mfα2*::*LEU2C* (lane 4); and *mfα1*::*URA3 mfα2*::*LEU2C* (lane 5). Plasmids: pBR-*MFα2* (lane 6); p*mfα2*::*LEU2A* (lane 7); and p*mfα2*::*LEU2C* (lane 8).

TABLE 2. α-Factor and mating phenotype of *MFα1* and MFα2 mutants

| Strain | Phenotype | |
|---|---|---|
| | α-Factor[a] | Mating[b] |
| *MFα1 MFα2* | +++ | +++ |
| *mfα1*::*LEU2A MFα2* | + | ++ |
| *mfα1*::*LEU2B MFα2* | + | ++ |
| *mfα1*::*LEU2C MFα2* | + | ++ |
| *mfα1*::*URA3 MFα2* | + | + |
| *MFα1 mfα2*::*LEU2A* | +++ | ++/+++ |
| *MFα1 mfα2*::*LEU2C* | +++ | ++/+++ |
| *MFα1 mfα2*::*URA3* | +++ | ++/+++ |
| *mfα1*::*LEU2A mfα2*::*URA3* | − | − |
| *mfα1*::*LEU2B mfα2*::*URA3* | − | − |
| *mfα1*::*URA3 mfα2*::*LEU2A* | − | − |
| *mfα1*::*URA3 mfα2*::*LEU2C* | − | − |

[a] α-Factor production was assayed by the halo assay. Symbols: (+++) wild-type halo—halo on *MAT***a** *sst 1-2 SST2* and *MAT***a** *SST1 sst2-1* lawns; (+) low level α-factor production—no halo on *MAT***a** *sst1-2 SST2* lawn, small halo on *MAT***a** *SST1 sst2-1* lawn; and (−) no halo on either tester lawn.

[b] Mating was assayed by patch tests and efficiency of mating experiments to strain 227 as described in the text. Symbols: (+++) wild-type mating; (++/+++) slightly reduced mating—difficult to see reduction by patch tests; 70 to 100% of wild-type levels by efficiency of mating experiments; (++) somewhat reduced mating—slightly less growth by patch tests; 25 to 40% of wild-type levels by efficiency of mating experiments; (+) low level of mating—papillations by patch test; 3% of wild-type level by efficiency of mating experiments; and (−) no mating.

**Effects of *mfα1* and *mfα2* mutations on mating.** Mating was assayed by patch tests and efficiency of mating experiments (10) as described above. The effects of the various mutant constructions on mating are shown in Tables 2 and 3.

The *mfα1*::*LEU2C* and *mfα2*::*URA3* mutants contain complete deletions of *MFα1* and *MFα2*, respectively, and therefore represent the null phenotypes for these genes. The *mfα1*::*LEU2C* mutant shows a 75% decrease in α mating ability in comparison to the *MFα1 MFα2* parental strain, whereas the *mfα2*::*URA3* mutant shows only an approximately 30% decrease in α-mating. This result suggests that *MFα1* is the more important of the two genes with respect to mating efficiency as well as with respect to α-factor production (as shown above).

Theoretically, all of the mutations constructed by the one-step gene disruption technique should be null mutations, due to large insertions into the coding region of the gene combined in some cases with complete or partial deletions of the gene. Therefore, one would expect all of the mutations in the same gene to result in the same phenotype. The three *mfα2* constructions show at most a slight decrease in mating ability (70 to 100% of the wild-type level of mating) which is consistent with this expectation. On the other hand, the *mfα1* mutants show quite different phenotypes with respect

TABLE 3. Efficiency of mating experiments[a]

| Strain | Mating frequency in: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Expt 1 | Expt 2 | Expt 3 | Expt 4 | Expt 5 | Expt 6 | Avg. |
| *MFα1 MFα2* | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| *mfα1::LEU2A MFα2* | 0.17 | 0.15 | 0.36 | 0.46 | NT | NT | 0.29 |
| *mfα1::LEU2B MFα2* | 0.20 | 0.32 | 0.62, 0.43 | 0.47, 0.55 | 0.26 | NT | 0.41 |
| *mfα1::LEU2C MFα2* | NT | NT | 0.37, 0.18 | 0.34, 0.21 | 0.27 | 0.12 | 0.25 |
| *mfα1::URA3 MFα2* | 0.04 | 0.05 | 0.02 | NT | 0.01 | NT | 0.03 |
| *MFα1 mfα2::LEU2A* | NT | 0.96 | 0.71, 0.82 | 0.34, 0.54 | 1.04 | 0.48 | 0.70 |
| *MFα1 mfα2::LEU2C* | NT | NT | 1.33 | 0.77 | NT | NT | 1.05 |
| *MFα1 mfα2::URA3* | NT | NT | NT | NT | 0.88, 0.93 | 0.62, 0.48 | 0.73 |
| *mfα1::LEU2A mfα2::URA3* | 0 | NT | NT | 0 | NT | NT | 0 |
| *mfα1::LEU2B mfα2::URA3* | 0 | NT | NT | NT | NT | NT | 0 |

[a] Efficiency of mating experiments were done as described in the text. Mating frequencies indicate the proportion of cells that form diploids with mating tester 227 (*MAT***a**) in comparison to the proportion of parental cells (W303-1B) that form diploids. A value of 0 indicates that the strain shows less than 0.001 the level of mating of the wild-type strain. In some cases, two isolates of the mutation were tested. NT, Not tested.

to α mating, although the α-factor production phenotypes are identical. The *mfα1::LEU2A* (insertion in the signal sequence), *mfα1::LEU2B* (partial deletion), and *mfα1::LEU2C* (complete deletion) mutants show mating frequencies of 25 to 40% of the wild-type level, whereas the *mfα1::URA3* (insertion into the spacer-α-factor region) mutant shows a mating frequency of only 3% of the wild-type level. Because the complete deletion of the *MFα1* gene must represent the actual null phenotype, these results suggest that the *mfα1::URA3* construction encodes a gene product that inhibits mating. Based on the structure of the *mfα1::URA3* construction, this mutant gene should produce a hybrid protein containing the *MFα1* signal sequence, the region of ca. 60 amino acids containing three glycosylation sites and a single spacer sequence. Translation should continue 13 amino acids into the *URA3*-containing fragment until a stop codon is reached. The phenotype of the *mfα1::URA3* mutant suggests that this hybrid protein interferes in some way with the α mating process.

Both *mfα1* and *mfα2* null mutations allow a significant level of α mating (25 to 40% and 70 to 100%, respectively), indicating that either of these gene products can function relatively well for α mating. To determine whether at least one α-factor gene is essential for α mating, *mfα1 mfα2* double mutants were also assayed. All of the *MATα mfα1 mfα2* double mutants that have been constructed are completely sterile (no rare matings have been detected), indicating that at least one α-factor gene product is required for α mating. As expected, the effect of the *mfα1 mfα2* double mutants is α-specific.

**α-Factor addition experiments.** The addition of exogenous α-factor has been shown to partially alleviate the mating defect of *MATα* strains containing mutations that result in defects in α-factor processing (5, 13, 14). Experiments to test for a similar alleviation of the mating defect of the α-factor mutants were done by adding purified α-factor to mating plates. The concentrations of α-factor added cover the ranges that induce *MAT***a** responses, including cell cycle arrest, agglutinin induction, and cell elongation (shmooing) (25). As an alternative method of providing exogenous α-factor, matings were done in the presence of a second, helper *MATα* strain. Mating of the helper *MATα* strain to the *MAT***a** mating tester strain was not detected in these experiments due to homozygosity for several auxotrophic markers in the resulting diploid.

A wild-type *MAT***a** *SST1 SST2* (RC618) strain and two strains that are supersensitive to α-factor (RC629-*MAT***a** *sst1-2 SST2* and RC631-*MAT***a** *SST1 sst2-1*) were used as mating testers. The *MAT***a** mating tester strains were observed under the microscope after exposure to α-factor to be certain that the *MAT***a** cell responses were being induced. The cell elongation response was observed at the appropriate α-factor concentrations (25).

The mating of *mfα1 mfα2* double mutants (*mfα1::LEU2A mfα2::URA3* and *mfα1::LEU2B mfα2::URA3*) in the presence of exogenous α-factor was compared with mating of the *MFα1 MFα2* parent (Table 4). In crosses to the wild-type *MAT***a** strain, exogenous α-factor is unable to alleviate the mating defect of the *MATα mfα1 mfα2* double mutants. In matings to the *MAT***a** *sst1-2 SST2* strain, the addition of α-factor at concentrations of $5 \times 10^{-8}$ M or greater allows some mating of the *mfα1 mfα2* double mutants. The addition of α-factor by including the helper *MATα* strain also results in some alleviation of the mating defect. The *MAT***a** *SST1 sst2-1* strain shows a low level of mating to the *MATα mfα1 mfα2* double mutants, even in the absence of exogenous α-factor. The addition of exogenous α-factor at concentrations of $5 \times 10^{-10}$ M or greater results in a higher level of mating. The addition of the helper *MATα* strain also allows some alleviation of the mating defect. In the mating of the wild-type *MATα MFα1 MFα2* strain to the supersensitive *MAT***a** strains, high levels of α-factor are somewhat inhibitory to mating. At an α-factor concentration of $5 \times 10^{-7}$ M, the mating level of the *MATα mfα1 mfα2* double mutants to the supersensitive *MAT***a** strains is approximately equivalent to the inhibited level of mating of the wild-type strains (as assayed by patch tests in which a severalfold difference in mating could be missed).

A more quantitative assay for the effects of exogenous α-factor on the mating of *mfα1 mfα2* double mutants was done by using the efficiency of mating assay in the presence of a helper *MATα* strain. The parental *MATα MFα1 MFα2* strain (W303-1B) and a *MATα mfα1 mfα2* double mutant (constructed in W303-1B) were mated to a *MAT***a** strain (J12-2B) either alone or in combination with a helper *MATα* strain (RC622) that produces as much or more α-factor than does W303-1B and therefore should provide a good source of α-factor. The mating of the *MATα* helper strain was scored separately from the *MFα1 MFα2* or *mfα1 mfα2* strain being tested. In this way, it was possible to determine whether the α-factor provided by strain RC622 has any effect on the mating defect of the *mfα1 mfα2* strain. The striking result of this experiment (Table 5) is that the presence of the α-factor provided by the helper *MATα* strain allows no alleviation of

TABLE 4. α-Factor addition experiments[a]

| MATa tester[c] | pH | Strain[d] | Mating in presence of α-factor (M) or helper strain[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | $5 \times 10^{-11}$ | $5 \times 10^{-10}$ | $5 \times 10^{-9}$ | $5 \times 10^{-8}$ | $5 \times 10^{-7}$ | Helper strain |
| *SST1 SST2* | 4.8 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | | *mfα1 mfα2* | − | − | − | − | − | − | − |
| | 6.0 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | | *mfα1 mfα2* | − | − | − | − | − | − | − |
| *sst1-2 SST2* | 4.8 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | + | ++ |
| | | *mfα1 mfα2* | − | − | − | − | p | + | p |
| | 6.0 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | + | ++ |
| | | *mfα1 mfα2* | − | − | − | − | p | + | − |
| *SST1 sst2-1* | 4.8 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | + | + |
| | | *mfα1 mfα2* | p | p | +/− | + | + | + | + |
| | 6.0 | *MFα1 MFα2* | ++ | ++ | ++ | ++ | ++ | + | + |
| | | *mfα1 mfα2* | p | p | +/− | +/− | + | + | +/− |

[a] Symbols: (++) wild-type mating; (+) less than wild-type mating; (+/−) low level of mating; (p) papillations—very low level of mating; and (−) no mating.
[b] Mating was tested by patch tests in the presence of α-factor or a *MATα* helper strain (RC622).
[c] *MATa* tester strains were RC618 (*SST1 SST2*), RC629 (*sst1-2 SST2*) and RC631 (*SST1 sst2-1*).
[d] The *MFα1 MFα2* strain tested is W303-1B. The *mfα1 mfα2* mutants tested (*mfα1::LEU2A mfα2::URA3* and *mαf1::LEU2B mfα2::URA3*) were constructed in W303-1B.

the mating defect of the *mfα1 mfα2* strain. This result confirms the more qualitative result described above, i.e., that the addition of physiological levels of α-factor does not alleviate the mating defect of the *mfα1 mfα2* double mutant.

## DISCUSSION

**Role of *MFα1* and *MFα2* in α-factor production and mating.** α-Factor is encoded within a small multigene family consisting of at least two genes, *MFα1* and *MFα2* (18, 31). *MFα1* and *MFα2* encode putative α-factor precursors with similar structures, suggesting that both can be processed to produce mature α-factor, although possibly with different efficiencies. The ability in yeast cells to replace the genomic copy of a gene with a mutant gene constructed in vitro makes it possible to examine the function of the members of a multigene family. Null mutations in *MFα1* and *MFα2* were constructed in *MATα* strains by using the one-step gene disruption technique (28). The α-factor production and mating phenotypes of the *mfα1*, *mfα2*, and *mfα1 mfα2* null mutants were tested (Tables 2 and 3).

TABLE 5. Effect of *MATα* helper strain on *mfα1 mfα2* Mating[a]

| *MATα* strain 1 | *MATα* strain 2 | Mating frequency of: | |
|---|---|---|---|
| | | Strain 1 | Strain 2 |
| *MFα1 MFα2* | | 1.0 | |
| *mfα1 mfα2* | | 0 | |
| | *MFα1 MFα2* | | 1.0 |
| *MFα1 MFα2* | *MFα1 MFα2* | 0.71 | 0.65 |
| *mfα1 mfα2* | *MFα1 MFα2* | 0 | 0.83 |

[a] Mating was tested by efficiency of mating experiments. *MATα* strain 1 is *MFα1 MFα2* (W303-1B) or *mfα1::LEU2A mfα2::URA3* (constructed in W303-1B). *MATα* strain 2 is the helper *MATα MFα1 MFα2* strain RC622. Mating (to *MATa* strain J12-2B) of W303-1B was scored on minimal plus adenine plates, and mating of RC622 was scored on minimal plus tryptophan plates. In each mating test, the total number of *MATα* cells was mixed with an equal number of *MATa* cells. When two *MATα* strains were present in the mating mixture, equal proportions of the two strains were added, resulting in reduced mating frequencies in comparison to the cases in which only a single *MATα* strain is present.

The *mfα1* mutants secrete a very low level of α-factor, consistent with previous results indicating that *MFα1* is responsible for the majority of α-factor production (2). The ability of the *mfα1* null mutants to produce a low level of α-factor, however, indicates that at least one other α-factor gene is active. The *mfα2* mutations by themselves have no detectable effect on α-factor production. The *mfα1 mfα2* double mutants do not secrete any detectable α-factor. These results indicate that *MFα1* and *MFα2* are the only active α-factor genes and that *MFα2* is responsible for the low level of α-factor produced by the *MATα mfα1 MFα2* strains.

A surprising result was that the mating phenotype of the *mfα1* mutants varies, depending on the structure of the mutant construction (Fig. 2). Several *mfα1* mutants (*mfα1::LEU2A*, *mfα1::LEU2B*, and *mfα1*::LEU2C), including a mutant in which the entire *mfα1* coding region is deleted, mate at 25 to 40% of the level of the parental strain. A fourth *mfα1* mutant (*mfα1::URA3*, which contains an insertion within the *mfα1* coding region) has a considerably more severe effect on α mating, showing only ca. 3% of the wild-type level of mating. All of the mutant constructions should result in totally nonfunctional gene products (due to large insertions into or deletions of the coding region of the mutated gene) and therefore would be expected to show the same phenotype. The ca. 10-fold lower level of mating of the *mfα1::URA3* mutant in comparison to the *mfα1::LEU2C* mutant, in which the entire *MFα1* gene is deleted, suggests that the *mfα1::URA3* gene product is inhibitory to α mating. A possible explanation for this result will be discussed below.

The phenotypes of three *mfα2* constructions analyzed were identical, resulting in 70 to 100% of the wild-type level of mating in *MATα* cells. The *MATα mfα1 mfα2* double mutants are totally sterile, no matter which *mfα1* and *mfα2* constructions are combined.

The sterility of the *mfα1 mfα2* double mutants implies that at least one of the α-factor structural genes is required for mating in *MATα* cells. The elimination of only one of the two genes has a relatively slight effect on mating, indicating

that *MFα1* and *MFα2* are functionally redundant. It is interesting that although *mfα1* mutations result in a large decrease in α-factor production (possibly as high as a 500-fold decrease), the level of mating is reduced only 4-fold by the complete deletion of *mfα1*. This result suggests that a low level of α-factor is sufficient for its role in mating and that wild-type *MATα* strains produce a considerable excess of α-factor. As might be expected, the effects of the *mfα1* and *mfα2* mutations are α-specific, thereby having no effect on **a**-factor production or mating when present in *MAT***a** cells.

The phenotypes of the α-factor mutants and the structure of the α-factor genes provide an explanation for the previous failure to identify α-factor structural gene mutations among mating-defective mutants. A mutation of either *MFα1* or *MFα2* alone should have little or no effect on mating; therefore, such mutations would not have been identified among the sterile (*ste*) mutants.

**Effect of addition of exogenous α-factor.** The sterility of the *MATα mfα1 mfα2* double mutants is consistent with the role of α-factor in the mating process that has been suggested by several types of experimental evidence. The addition of exogenous α-factor has been shown to partially alleviate the α mating defects of two mutants (*ste13* and *kex2*) that exhibit defects in α-factor processing (5, 13, 14). The effect of exogenous α-factor addition on the α mating of the *mfα1 mfα2* double mutants was investigated.

The addition of exogenous α-factor at concentrations that induce *MAT***a** cell responses (25) was tested for the alleviation of the mating defect of *MATα mfα1 mfα2* double mutants (Table 4). The α-factor addition had no effect on the mating of the *MATα mfα1 mfα2* mutants to a wild-type *MAT***a** strain. As an alternative method of providing physiological levels of α-factor, a helper *MATα* strain was added to the mating mixture. In this experiment, quantitative efficiency-of-mating experiments were done; therefore, even a slight alleviation of the mating defect could have been detected. No alleviation of the *MATα mfα1 mfα2* mating defect was seen (Table 5). The results of these experiments indicate that the addition of α-factor at concentrations that are capable of eliciting physiological responses to α-factor in *MAT***a** cells does not alleviate the mating defect of the *MATα mfα1 mfα2* double mutants.

Some alleviation of the mating defect was seen (Table 4) in crosses to *MAT***a** strains that contain mutations that result in supersensitivity to α-factor (*sst1-2* and *sst2-1*) (6). In matings to a *MAT***a** *sst1-2* strain, high concentrations of α-factor allowed some alleviation of the mating defect of the *mfα1 mfα2* double mutants. The supersensitivity of the *sst1* mutants has been attributed to a defect in the degradation of α-factor (6, 7, 21, 37); therefore, the addition of α-factor to the *MAT***a** *sst1-2* strain should be equivalent to adding high levels of α-factor to a wild-type *MAT***a** strain. In matings to a *MAT***a** *sst2-1* strain, a low level of mating was seen in the absence of exogenous α-factor, and relatively low concentrations of α-factor resulted in an increase in mating above this background level. The ability of *MAT***a** *sst2-1* strains to mate with mutants unable to produce α-factor (*ste13* or *kex2*) has been observed previously (5). One interpretation of this ability is that *sst2-1* strains exhibit some of the pheromone responses in the absence of α-factor and therefore are able to mate with strain that are defective in α-factor production. Although the molecular defect of the *sst2-1* mutant is unknown, the addition of exogenous α-factor to this strain may again be equivalent to adding high levels of α-factor to a wild-type strain. Thus, the results with the *sst1-2* and *sst2-1* strains can be interpreted to indicate that the addition of α-factor at very high concentrations can alleviate the mating defect of the *MATα mfα1 mfα2* mutants.

The results with the *MATα mfα1 mfα2* double mutants are in contrast to the effect of exogenous α-factor addition on mating by *MATα ste13* or *MATα kex2* mutants, for which the α-factor addition allowed partial alleviation of the mating defect in crosses to *MAT***a** *SST*$^+$ strains (5). In addition, the alleviation of the *ste13* or *kex2* mating defects was seen at lower levels of α-factor in crosses to *MAT***a** *sst1* strains than I have observed for the *MATα mfα1 mfα2* mutants.

The lack of alleviation of the *MATα mfα1 mfα2* mating defect by the addition of physiological levels of α-factor can be interpreted in several ways. One interpretation is that α-factor action is partly dependent on the relative positions of the *MAT***a** and *MATα* cells with respect to one another during the pheromone secretion process. Although the addition of exogenous α-factor would induce *MAT***a** responses, the requirement for correct directionality of the response would not be met, and alleviation of the mating defect would not be observed. Under this interpretation, however, exogenous α-factor should be unable to alleviate the mating defect of *MATα ste13* or *MATα kex2* as well as *MATα mfα1 mfα2* mutants.

An alternative interpretation that I favor is that the *MFα1* and *MFα2* gene products play a role in mating in addition to that of α-factor production. The addition of exogenous α-factor would be able to produce the appropriate *MAT***a** responses but would be unable to compensate for the additional role of the *MFα1* and *MFα2* gene products. The *MATα ste13* and *MATα kex2* mutants, which are producing the *MFα1* and *MFα2* gene products but are defective in α-factor precursor processing, would be able to accomplish this additional role; therefore, the α-factor addition would be able to alleviate the mating defect. I would like to emphasize, however, that the lack of alleviation of the *MATα mfα1 mfα2* mating defect by exogenous α-factor concentrations that induce *MAT***a** responses is a negative result and must be interpreted cautiously.

***MFα1* and *MFα2* function.** The differences in levels of α-factor produced by *MFα1* versus *MFα2* could be due to differences in mRNA levels, differences in the number of α-factor copies in the two genes, less efficient processing of the *MFα2* versus the *MFα1* precursor, or a combination of these reasons. Work in progress indicates that the *MFα1* message is present at significantly higher levels in *MATα* cells than the *MFα2* message, as indicated by Northern analysis and β-galactosidase gene fusion experiments. This result is consistent with results of Brake et al. (2), which indicate that *MFα1* encodes that major α-factor precursor. Structural differences between the *MFα1* and *MFα2* precursors, however, may also play a role in the different phenotypes of the *mfα1* and *mfα2* mutants.

One of the interesting results of Julius et al. indicates that the signal sequence of *MFα1* is not cleaved during transport through the secretory pathway (15). They speculate that the *MFα1* signal sequence may act as a membrane anchor sequence for the *MFα1* precursor. The spacer sequences separating the α-factor repeats of the α-factor precursors contain lysine-arginine dipeptides that are likely to act as proteolytic processing signals in a manner similar to that found in higher organisms (for a review, see references 8, 19, 26, 33, and 34). Recent results suggest that the *KEX2* gene encodes the lysine-arginine-cleaving endopeptidase responsible for this step (14). Because the signal sequence is not cleaved, processing of the *MFα1* precursor by the lysine-

arginine endopeptidase might result in exposure of the amino-terminal half of the precursor on the surface of the cell. If this membrane protein exists, it might play the role in mating suggested for the α-factor precursors in addition to the role of simply producing α-factor. The role of this putative membrane protein might also explain the inhibition of α mating by the *mfα1::URA3* gene product. As mentioned above, this gene product would be a hybrid protein consisting of the amino-terminal half of the *MFα1* precursor followed by several amino acids encoded by the end of the *URA3* fragment that was inserted into the *MFα1* gene. If this hybrid protein was not recognized by the lysine-arginine endopeptidase, a mutant membrane protein would be exposed on the surface of the cell and might be inhibitory to mating.

If the *MFα1* gene product does play a role in mating in addition to that of producing α-factor, mutations that alter the structure of the *MFα1* gene product but still allow α-factor production might result in a mating defect. If the lack of alleviation of the mating defect is due simply to a need for correct positioning of *MAT***a** and *MATα* cells during the secretion process, an *MFα1* gene that has been altered in this way should be able to alleviate the mating defect of the *MATα mfα1 mfα2* mutants. *MFα1* constructions of this sort are being made to test these possibilities.

## ACKNOWLEDGMENTS

I thank Timothy Hoey and Troy Hailparn for technical assistance, Rodney Rothstein for discussions on the one-step gene disruption technique, Helen Campbell for preparation of the figures, Marina Hatch and Heather von Manowski for help in preparation of the manuscript, and Ira Herskowitz, Mary Rykowski, Anita Hopper, Lee Venolia, Laurel Eckhardt, John Hill, Christine Dietzel, Timothy Hoey, Rodney Rothstein, and Shari Caplan for comments on the manuscript.

This work was supported by a Public Health Service research grant from the National Institutes of Health.

## LITERATURE CITED

1. **Betz, R., W. Duntze, and T. R. Manney.** 1981. Hormonal control of gametogenesis in the yeast *Saccharomyces cerevisiae*. Gamete Res. **4**:571–584.
2. **Brake, A. J., D. J. Julius, and J. Thorner.** 1983. A functional prepro-α-factor gene in *Saccharomyces* yeasts can contain three, four, or five repeats of the mature pheromone sequence. Mol. Cell. Biol. **3**:1440–1450.
3. **Broach, J. R., J. N. Strethern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN*1 gene. Gene **8**:121–133.
4. **Bucking-Throm, E., W. Duntze, L. H. Hartwell, and T. R. Manney.** 1973. Reversible arrest of haploid yeast cells at the initiation of DNA synthesis by a diffusible sex factor. Exp. Cell Res. **76**:99–110.
5. **Chan, R. K., L. M. Melnick, L. C. Blair, and J. Thorner.** 1983. Extracellular suppression allows mating by pheromone-deficient mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **155**:903–906.
6. **Chan, R. K., and C. A. Otte.** 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a** factor and α factor pheromones. Mol. Cell Biol. **2**:11–20.
7. **Ciejek, E., and J. Thorner.** 1979. Recovery of *Saccharomyces cerevisiae* **a** cells from G1 arrest by α factor pheromone requires endopeptidase action. Cell **18**:623–635.
8. **Comb, M., P. H. Seeburg, J. Adelman, L. Eiden, and E. Herbert.** 1982. Primary structure of the human met- and leu-enkephalin precursor and its mRNA. Nature (London) **295**:663–666.
9. **Duntze, W., V. MacKay, and T. R. Manney.** 1970. *Saccharomyces cerevisiae*: a diffusible sex factor. Science **168**:1472–1473.
10. **Dutcher, S. K., and L. H. Hartwell.** 1982. The role of *S. cerevisae* cell division cycle genes in nuclear fusion. Genetics **100**:175–184.
11. **Hartwell, L. H.** 1973. Synchronization of haploid yeast cell cycles, a prelude to conjugation. Exp. Cell Res. **76**:111–117.
12. **Hartwell, L. H.** 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. J. Cell Biol. **85**:811–822.
13. **Julius, D., L. Blair, A. Brake, G. Sprague, and J. Thorner.** 1983. Yeast α factor is processed from a larger precursor polypeptide: the essential role of a membrane-bound dipeptidyl aminopeptidase. Cell **32**:839–852.
14. **Julius, D., A. Brake, L. Blair, R. Kunisawa, and J. Thorner.** 1984. Isolation of the putative structural gene for the lysine-arginine-cleaving endopeptidase required for processing of yeast prepro-α-factor. Cell **37**:1075–1089.
15. **Julius, D., R. Schekman, and J. Thorner.** 1984. Glycosylation and processing of prepro-α-factor through the yeast secretory pathway. Cell **36**:309–318.
16. **Kreil, G.** 1981. Transfer of proteins across membranes. Annu. Rev. Biochem. **50**:317–348.
17. **Kurjan, J., B. D. Hall, S. Gillam, and M. Smith.** 1980. Mutations at the yeast *SUP*4 $tRNA^{Tyr}$ locus: DNA sequence changes in mutants lacking suppressor activity. Cell **20**:701–709.
18. **Kurjan, J., and I. Herskowitz.** 1982. Structure of a yeast pheromone gene (*MF*α): a putative α-factor precursor contains four tandem copies of mature α-factor. Cell **39**:933–943.
19. **Lund, P. K., R. H. Goodman, P. C. Dee, and J. F. Habener.** 1982. Pancreatic preproglucagon DNA contains two glucagon-related coding sequences arrayed in tandem. Proc. Natl. Acad. Sci. U.S.A. **79**:345–349.
20. **MacKay, V. L., and T. R. Manney.** 1974. Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae*. I. Isolation and phenotypic characterization of nonmating mutants. Genetics **76**:255–271.
21. **Maness, P. F., and G. M. Edelman.** 1978. Inactivation and chemical alteration of mating factor α by cells and spheroplasts of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1304–1308.
22. **Manney, T. R., W. Duntze, and R. Betz.** 1981. The isolation, characterization and physiological effects of the *Saccharomyces cerevisiae* sex pheromones, p. 21–51. *In* D. O. O'Day and P. A. Horgen (ed.), Sexual interactions in eukaryotic microbes. Academic Press, Inc., New York.
23. **Manney, T. R., and V. Woods.** 1976. Mutants of *Saccharomyces cerevisiae* resistant to the α mating-type factor. Genetics **82**:639–644.
24. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–79.
25. **Moore, S. A.** 1983. Comparison of dose-responsive curves for alpha factor-induced agglutinin, cell division arrest, and projection formation of *Saccharomyces cerevisiae MAT***a** yeast cells. J. Biol. Chem. **258**:13849–13856.
26. **Nakanishi, S., A. Inoue, T. Kita, M. Nakamura, A. C. Y. Chang, S. N. Cohen, and S. Numa.** 1979. Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. Nature (London) **278**:423–427.
27. **Nasmyth, K. A., and K. Tatchell.** 1980. The structure of transposable yeast mating type loci. Cell **19**:753–764.
28. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
29. **Sakurai, A., K. Sakata, S. Tamura, K. Aizawa, N. Yanagishima, and C. Shimoda.** 1976. Structure of the peptidyl factor inducing sexual agglutination in *S. cerevisiae*. Agric. Biol. Chem. **40**:1057–1058.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
31. **Singh, A., E. Y. Chen, J. M. Lugovoy, C. N. Chang, R. A. Hitzman, and P. H. Seeburg.** 1983. *Saccharomyces cerevisiae* contains two discrete genes coding for the α-factor pheromone. Nucleic Acids Res. **11**:4049–4063.

32. **Sprague, G. F., Jr., J. Rine, and I. Herskowitz.** 1981. Control of yeast cell type by the mating type locus. II. Genetic interaction between *MAT*α and unlinked α-specific *STE* genes. J. Mol. Biol. **153**:323–335.
33. **Steiner, D. F., P. S. Quinn, S. J. Chan, J. Marsh, and H. S. Trager.** 1980. Processing mechanisms in the biosynthesis of proteins. Ann. N.Y. Acad. Sci. **343**:1–16.
34. **Stern, A. S., B. N. Jones, J. E. Shively, S. Stein, and S. Udenfriend.** 1981. Two adrenal opioid polypeptides: proposed intermediates in the processing of proenkephalin. Proc. Natl. Acad. Sci. U.S.A. **78**:1962–1966.
35. **Stotzler, D., and W. Duntze.** 1976. Isolation and characterization of four related peptides exhibiting α-factor activity from *Saccharomyces cerevisiae*. Eur. J. Biochem. **65**:257–262.
36. **Stotzler, D., H. Kiltz, and W. Duntze.** 1976. Primary structure of α-factor peptides from *Saccharomyces cerevisiae*. Eur. J. Biochem. **69**:397–400.
37. **Tanaka, T., and H. Kita.** 1977. Degradation of mating factor by α-mating type cells of *Saccharomyces cerevisiae*. J. Biochem. **82**:1689–1693.
38. **Thorner, J.** 1981. Pheromonal regulation of development in *Saccharomyces cerevisiae*, p. 143–180. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast saccharomyces: life cycle and inheritance. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
39. **Yanagishima, N., and K. Yoshida.** 1981. Sexual interactions in *Saccharomyces cerevisiae* with special reference to the regulation of sexual agglutinability, p. 261–295. *In* D. O. O'Day and P. A. Horgen (ed.), Sexual interactions in eukaryotic microbes. Academic Press, Inc., New York.