Pheromones and Pheromone Receptors Are the Primary Determinants of Mating Specificity in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Saccharomyces cerevisiae has two haploid cell types, **a** and α, each of which produces a unique set of proteins that participate in the mating process. We sought to determine the minimum set of proteins that must be expressed to allow mating and to confer specificity. We show that the capacity to synthesize α-factor pheromone and **a**-factor receptor is sufficient to allow mating by *matα1* mutants, mutants that normally do not express any α- or **a**-specific products. Likewise, the capacity to synthesize **a**-factor receptor and α-factor pheromone is sufficient to allow **a** *ste2 ste6* mutants, which do not produce the normal **a** cell pheromone and receptor, to mate with wild-type **a** cells. Thus, the **a**-factor receptor and α-factor pheromone constitute the minimum set of α-specific proteins that must be produced to allow mating as an α cell. Furthermore, the production of these two proteins dictates the mating specificity exhibited by that cell. Further evidence that the pheromones and pheromone receptors are important determinants of mating specificity comes from studies with *matα2* mutants, cells that simultaneously express both pheromones and both receptors. We created a series of strains that express different combinations of pheromones and receptors in a *matα2* background. These constructions reveal that *matα2* mutants can be made to mate as either **a** cells or as α cells by causing them to express only the pheromone and receptor set appropriate for a particular cell type. Moreover, these studies show that the inability of *matα2* mutants to respond to either pheromone is a consequence of two phenomena: adaptation to an autocrine response to the pheromones they secrete and interference with response to α factor by the **a**-factor receptor.

HAPLOID cells of the yeast *Saccharomyces cerevisiae* exhibit either of two cellular phenotypes, the mating types **a** and α. These cells can reproduce vegetatively by a mitotic cell cycle. However, when cells of opposite mating type are co-cultured, they exit the cell cycle and participate in a mating process that results in cell and nuclear fusion to create an **a**/α diploid cell (reviewed by SPRAGUE, BLAIR AND THORNER 1983). Mating occurs only between cells of opposite type; each haploid cell type produces several unique proteins, including pheromones, receptors, and agglutinins, that participate in the mating process and may contribute to the specificity of mating. The reciprocal action of cell-type-specific peptide pheromones initiates mating. In particular, α cells secrete α factor, which binds to a receptor present only on the surface of **a** cells (JENNESS, BURKHOLDER and HARTWELL 1983), and **a** cells secrete **a** factor, which interacts with a receptor present only on the surface of α cells (HAGEN, MCCAFFREY and SPRAGUE 1986; BENDER and SPRAGUE 1986; NAKAYAMA, MIYAJIMA and ARAI 1987). In response to pheromone, a cell changes its pattern of gene expression (HAGEN and SPRAGUE 1984; STETLER and THORNER 1984; HARTIG *et al.* 1986; KRONSTAD, HOLLY and MACKAY 1987; VAN ARSDELL, STETLER and THORNER 1987), increases production of agglutinin (FEHRENBACHER, PERRY and THORNER 1978; BETZ, DUNTZE and MANNEY 1978), prepares for cell and nuclear fusion (ROSE, PRICE and FINK 1986; MCCAFFREY *et al.* 1987; TRUEHEART, BOEKE and FINK 1987), arrests cell division in the G1 phase of the cell cycle (BUCKING-THROM *et al.* 1973; WILKINSON and PRINGLE 1974), and projects part of itself toward its mating partner (projection formation) (AHMAD 1953; LEVI 1956; RINE 1979). Cell and nuclear fusion of the mating partners then creates an **a**/α zygote.

The differential production of pheromones, receptors, and agglutinins in **a** and α cells is achieved through the action of regulatory proteins encoded by the mating-type locus (*MAT*). Of the two *MAT* alleles, *MAT***a** and *MATα*, the latter is the determinant of whether a cell exhibits the **a** or α phenotype. *MATα* encodes two proteins, MATα1 and MATα2, that control transcription of known α-specific and **a**-specific genes (Figure 1) (for review see SPRAGUE, BLAIR and THORNER 1983; HERSKOWITZ 1986; NASMYTH and SHORE 1987). MATα1 activates transcription of the α-specific gene set, which includes the α-factor struc-

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TABLE 1

Gene-function relationships

Gene	Function
A. Genes required for the α cell phenotype[a]	
STE3	**a**-factor receptor structural gene
MFα1, MFα2	α-factor structural genes
STE13	α-factor maturation
KEX2	α-factor maturation
B. Genes required for the **a** cell phenotype[b]	
STE2	α-factor receptor structural gene
MFa1, MFa2	**a**-factor structural genes
STE6	**a**-factor maturation
STE14	**a**-factor maturation
STE16	**a**-factor maturation
BAR1	α-factor degradation

[a] The references for the assignment of function to each of these genes are given in the text. Transcription of *STE3* (SPRAGUE, JENSEN and HERSKOWITZ 1983) and *MFα1* and *MFα2* (FIELDS and HERSKOWITZ 1985) is limited to the α cell type. *STE13* and *KEX2* are transcribed in all three cell types (LEIBOWITZ and WICKNER 1976; JULIUS *et al.* 1983; G. SPRAGUE and I. HERSKOWITZ, unpublished observations).

[b] The references for the assignment of function to each of these genes are given in the text. Transcription of *STE2* (HARTIG *et al.* 1986), *MFa1* and *MFa2* (MICHEALIS and HERSKOWITZ 1988), *STE6* (WILSON and HERSKOWITZ 1984), and *BAR1* (KRONSTAD, HOLLY and MACKAY 1987) is limited to the **a** cell type. *STE16* (*RAM1*) is expressed in all three cell types (POWERS *et al.* 1986). Expression of *STE14* has not yet been examined.

tural genes and the **a**-factor receptor structural gene, whereas MATα2 represses transcription of the **a**-specific gene set, which includes the **a**-factor structural genes and α-factor receptor structural gene. Thus, *MATα* cells express only the α-specific gene set. We presume that any as yet unidentified α- or **a**-specific genes will also be regulated in this fashion by MATα1 or MATα2. Because *MAT***a** cells lack the two *MATα*-encoded regulators, they express only the **a**-specific gene set.

The importance of the particular α- and **a**-specific proteins in the mating process has been investigated through the use of structural gene mutations (see Table 1 for summary of gene functions). These studies reveal that the pheromones and receptors are required for mating: strains harboring mutations in *STE3* (**a**-factor receptor)(MACKAY and MANNEY 1974; HAGEN, MCCAFFREY and SPRAGUE 1986), *STE2* (α-factor receptor) (MACKAY and MANNEY 1974; HARTWELL 1980; JENNESS, BURKHOLDER and HARTWELL 1983; BLUMER, RENEKE and THORNER 1988; MARSH and HERSKOWITZ 1988; KONOPKA, JENNESS and HARTWELL 1988), *MFα1* and *MFα2* (α factor) (KURJAN and HERSKOWITZ 1982; SINGH *et al.* 1983; KURJAN 1985), *MFa1* and *MFa2* (**a** factor) (MICHAELIS and HERSKOWITZ 1988), *STE13* or *KEX2* (genes required for maturation of α factor) (LEIBOWITZ and WICKNER 1976; SPRAGUE, RINE and HERSKOWITZ 1981; JULIUS *et al.* 1983, 1984), or *STE6, STE14,* or *STE16* (genes required for maturation of **a** factor) (BLAIR 1979; RINE 1979; POWERS *et al.* 1986; WILSON and HERSKOWITZ 1987) typically show a mating efficiency that is reduced by six orders of magnitude compared with wild-type strains. Surprisingly, the agglutinins do not have essential roles in mating. The structural genes for these proteins have not been identified with certainty, but the *AGα1* gene may encode the α-agglutinin. Strains harboring mutations in these gene show only a modest reduction in mating efficiency (two- to tenfold) compared with wild type (SUZUKI and YANAGISHIMA 1985; J. KURJAN and P. LIPKE, personal communication). *In toto,* these studies have identified some α- and **a**-specific components that are required for mating, but they leave unanswered two questions. (1) What combination of α- or **a**-specific functions is sufficient to allow mating? That is, are there as yet unidentified α- or **a**-specific functions that are required for mating? (2) How is mating specificity achieved? Under standard mating conditions there are many **a** and α cells present that have prepared for mating by responding to the pheromone produced by the opposite mating type. Nonetheless, in this mix **a** and α cells only mate with cells of the opposite mating type. Given that the agglutinins apparently do not have essential roles in mating and that they are the only known proteins involved in cell contact, where does specificity arise? One possibility is that there are unidentified α- and **a**-specific functions that contribute to mating specificity. A second possibility is that the pheromones and receptors dictate specificity. For example, perhaps the sending and receiving of pheromone signals establishes directional cues that identify mating partners.

We have investigated these issues from several perspectives. First, we determined whether the provision of known α-specific functions allows *matα1* mutants to mate as α. These mutants fail to activate transcription of α-specific genes, but still repress **a**-specific genes, and hence express neither α- nor **a**-specific genes (Figure 1). It has been shown previously that expression of receptor (from the heterologous *GAL1* promoter) is sufficient to allow response to pheromone by these mutants (BENDER and SPRAGUE 1986; NAKAYAMA, MIYAJIMA and ARAI 1987). Therefore we have asked whether simultaneous expression of *STE3* (**a**-factor receptor structural gene) and *MFα1* (α-factor structural gene) restores mating competence to *matα1* mutants. Second, in a complementary set of experiments, we have determined what functions must be removed from a *matα2* mutant to allow response to pheromone and to restore mating competence. In *matα2* mutants, both α- and **a**-specific proteins are present because of the failure to repress **a**-specific genes (Figure 1); the simultaneous expression of the α- and **a**-specific gene sets apparently leads to functional antagonisms that result in an inability to re-

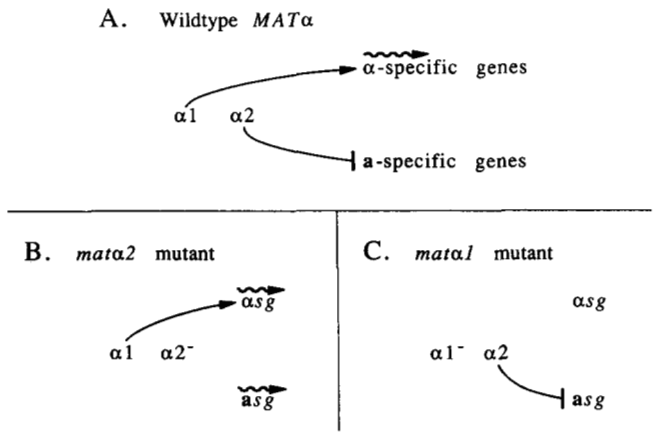

FIGURE 1.—Control of **a**- and α-specific gene expression. Expression of genes that are controlled by the two products of *MATα* is shown for wild-type *MATα* cells (A), *matα2* mutant cells (B), and *matα1* mutant cells (C). Wavy lines indicate gene expression, lines with arrowheads indicate stimulation of gene expression; lines with terminal bars indicate inhibition of gene expression. The terms **a**-specific genes (**a***sg*) and α-specific genes (*αsg*) indicate genes whose transcription is limited to **a** or α cells. There are examples of genes that are required for mating by only one cell type but are nonetheless expressed in both **a** and α cells (*e.g.*, *STE13* and *STE16*), but these genes are not **a**- or α-specific genes in the sense defined here. See text and Table 1 for further details.

spond to either pheromone and in a defect in mating. This view is derived in part from the finding that *matα2* mutants that also carry a mutant allele of *STE3* mate efficiently as **a** cells (STRATHERN, HICKS and HERSKOWITZ 1981; SPRAGUE, RINE and HERSKOWITZ 1981). Apparently the **a**-factor receptor is a key component in the antagonism of the **a** cell phenotype in the setting of a *matα2* mutant. To understand the roles that the pheromones and receptors play in determining the pheromone response and mating phenotypes of *matα2* mutants, we have engineered a series of isogenic strains that express various combinations of α- and **a**-specific products. Finally, we have determined whether the specificity of mating can be perturbed if the ability of an **a** cell to synthesize its normal pheromone and receptor is disrupted, and instead it is made to synthesize the inappropriate pheromone and receptor.

MATERIALS AND METHODS

Plasmids, strains and media: Relevant strains are listed in Table 2. Appropriate alleles of mating-related genes were introduced by the one-step gene replacement method of ROTHSTEIN (1983). *matα2-Δ1* contains an *Xba*I deletion that removes 53 codons from the 3′ end of *matα2*. (The sequence of *matα2* is reported in ASTELL *et al.* 1981.) *ste3*::*LEU2* is described in HAGEN, MCCAFFREY and SPRAGUE (1986). *bar1*::*LEU2* is described in KRONSTAD, HOLLY and MACKAY (1987). *ste13*::*LEU2* has approximately 1.6 kb of *STE13* coding sequence replaced with *LEU2* DNA (G. SPRAGUE and I. HERSKOWITZ, unpublished data). *matα1-189* is described in TATCHELL *et al.* (1981). The *ura3* mutation in SY1229 was selected using 5-fluoroorotic acid (BOEKE *et al.* 1987). The *lys2* mutations in strains SY1240 and SY1251 were selected using α-aminoadipic acid (CHATTOO *et al.* 1979). Strain SY1263 was made *MATα* by *HO*-promoted mating-type interconversion using plasmid *GAL10-HO* (JENSEN and HERSKOWITZ 1984).

YCp50 contains *CEN4* and *ARS1* on a *URA3*-based plasmid (STINCHCOMB, MANN and DAVIS 1982). pSL602 contains a 4-kb *Hin*dIII fragment of *MATα* on YCp50 (kindly provided by J. MARGOLSKEE). pSL552 contains *STE3* under *GAL1* promoter control on YCp50 (BENDER and SPRAGUE 1986). pSL113 contains an inversion between the *Xho*I and *Sal*I sites of YEp13, a *LEU2* 2 μm-based plasmid (BROACH, STRATHERN and HICKS 1979). Into this plasmid we cloned a 1.7-kb *Bgl*II-*Sal*I fragment containing *TPI-MFα1* (kindly provided by V. MACKAY) and a 4.8-kb fragment containing *LYS2*, to generate pSL801. The 4.8 *LYS2* fragment was derived from pDA620-R (BARNES and THORNER 1986). A *Hin*dIII *LYS2* fragment from this plasmid was cloned into a version of pBR322 in which the *Hin*dIII-*Eco*RI segment was substituted by the *Hin*dIII-*Eco*RI polylinker from M13 phage mp18. This manipulation enabled the *LYS2* segment to be cloned into pSL113 as a *Sal*I fragment.

The media used were YPD (SHERMAN, HICKS and FINK 1982), YPG (YPD containing 3% galactose instead of 2% glucose), SD-His or SD-Ura (synthetic glucose medium supplemented with adenine, L-arginine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, L-lysine, and uracil or L-histidine) (SHERMAN, HICKS and FINK 1982), SD-Ura-Lys (SD-Ura medium that also lacks L-lysine) and SG-Ura-Lys (same as SD-Ura-Lys, but containing galactose instead of glucose). SD + X is synthetic glucose medium supplemented with the indicated amino acids. Plates contained 2% Bacto agar. Yeast cells were grown at 30°.

Quantitative matings: Quantitative mating were performed essentially as described by HARTWELL (1980). In particular, for the matings presented in Table 3, cells of mating-type tester strain SY1229 were grown to stationary phase in YPD, pelleted by centrifugation, and resuspended at approximately 20 Klett units (KU; 1 KU (green filter) = 10^5 cells/ml) in YPG. Simultaneously, plasmid-bearing cells whose mating efficiency was to be determined (*matα1-189*, *MAT***a**, and *MAT***a** *bar1*::*LEU2 ste2-10*::*LEU2 ste6*::*lacZ*) were grown in galactose-containing plasmid-selective minimal medium (SG-Ura or SG-Ura-Lys) to approximately 100 KU, pelleted by centrifugation, resuspended in YPG at approximately 15 KU, and allowed to double twice (approximately 7 hr). An aliquot of each culture was then plated on SD + His + Leu to check for reversion and contamination. One milliliter (50 KU) of plasmid-bearing cells was mixed by vortexing with 1 ml (50 KU) of SY1229. One milliliter of each mating mix was filtered onto a nitrocellulose filter (Millipore, type HA 0.45 μm) and incubated for 6 hr on YPG plates. Cells from the filters were then resuspended in water, sonicated, diluted, and titered on SD + His + Leu and on YPD plates. The absolute mating efficiency was defined as the ratio of the titer of diploids (from the SD + His + Leu plates) to the titer of the total number of cells in the mating mix (from the YPD plates).

In the matings involving W303-1b or YY1154 with tester strains EG123, cells were prepared essentially as described above except glucose was always the carbon source. The cells were mated for 4.5 hr on YPD plates, resuspended in water, sonicated, and plated on SD-His (to titer diploids) and YPD plates (to titer the total number of cells in the mating mix).

In the mating between YY1129 and SY1229, α factor was provided by inclusion of YY506 cells (α cells that do

TABLE 2

Strains

Strain	Genotype	Source
DC5	*MAT***a** *leu2-3,-112 his3 can1 gal2*	J. STRATHERN
EG123	*MAT***a** *leu2 ura3 trp1 his4-519 can1* Gal$^-$	K. TATCHELL
246-1-1	*MATα leu2 ura3 trp1 his4-519 can1* Gal$^-$	K. TATCHELL
23α-189	*matα1-189* (other markers as in 246-1-1)	K. TATCHELL
W303-1b	*MATα leu2-3,-112 ura3-1 trp1-1 his3-11,-15 ade2-1 can1-100*	J. KURJAN
T2-4d	*MATα mfα1::LEU2C mfα2::LEU2C* (other markers as in W303-1b)	J. KURJAN
SY1229	*MAT***a** *leu2-3,-112 his3 ura3 can1 gal2*	*ura3* derivative of DC5; D. HAGEN
SY1263	*MATα leu2-3,-112 his3 ura3 can1 gal2*	*MATα* derivative of SY1229
SY1271	*MATα mfα1::LEU2C mfα2::LEU2C lys2* (other markers as in W303-1b)	*lys2* derivative of T2-4d
YY506	*MATα ste3::LEU2 leu2-3,-112 his4 can1 gal2*	HAGEN, MCCAFFREY and SPRAGUE (1986)
YY1154	*TPI-MFα1 LYS2 LEU2 /MATα mfα1::LEU2C mfα2::LEU2C lys2* (other markers as in W303-1b)	pSL801 (YEp13)[a]/SY1271
SY925	*MAT***a** *ste2-10::LEU2 leu2 ura3 met14 trp1am his6 ade1 ade2-1oc cry1* or *CRY1*	BENDER and SPRAGUE (1986)
	All subsequent strains are isogenic to SY925[b]	
SY987	*MAT***a** *ste2-10::LEU2 ste6::lacZ*	BENDER and SPRAGUE (1986)
SY1103	*MATα ste2-10::LEU2 ste6::lacZ*	SY987 made *MATα*
SY1153	*matα2-Δ1 ste2-10::LEU2 ste6::lacZ*	SY987 made *matα2-Δ1*
SY1154	*MAT***a**	SY925 made *STE2*
SY1156	*MAT***a** *ste3::LEU2*	SY1154 made *ste3::LEU2*
SY1159	*MAT***a** *bar1::LEU2*	SY1154 made *bar1::LEU2*
SY1164	*MAT***a** *bar1::LEU2 ste2-10::LEU2 ste6::lacZ*	Segregant from YY969 × SY1159
SY1167	*matα2-Δ1 bar1::LEU2 ste2-10::LEU2*	Segregant from YY969 × SY1159
SY1169	*matα2-Δ1 bar1::LEU2 ste2-10::LEU2 ste6::lacZ*	Segregant from YY969 × SY1159
SY1176	*matα2-Δ1 ste2-10::LEU2*	Segregant from YY969 × SY1159
SY1179	*matα2-Δ1 ste6::lacZ*	Segregant from YY969 × SY1159
SY1180	*matα2-Δ1 ste3::leu2*	Segregant from YY969 × SY1156
SY1184	*matα2-Δ1 bar1::LEU2 ste6::lacZ*	Segregant from YY969 × SY1159
SY1187	*MATα*	Segregant from SY1103 × SY1156
SY1195	*matα2-Δ1 ste3::LEU2 bar1::LEU2*	Segregant from YY987 × SY1156
SY1197	*matα2-Δ1 bar1::LEU2*	Segregant from YY969 × SY1159
SY1212	*matα2-Δ1 ste13::LEU2*	SY1256 made *ste13::LEU2*
SY1228	*matα2-Δ1 ste13::LEU2 ste6::lacZ*	SY1179 made *ste13::LEU2*
SY1234	*matα2-Δ1 ste3::LEU2 ste13::LEU2*	Segregant from YY1059 × SY1156
SY1236	*MAT***a** *ste3::LEU2 ste13::LEU2*	Segregant from YY1059 × SY1156
SY1240	*MAT***a** *bar1::LEU2 ste2-10::LEU2 ste6::lacZ lys2*	*lys2* derivative of SY1164
SY1241	*matα1-189*	SY1187 made *matα1-189*
SY1242	*matα2-Δ1 ste3::LEU2 ste13::LEU2 ste6::lacZ*	Segregant from YY969 × SY1236
SY1251	*matα1-189 lys2*	*lys2* derivative of SY1241
SY1256	*matα2-Δ1*	Segregant from YY969 × SY1159
YY969	*MATα URA3 /matα2-Δ1 ste2-10::LEU2 ste6::lacZ*	pSL602 (YCp50)/SY1153
YY987	*MATα URA3 /matα2-Δ1 bar1::LEU2 ste6::lacZ*	pSL602 (YCp50)/SY1184
YY1059	*MATα URA3 /matα2-Δ1 ste13::LEU2*	pSL602 (YCp50)/SY1212
YY1101	*MATα URA3 /matα1-189 lys2*	pSL602 (YCp50)/SY1251
YY1102	*URA3 /matα1-189 lys2*	YCp50/SY1251
YY1104	*GAL1-STE3 URA3 /matα1-189 lys2*	pSL552 (YCp50)/SY1251
YY1121	*URA3, TPI-MFα1 LYS2 LEU2 /matα1-189 lys2*	YCp50, pSL801 (YEp13)/SY1251
YY1091	*GAL1-STE3 URA3, TPI-MFα1 LYS2 LEU2 /matα1-189 lys2*	pSL552 (YCp50), pSL801 (YEp13)/SY1251
YY1129	*URA3 /MAT***a**	YCp50/SY1154
YY1110	*URA3 /MAT***a** *bar1::LEU2 ste2-10::LEU2 ste6::lacZ lys2*	YCp50/SY1240
YY1087	*GAL1-STE3 URA3, TPI-MFα1 LYS2 LEU2 /MAT***a** *bar1::LEU2 ste2-10::LEU2 ste6::lacZ lys2*	pSL552 (YCp50), pSL801 (YEp13)/SY1240

[a] Whether the plasmid is a derivative of YCp50 or YEp13 is indicated in parentheses.

[b] The genotype at the mating-type locus is given for all strains. For *STE2*, *STE3*, *STE6*, *STE13* and *BAR1*, a designation is given only if the gene is mutant. In addition, newly introduced mutations in the *LYS2* gene are also noted. For the YY plasmids the chromosomal *ura3* genotype is given to emphasize that there is a selection for maintenance of *URA3*-based plasmids.

not mate because of a mutation in *STE3*, but that do secrete α factor). In these experiments, YY506 cells were grown as the SY1229 cells were grown and then filtered with an equal number of YY1129 and SY1229 cells. The matings were done as described above, except that the mating efficiency was defined as the ratio of the titer of diploids to 0.67× the total titer of cells.

In the matings described above that involved plasmid-bearing strains, the proportion of cells actually containing the plasmids was determined at the beginning of the mating by titering the number of cells able to form colonies on selective medium (*e.g.*, SG-Ura) and comparing that value with the number of cells able to form colonies on nonselective medium (*e.g.*, SG-Ura + Ura). For YCp50-based plasmids the proportion of plasmid-bearing cells was typically 0.7 to 0.9, and for YEp13-based plasmids the proportion was typically 0.4 to 0.7. Mating efficiencies were not normalized to account for these proportions because the generation in which the plasmids were lost is not known nor is the half-life of receptor activity or pheromone production in the plasmid-free cells.

The matings for Table 4 were performed as follows. Cells from stationary phase YPD cultures were diluted to approximately 12 KU into YPD and allowed to double twice. Cells were plated on SD-His to assay for revertants and contaminants. One milliliter (50 KU) of cells whose mating efficiency was to be determined was mixed by vortexing with 1 ml (50 KU) of the appropriate tester cells. The mating mixes were filtered through nitrocellulose filters and the filters were incubated 4.5 hr on YPD plates. Cells on the filters were then resuspended in water, sonicated, diluted, and plated on SD-His (to titer diploids) and on YPD (to titer the total number of cells in the mating mix).

Cell cycle arrest: Cells from stationary phase YPD cultures were diluted to approximately 12 KU in YPD and allowed to double twice. The cultures were then incubated with an equal volume of either cell-free filtrates obtained from stationary phase YPD cultures of strains DC5 (as a source of **a** factor) or 23α-189 (*matα1*; therefore no pheromone present). In some instances the 23α-189 filtrate contained 2.5 μg/ml α factor (Sigma). After 90 min, aliquots of cultures were pelleted by centrifugation, resuspended by vortexing in 3% formaldehyde saline solution, and sonicated to separate clumped cells. At least 200 cells from each culture were scored for the presence or absence of buds using phase contrast microscopy. Cells in the G_1 phase of the cell cycle are unbudded.

Transcript analysis: Cultures prepared for cell cycle arrest were also used for transcript analysis. Cells were harvested 30 min after exposure to pheromone. RNA was isolated as described in SPRAGUE, JENSEN and HERSKOWITZ (1983). *FUS1* and *URA3* RNAs were probed as described in BENDER and SPRAGUE (1986). Quantitation of the RNAs was by densitometric scanning of autoradiograms.

RESULTS

The a-factor receptor and α factor are the only α-specific products needed for mating: A *matα1* mutant expresses neither α- nor **a**-specific products (see Figure 1). To ask which α-specific products are needed for mating, *matα1* mutants engineered to express the **a**-factor receptor and/or α factor were tested for mating competence. To express the **a**-factor receptor in a *matα1* mutant, *STE3* was placed under the control of the *GAL1* promoter on the single-copy plasmid YCp50 (BENDER and SPRAGUE 1986). To produce α factor, *MFα1* was expressed under the control of the *TPI* promoter (triose phosphate isomerase) on a derivative of plasmid YEp13 (*TPI-MFα1* was the gift of V. MACKAY). The mating ability of *matα1* cells containing the *GAL1-STE3* and/or the *TPI-MFα1* plasmid was compared with the mating ability of the same cells harboring either YCp50 alone or YCp50 containing the wild-type *MATα1* gene.

As shown in Table 3, *matα1* cells that contained the *MATα* plasmid, and therefore expressed all α-specific genes, mated efficiently, whereas *matα1* cells with the control plasmid, mated with a relative efficiency of 10^{-6} (strains YY1101 and YY1102). The expression of *STE3* alone did not increase the mating ability of the *matα1* mutant, and the expression of *MFα1* alone increased mating by about one order of magnitude (strains YY1104 and YY1121). The expression of both *STE3* and *MFα1*, however, increased the mating efficiency more than four orders of magnitude to about 6% (strain YY1091). This mating efficiency, though substantial, should be further normalized to the efficiency observed when the *STE3* or *MFα1* plasmids were tested for the ability to restore mating to strains harboring defects in the receptor structural gene alone or in the α-factor structural genes alone. The *GAL1-STE3* plasmid complemented a *ste3* chromosomal mutation with 100% efficiency (data not shown), but the *TP1-MFα1* plasmid complemented an *mfα1 mfα2* double mutant only well enough to allow 30% of the cells to mate (Table 3). These data suggest that a more accurate estimate of the mating efficiency of the *matα1* mutants containing the *GAL1-STE3* and *TPI-MFα1* genes is 20% (6÷30%), nearly indistinguishable from wild-type mating. We conclude that the **a**-factor receptor and α factor are the only α-specific products needed for mating, although other α-specific products might make minor contributions to mating. In parallel experiments, J. KURJAN has reached the same conclusion (personal communication).

Wild-type *MAT*a cells can mate with *MAT*a cells that express α-factor pheromone and a-factor receptor: The ability of *matα1* mutants containing *GAL1-STE3* and *TPI-MFα1* to mate efficiently raises the question of how the specificity of mating is conferred, given that the pheromones and receptors are not thought to be involved in cell contact. One possibility is that other α- or **a**-specific products may function to prevent mating between cells of the *same* mating type. A second possibility is that the reciprocal detection of pheromone by potential mating partners in some fashion establishes specificity. To distinguish between these possibilities, and, more generally, to determine if normal mating specificity could be perturbed, we asked whether **a** cells could be made to mate with

TABLE 3

Expression of *STE3* and *MFα1* is sufficient to allow mating as α

Strain[a]	Plasmid and relevant genes[b]	Chromosomal genotype	Mating efficiency with **a** cells[c]
YY1101	pSL602 (YCp50); *MATα*	*matα1*	1
YY1102	YCp50; none	*matα1*	1×10^{-6}
YY1104	pSL552 (YCp50); *GAL1-STE3*	*matα1*	2×10^{-6}
YY1121	pSL801 (YEp13) and YCp50; *TPI-MFα1*	*matα1*	2×10^{-5}
YY1091	pSL801 (YEp13) and pSL552; *TPI-MFα1*, *GAL1-STE3*	*matα1*	6×10^{-2}
W303-1b	None	*MFα1 MFα2*	1
YY1154	pSL801 (YEp13); *TPI-MFα1*	*mfα1 mfα2*	3×10^{-1}
YY1129	YCp50; none	*MAT***a**	2×10^{-6}
YY1129	YCp50, none	*MAT***a** +α factor	2×10^{-6}
YY1110	YCp50; none	*MAT***a** *bar1 ste2 ste6*	1×10^{-5}
YY108	pSL801 (YEp13) and pSL552 (YCp50); *GAL1-STE3*, *TPI-MFα1*	*MAT***a** *bar1 ste2 ste6*	5×10^{-3}

[a] All strains were isogenic except W303-16 and YY1154, which were isogenic to each other.

[b] Whether the plasmid is a derivative of YCp50 or YEp13 is indicated in parentheses.

[c] Mating efficiencies were determined as described in MATERIALS AND METHODS. The **a** tester strain was SY1229 except for matings involving W303-1b and YY1154. For these two strains the **a** tester was EG123. The mating efficiency for W303-1b is the average of two experiments; for YY1102, YY1104, YY1121, YY1129 + α factor, and YY1110, the average of three experiments; for YY1154, the average of four experiments; for YY1101 and YY1129, the average of six experiments; and for YY1091 and YY1087, the average of nine experiments. Mating efficiencies involving W303-1b and YY1154 were normalized to the efficiency observed in the mating of W303-1b with EG123 (absolute mating efficiency of 2×10^{-1}). Mating efficiencies for all other strains were normalized to the efficiency observed in the mating of YY1101 with SY1229 (absolute mating efficiency of $4 \times 10^{-1} \pm$ a standard error of 1.5×10^{-1}).

other **a** cells. We engineered an **a** strain to produce the species of pheromone and pheromone receptor normally expressed only in an α strain. The strain carries mutations in the **a**-specific *STE2* and *STE6* genes so that the α-factor receptor and active **a** factor would not be produced. In addition, the strain carries a mutation in *BAR1*, which encodes an α-factor protease (CIEJEK and THORNER 1979; SPRAGUE and HERSKOWITZ 1981; MANNEY 1983; MACKAY *et al.* 1988), so production of α factor would not be prevented. Plasmids containing *GAL1-STE3* and *TPI-MFα1* were introduced into the *MAT***a** *ste2 ste6 bar1* strain, and the ability of this strain to mate with a wild-type **a** strain was determined.

Wild-type **a** cells mated with other **a** cells at a frequency of about 10^{-6} (Table 3), even in the presence of exogenous α factor (provided from helper, nonmating α cells, see MATERIALS AND METHODS). In contrast, the *MAT***a** *ste2 ste6 bar1* strain carrying the *STE3* and *MFα1* plasmids mated with other **a** cells at a frequency of 5×10^{-3}, a substantial level of mating. The most relevant comparison is with the *matα1* mutant harboring the same plasmids, which mated with **a** cells at a frequency of 6×10^{-2} (Table 3). By this comparison, the **a** × **a** mating involving the engineered **a** strain is reduced only about tenfold from the expected maximum frequency. Thus, although there may be an **a**-specific product that partially inhibits its **a** × **a** mating, the ability to send and receive pheromone signals appears to be an important determinant for the specificity of mating.

Pheromones and pheromone receptors control the mating specificity of *matα2* mutants: *matα2* mutants express both the α- and **a**-specific gene sets (see Figure 1). Although the initial expectation might be that the mutants would be promiscuous and mate with both **a** and α cells, in fact they exhibit a nonmating phenotype and are unable to respond to either pheromone. As mentioned in the introduction, it has been proposed that antagonisms between α- and **a**-specific gene products account for these phenotypes. To determine what functions must be removed to restore the capacity to mate or to respond to pheromone in a *matα2* background, we have created mutations in the receptor structural genes and/or in genes required for pheromone production. All strains used were isogenic; the mutations were introduced by substitutive transformation (ROTHSTEIN 1983) using disrupted alleles of the appropriate genes. The *ste2* and *ste3* mutations abolish receptor function (HAGEN, MCCAFFREY and SPRAGUE 1986; BENDER and SPRAGUE 1986; NAKAYAMA, MIYAJIMA and ARAI 1987), and strains harboring *ste6* or *ste13* mutations secrete very little active **a** factor or α factor (WILSON and HERSKOWITZ 1987; SPRAGUE, RINE and HERSKOWITZ 1981; JULIUS *et al.* 1983). In some experiments, a *ste14* mutant allele, which totally abolishes **a**-factor production (WILSON and HERSKOWITZ 1987), was used. These studies again point to the receptors and pheromones as the central players for determining mating competence and specificity. We first describe experiments that examine the mating capacity of the *matα2* derivatives and then turn to a detailed look at the ability of *matα2* mutants and their derivatives to respond to pheromones.

TABLE 4

Mating efficiencies of *matα2* mutants and their derivatives

Strain	Genotype	αF[a]	αF Rec	aF	aF Rec	Mating efficiency with α cells[b]	Mating efficiency with a cells[c]
SY1154	*MAT***a**	−	+	+	−	1	8×10^{-7}
SY1187	*MATα*	+	−	−	+	6×10^{-7}	1
SY1256	*matα2*	±	+	+	+	2×10^{-2}	6×10^{-7}
SY1180	*matα2 ste3*	±	+	+	−	5×10^{-1}	1×10^{-7}
SY1212	*matα2 ste13*	−	+	+	+	2×10^{-2}	8×10^{-8}
SY1234	*matα2 ste13 ste3*	−	+	+	−	7×10^{-1}	1×10^{-7}
SY1197	*matα2 bar1*	+	+	+	+	6×10^{-4}	2×10^{-5}
SY1167	*matα2 bar1 ste2*	+	−	+	+	1×10^{-3}	4×10^{-5}
SY1184	*matα2 bar1 ste6*	+	+	−	+	$<1 \times 10^{-7}$	9×10^{-3}
SY1169	*matα2 bar1 ste2 ste6*	+	−	−	+	$<6 \times 10^{-8}$	4×10^{-2}

[a] Abbreviations: αF, α factor; αF Rec, α-factor receptor; **a**F, **a** factor; **a**F Rec, **a**-factor receptor. ± indicates the very slight amount of α factor that is secreted by *BAR1* strains that are otherwise competent to synthesize α factor.

[b] The α-tester strain was 246-1-1. All mating efficiencies are normalized to the efficiency of mating of strains SY1154 and 246-1-1, which was 3.9×10^{-1} in one experiment and 4.8×10^{-1} in a second experiment (see MATERIALS AND METHODS for details). The values reported are the average of two determinations. Matings with efficiencies less than 10^{-5} varied no more than 50% about the mean. Matings with efficiencies greater than 10^{-5} varied no more than 20% about the mean.

[c] The **a** tester strain was EG123. All mating efficiencies are normalized to the efficiency of mating of strains SY1187 and EG123, which was 6.1×10^{-1} in one experiment and 7.8×10^{-1} in a second experiment. The values reported are the average of two determinations. Matings with efficiencies less than 10^{-5} varied no more than 50% about the mean. Matings with efficiencies greater than 10^{-5} varied no more than 20% about the mean.

Previous work had established the requirements to allow *matα2* mutants to mate efficiently with α cells—the production of **a**-factor receptor must be prevented (by a *ste3* mutation) (SPRAGUE, RINE and HERSKOWITZ 1981; STRATHERN, HICKS and HERSKOWITZ 1981). *matα2* mutants have an inherent capacity to mate more efficiently with α cells than with **a** cells, and the introduction of a *ste3* mutation augments their ability to mate with α cells. In our genetic background, the mating efficiency of a *matα2* mutant (SY1256) was about 2×10^{-2} with α cells and 6×10^{-7} with **a** cells (Table 4). The *matα2 ste3* double mutants mated with α cells at essentially the same frequency as wild-type **a** cells mate with α cells (Table 4). Thus, removal of a single α-specific function, the **a**-factor receptor, allows the *matα2* mutant to mate as if it were a wild-type **a** cell. In contrast, removal of the capacity to produce α factor by the introduction of a mutant allele of *STE13* (a gene whose product is needed for α-factor maturation) had little effect on the mating ability of the *matα2* mutant (Table 4).

Because introduction of a mutation in an α-specific gene (*STE3*) enabled *matα2* mutants to mate as **a** cells, we sought to determine whether introduction of mutations in **a**-specific genes involved in pheromone metabolism and reception would enable *matα2* mutants to mate as α cells. *matα2* mutants mate as α cells with a frequency of about 6×10^{-7}. Part of the explanation for this low mating frequency is that *matα2* mutants do not secrete detectable levels of α factor due to the expression of the **a**-specific *BAR1* gene (SPRAGUE and HERSKOWITZ 1981). Indeed, the ability of the *matα2* mutant to mate as α increased by one order of magnitude when a *bar1* mutation was introduced (SY1197; Table 4). The introduction of a mutant allele of *STE2* (α-factor receptor) had little effect on the mating ability of the *matα2 bar1* strain (SY1167). This is a somewhat surprising result given the ability of *ste3* mutations (**a**-factor receptor) to allow *matα2* mutants to mate as **a**. On the other hand, the introduction of a mutant allele of *STE6*, which is required for **a**-factor maturation, allowed the *matα2 bar1* strain to mate as α at a frequency of 9×10^{-3} (SY1184). Finally, a *matα2 bar1 ste6 ste2* quadruple mutant had a slightly better mating efficiency (4×10^{-2}; SY1169, Table 4). Thus, a reasonable capacity to mate as α can be conferred to a *matα2* mutant by allowing it to secrete detectable α factor and by disrupting its ability to produce **a** factor. The observed mating is not at wild-type levels, however. It appears that, as was true in the case of the **a** × **a** matings described above, an unidentified **a**-specific product partially interferes with the ability to mate with **a** cells. We conclude that the ability of *matα2* mutants to mate either as **a** cells or as α cells can be unmasked by elimination of known α- or **a**-specific functions.

***matα2* mutants exhibit a partial response to the pheromone they secrete:** As noted in the introduction, *matα2* mutants express both pheromone receptors and both pheromones, although the amount of secreted α factor that can be detected is slight, due to the action of the BAR1 product. Because one of the responses to pheromone is cell cycle arrest in G_1, the initial expectation is that *matα2* mutants would be

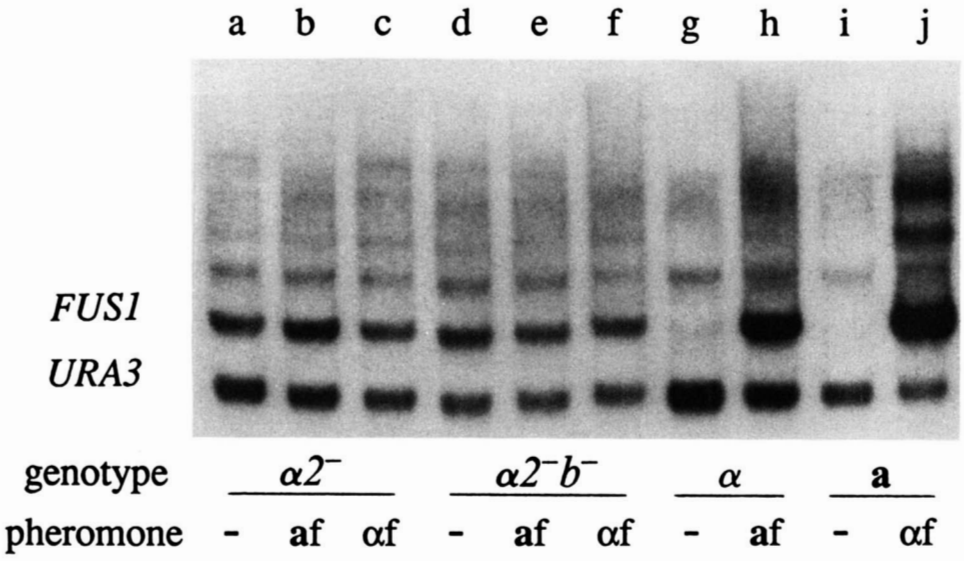

FIGURE 2.—*FUS1* transcript levels in wild-type cells and in *matα2* mutants. RNA was isolated from strains SY1256 (*matα2*; lanes a–c), SY1197 (*matα2 bar1*; lanes d–f), SY1187 (*MATα*; lanes g and h), and SY1154 (*MAT***a**; lanes i and j). The strains had been treated with **a** factor, α factor, or no pheromone, as indicated. For each lane, 20 μg of total RNA was fractionated by agarose electrophoresis and transferred to nitrocellulose. *FUS1* and *URA3* transcripts were visualized by hybridization with radioactive RNA probes followed by autoradiography. The positions of the *FUS1* and *URA3* transcripts are indicated.

inviable. Two explanations for their viability can be offered: the pheromone response pathway may have adapted to the continuous supply of pheromone (MOORE 1984) or the receptors may interfere with each other's function. In this and the subsequent section we present evidence for both phenomena.

To determine whether *matα2* cells show *any* response to the pheromones they produce, we measured the amount of transcript from *FUS1*, a gene whose transcription is greatly enhanced in response to either pheromone (MCCAFFREY *et al.* 1987; TRUEHEART, BOEKE and FINK 1987). Figure 2 shows that the amount of *FUS1* transcript in a *matα2* mutant (lane a) was intermediate to the basal and pheromone-induced *FUS1* transcript levels found in isogenic, wild-type **a** or α strains (lanes g–j). Moreover, the amount of *FUS1* transcript in the *matα2* mutants did not change in response to added pheromone (lanes b and c). *FUS1* transcript levels in a *matα2 bar1* mutant, which secretes wild-type quantities of α factor (SPRAGUE and HERSKOWITZ 1981) as well as **a** factor, were also comparable to those seen for the *matα2* mutants (lanes d–f; summarized in Table 5).

Strains that do not produce either pheromone (*e.g.*, *matα2 ste13 ste6*) showed low *FUS1* transcript levels (Figure 3A, lane f and Table 5, SY1228), demonstrating that the elevated *FUS1* levels seen in *matα2* mutants indeed reflect an autocrine response. Thus, *matα2* mutants have responded partially to the pheromone they secrete, indicating that the receptors have at least some capacity to function.

These data suggest that adaptation to the continuous supply of pheromone is part of the explanation for the viability of *matα2* mutants. This suggestion was made more firm by examination of *FUS1* transcript levels in *matα2 ste2* and *matα2 ste3 bar1* strains. These strains express one cognate receptor-pheromone set but do not express the other receptor, so there is no possibility that interference between the receptors can contribute to the phenotype. Both strains showed levels of *FUS1* transcript similar to the induced levels seen for wild-type strains, and the *FUS1* transcript levels did not change when pheromone was added (Figure 3A, lanes d and e; Figure 3B, lanes d and e; summarized in Table 5, strains SY1176 and SY1195). *matα2 ste2 ste6* and *matα2 ste3 ste13* strains, which no longer synthesize the cognate pheromone to the receptor they synthesize (*STE6* is required for **a**-factor maturation and *STE13* for α-factor maturation), had low levels of *FUS1* transcript (Figure 3B, lanes k–n; Table 5, strains SY1153 and SY1234), again demonstrating the autocrine nature of the intermediate *FUS1* transcript levels.

The a-factor receptor interferes with response to α factor: If adaptation to pheromone is the explanation for the viability of *matα2* mutants, and if there is not interference between α- and **a**-specific products, we expected that a *matα2* mutant that did not itself produce *either* pheromone ought to be able to respond to either pheromone added exogenously. To determine whether this expectation proved true, we assayed two facets of the pheromone response of a *matα2 ste6 ste13* strain, namely transcriptional induction of *FUS1* and cell cycle arrest. As shown in Figure 3A (lanes f–i) and Table 5 (strain SY1228), the *matα2 ste6 ste13* mutant had a low basal level of *FUS1* transcript that was greatly enhanced by the addition of either pheromone. However, although the mutant responded to both pheromones, it did not arrest fully in response to either pheromone, and it arrested relatively more poorly in response to α factor than to **a** factor (Table 5). Perhaps interference between the α-factor and **a**-factor receptors prevents full response to pheromone. The experiments described below dem-

TABLE 5

Response of *matα2* mutants and their derivatives to pheromone

A. Response to **a** factor

Strain	Genotype	Phenotype[a]				*FUS1/URA3*[b]		Percent unbudded[c]	
		αF	αF Rec	aF	aF Rec	Basal	+aF	Basal	+aF
SY1187	*MATα*	+	−	−	+	0.1	2	32 (28, 35)	78 (72, 83)
SY1153	*matα2 ste2 ste6*	±	−	−	+	0.2	2	39 (41, 37)	65 (55, 75)
SY1228	*matα2 ste13 ste6*	−	+	−	+	0.1	3	32 (31, 32)	62 (60, 63)
SY1179	*matα2 ste6*	±	+	−	+	0.4	2	33 (31, 35)	59 (55, 63)
SY1164	*matα2 bar1 ste6*	+	+	−	+	0.6	4	45 (51, 39)	71 (72, 69)
SY1176	*matα2 ste2*	±	−	+	+	1	0.9	42 (38, 46)	44 (35, 52)
SY1212	*matα2 ste13*	−	+	+	+	1	0.9	43 (41, 45)	46 (40, 52)
SY1256	*matα2*	±	+	+	+	1	1	40 (32, 48)	46 (78, 54)
SY1197	*matα2 bar1*	+	+	+	+	1	1	37 (32, 43)	42 (38, 45)

B. Response to α factor

Strain	Genotype	Phenotype				*FUS1/URA3*		Percent unbudded	
		αF	αF Rec	aF	aF Rec	Basal	+αF	Basal	+αF
SY1154	*MAT***a**	−	+	+	−	<0.1	10	31 (32, 30)	79 (81, 77)
SY1242	*matα2 ste13 ste6 ste3*	−	+	−	−	0.3	9	40 (39, 40)	83 (84, 82)
SY1234	*matα2 ste13 ste3*	−	+	+	−	0.1	8	36 (38, 34)	81 (81, 81)
SY1228	*matα2 ste13 ste6*	−	+	−	+	0.1	5	32 (31, 32)	51 (50, 53)
SY1212	*matα2 ste13*	−	+	+	+	1	2	43 (41, 45)	51 (45, 57)
SY1180	*matα2 ste3*	±	+	+	−	1	4	45 (45, 46)	74 (76, 72)
SY1179	*matα2 ste6*	±	+	−	+	0.4	2	33 (31, 35)	37 (31, 43)
SY1256	*matα2*	±	+	+	+	1	1	40 (32, 48)	42 (42, 42)
SY1195	*matα2 bar1 ste3*	+	+	+	−	5	5	57 (54, 60)	53 (50, 56)
SY1184	*matα2 bar1 ste6*	+	+	−	+	0.6	0.5	45 (51, 39)	46 (48, 44)
SY1197	*matα2 bar1*	+	+	+	+	1	1	37 (32, 43)	39 (41, 36)

[a] Phenotype symbols are as defined for Table 4.

[b] The ratio of *FUS1* to *URA3* transcript was determined as described in MATERIALS AND METHODS. Values presented are the average of two determinations, which were in close agreement.

[c] The percent unbudded cells in a culture was determined with and without pheromone treatment as described in MATERIALS AND METHODS. The average of two determinations is presented, followed by the values for each experiment in parentheses.

onstrate that receptor interference contributes to the phenotype of *matα2* mutants.

To investigate more fully the relative contributions of pheromone adaptation and receptor interference to the phenotype of *matα2* mutants, we used a complete set of isogenic *matα2* mutants, each of which is deficient in the ability to produce one or both pheromones and/or one or both pheromone receptors. The response of each strain to added pheromone was assayed, and the results are shown in Figure 3 and Table 5.

For a *matα2* mutant to respond to **a** factor, the only requirement (other than that it produces the **a**-factor receptor) is that the strain does not produce **a** factor: *matα2 ste6, matα2 ste2 ste6,* and *matα2 ste13 ste6* strains each responded to added **a** factor; but *matα2, matα2 ste2,* and *matα2 ste13* strains failed to respond. Even a *matα2 bar1 ste6* strain, which secretes high levels of α factor and expresses the α-factor receptor (but does not produce **a** factor) responded to added **a** factor. This is a surprising finding because a simple expectation would be that the strain has adapted to the α factor it secretes and that adaptation to one pheromone should result in adaptation to a heterologous pheromone. The ability of the *matα2 bar1 ste6* strain to respond to **a** factor could indicate that adaptation can be pheromone-specific. For example, perhaps adaptation to **a** factor by *matα2* cells requires a greater dose of pheromone than does adaptation to α factor. It is known that adaptation to α factor is dose dependent (MOORE 1984), but the dose response relationship is not predictable in these autocrine situations. A second possibility is that the strain is not adapted even to α factor. Rather, the *matα2 bar1 ste6* strain may not respond appreciably to the α factor it secretes due to interference by an α-specific product, perhaps the **a**-factor receptor. Evidence that the **a**-factor receptor does indeed interfere with response to α factor emerges from our analysis of what is required for a *matα2* mutant to respond to added α factor.

Analysis of the data presented in Figure 3 and Table 5 leads to two generalizations concerning the requirements for response to α factor. The first is that a *matα2* mutant that secretes wild-type levels of α factor (due to a *bar1* mutation) cannot respond to added α factor. This is analogous to the generalization regard-

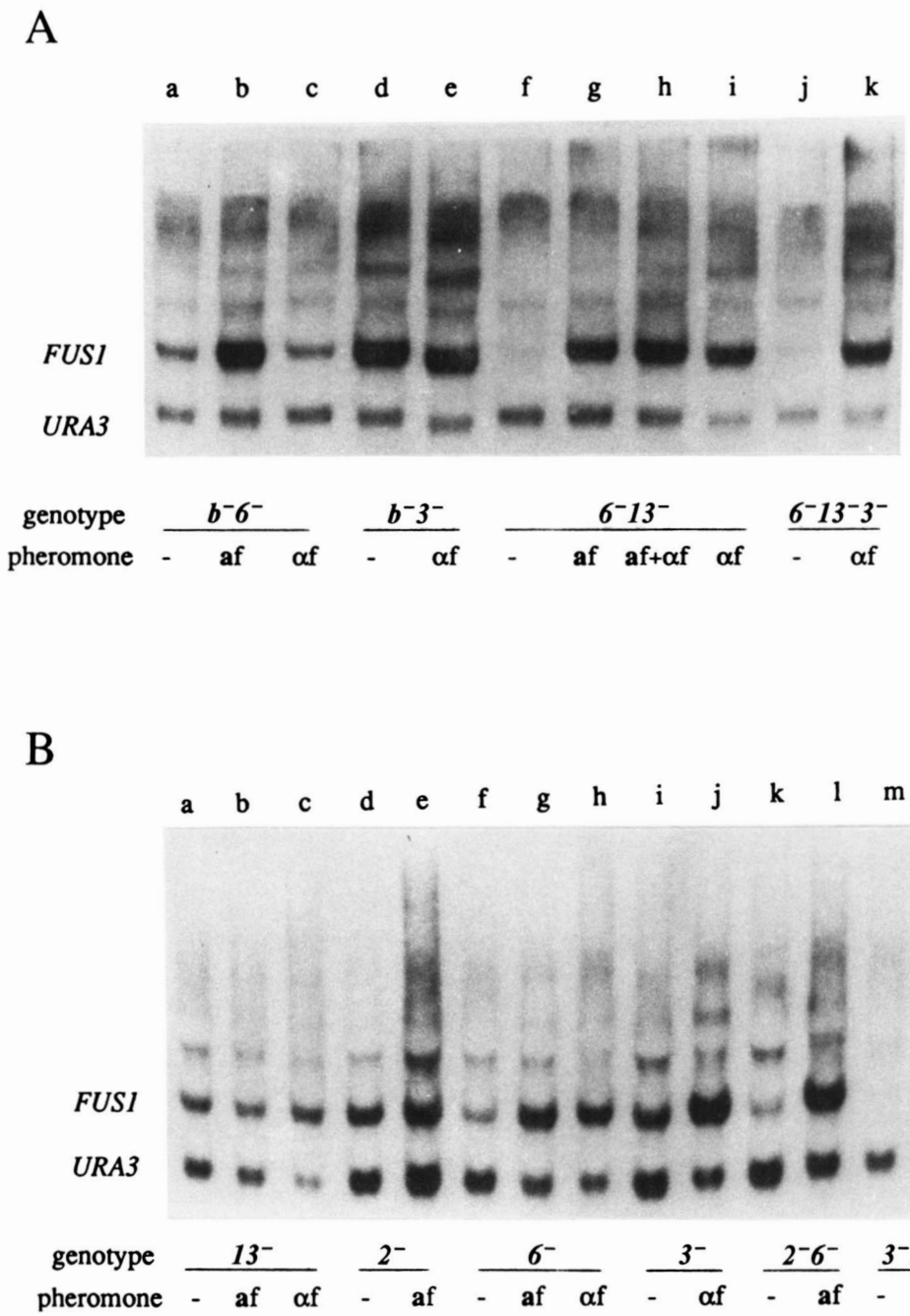

FIGURE 3.—*FUS1* transcript levels in isogenic derivatives of a *matα2* mutant. RNA was isolated and analyzed as described for Figure 2. (A) The strains used were: SY1184 (*matα2 bar1 ste6*, lanes a–c), SY1195 (*matα2 bar1 ste3*, lanes d and e), SY1228 (*matα2 ste6 ste13*, lanes f–i), and SY1242 (*matα2 ste6 ste13 ste3*, lane j and k). (B) The strains used were: SY1212 (*matα2 ste13*, lanes a–c), SY1176 (*matα2 ste2*, lanes d and e), SY1179 (*matα2 ste6*, lanes f–h), SY1180 (*matα2 ste3*, lanes i and j), SY1153 (*matα2 ste2 ste6*, lanes k and l), and SY1234 (*matα2 ste3 ste13*, lanes m and n).

ing response to **a** factor. The second generalization is that among pairs of isogenic strains differing at the *STE3* locus, response to α factor is more vigorous for the strain that carries the mutant allele of *STE3*. Two comparisons illustrate the point. (1) A *matα2 ste3* mutant (SY1180) responded well to added α factor, whereas a *matα2* mutant (SY1256) did not. (2) A *matα2 ste13 ste3* strain (SY1234) responded to α factor much better than did a *matα2 ste13* (SY1212) strain. In each of these pairs, the inability of the *STE3* strain to respond to α factor could once again have two explanations: the **a**-factor receptor could interfere with response to α factor or adaptation to **a** factor could also result in adaptation to α factor.

Two other comparisons confirm that inability to show full response to α factor results from interference by the **a**-factor receptor, especially when response is assessed by the ability to arrest in the G1 phase of the cell cycle (Table 5B). (1) A *matα2 ste13 ste6* strain (SY1228) responded to added α factor better if it also carried a mutant allele of *STE3* (SY1242). (2) A *matα2 ste3* mutant (SY1180) responded more fully to α factor than did a *matα2 ste6* strain (SY1179). In both these comparisons, the realization that removal of STE3 and STE6 functions is not equivalent, even though both the *ste3* and *ste6* mutations would block autocrine response to **a** factor, implies that receptor interference rather than phero-

mone adaptation accounts for the defect in response to α factor. To demonstrate that the incomplete response to α factor shown by *matα2 ste6* cells is not a peculiar feature of the *ste6* mutation, we also disrupted **a**-factor production via a mutation in *STE14*, a second gene that is required for **a**-factor maturation (WILSON and HERSKOWITZ 1987). The *matα2 ste14* strain behaved like the *matα2 ste6* strain (data not shown).

A final and striking example of the **a**-factor receptor interfering with response to α factor is the contrast in the behavior of *matα2 bar1* and *matα2 bar1 ste3* mutants. *matα2 bar1* mutants were indistinguishable from *matα2* mutants in the level of *FUS1* transcript detected, whereas the *matα2 bar1 ste3* cells expressed *FUS1* at a high level, nearly the level seen in wild-type **a** cells treated with α factor (Table 5, strains SY1197, SY1256, and SY1195). In addition, *matα2 bar1 ste3* cells displayed extreme projection formation, reminiscent of that shown by wild-type cells responding to pheromone, and had a generation time 50% longer than *matα2* cells (data not shown). The introduction of a *ste3* mutation apparently allowed the *matα2 bar1* cells to respond more strongly to their own α factor; nonetheless the cells had adapted sufficiently to the α factor to be able to undergo cell division, albeit at a reduced rate.

DISCUSSION

Specificity of mating: To investigate the roles that the known **a**- and α-specific products play in the mating process, we engineered isogenic strains that express various combinations of the pheromones and pheromone receptors. We had previously shown that the **a**-factor receptor is the only α-specific product required for response to **a** factor (BENDER and SPRAGUE 1986; see also NAKAYAMA, MIYAJIMA and ARAI 1987). In this work, we have shown that the **a**-factor receptor and α factor are the only α-specific products needed for mating: the expression of *STE3* and *MFα1* allows *matα1* cells to mate with **a** cells at a normalized mating efficiency of 20%. The mating efficiency is similar to the mating efficiency of mutants defective solely in agglutination (SUZUKI and YANAGISHIMA 1985; J. KURJAN and P. LIPKE, personal communication), implying that the α-agglutinin is the only α-specific function lacking in these *matα1* cells. This interpretation further suggests that any as yet unidentified α-specific products that may exist do not play significant roles in mating.

These findings raise the issue of how mating specificity is achieved. If the agglutinins are not needed for mating, what determines that **a** cells mate with α cells but not with other **a** cells? Why do not two cells that are both responding to pheromone and in contact with each other mate? We imagined that there might exist α- and **a**-specific products (perhaps the agglutinins) whose role is to prevent mating between cells of the same mating type. We found, however, that **a** cells can mate with other **a** cells to a modest extent if one of the mating partners expresses the α set of pheromone and receptor instead of its normal (**a**) set. Thus, there are no **a**-specific products that create an absolute block to mating between **a** cells. It is interesting to note, however, that the expression of *STE3* and *MFα1* allows a *matα1* mutant to mate better (~10×) than it allows a *MAT***a** *bar1 ste2 ste6* strain to mate with an **a** strain. It is likely that an **a**-specific product partially inhibits mating with **a** cells.

Further evidence that the pheromones and receptors are important determinants of mating specificity comes from work with *matα2* mutants, cells that express both the α- and **a**-specific gene sets. By introducing appropriate mutant alleles of α- and **a**-specific genes involved in pheromone metabolism and reception, *matα2* mutants can be made to mate either as **a** cells or as α cells. However, the mating efficiency of α cells is about 20-fold less than the mating efficiency of wild-type α cells, again implying that an **a**-specific product, present by virtue of the *matα2* mutation, partially inhibits mating with **a** cells. We conclude that the species of pheromone and pheromone receptor that a cell makes are primary factors in determining with whom that cell with mate. The α- and **a**-specific products other than those involved in pheromone production and pheromone response do not preclude mating between cells of the same mating type.

These conclusions imply that the acts of emitting and receiving a pheromone signal establish cues that are essential if the mating process is to proceed efficiently. This suggestion is bolstered by the finding that exogenously-supplied pheromone does not fully complement pheromone-deficient mutants (CHAN *et al.* 1983; KURJAN 1985; MICHAELIS and HERSKOWITZ 1988). Perhaps the geometry of pheromone presentation is important because a very high local concentration of pheromone is required for a late step in mating. Or perhaps there is direct cell surface interaction between mating partners that is mediated by spatially oriented pheromones and receptors.

Phenotype of *matα2* mutants: Because *matα2* mutants express both the α- and **a**-specific gene sets, their phenotype presents two puzzles. First, an initial expectation is that *matα2* mutants would be capable of mating with both **a** and α cells. Instead, the mutants have a nonmating phenotype. Second, given that *matα2* cells express both sets of pheromones and pheromone receptors and given that one response to pheromone is cell cycle arrest, it is initially surprising that these cells are viable. Our experiments provide an explanation for these puzzles. Two phenomena—adaptation to pheromone and interference between receptors—create a cell that is refractory to phero-

mone and therefore viable but unable to mate. In particular, we find that *matα2* cells constitutively express levels of the pheromone-inducible *FUS1* transcript that are intermediate to the basal and fully induced levels seen in wild-type cells. Thus, *matα2* cells behave, in at least one respect, as though they have responded partially and adapted to the pheromone that they secrete. Consistent with this interpretation, a *matα2 ste13 ste6* mutant, which does not produce active α factor or **a** factor, has a low basal level of *FUS1* transcript, and the *FUS1* transcript in these cells is pheromone-inducible. At first glance it seems, then, that the inability of *matα2* mutants to respond to exogenously supplied pheromone can be understood solely in terms of adaptation to the pheromone they secrete. However, although *matα2 ste13 ste6* cells clearly respond to both pheromones, they fail to exhibit the degree of cell cycle arrest shown by wild-type **a** or α cells, suggesting that interference between **a**- and α-specific products prevents full response to either pheromone. In fact, we find that an α-specific product, the **a**-factor receptor, actually interferes with the response of *matα2* cells to α factor.

Adaptation and receptor interference must also be relieved in order to enable *matα2* mutants to mate. In particular, the **a**-factor receptor must be inactive (*ste3*) to allow a *matα2* cell to mate as an **a** cell, and **a**-factor production must be prevented (*ste6*) to allow a *matα2* cell to mate as an α cell.

Despite the insight our studies provide into the phenotype of *matα2* mutants, there are still aspects of their phenotype that remain unexplained. Comparisons among the isogenic *matα2* mutants we generated reveal that reciprocal changes in the species of pheromones or receptors that can be synthesized do not have equivalent consequences for the phenotype of the *matα2* cells. For example, a strain that synthesizes only the pheromone and receptor characteristic of an **a** cell (*matα2 ste13 ste3*) mates at wild-type efficiency with an α cell, whereas a cell that synthesizes only pheromone and receptor characteristic of an α cell (*matα2 bar1 ste6 ste2*) mates with an efficiency of 4×10^{-2} with an **a** cell. As noted above, this difference suggests that an **a**-specific product present in the *matα2 bar1 ste2 ste6* strain partially blocks mating with **a** cells. This product could be the **a**-factor precursor present in *ste6* (and *ste14*) mutants, **a**-agglutinin, or an unidentified **a**-specific protein. A second comparison illustrates that the two receptors differ in the degree to which they interfere with mating and pheromone response when a cell is producing the cognate pheromone. In particular, expression of the **a**-factor receptor in a cell that otherwise produces the pheromone and receptor characteristic of an **a** cell reduces the ability to mate with α cells by 30-fold (compare *matα2 ste13 ste3* with *matα2 ste13*). In contrast, expression of the α-factor receptor in a cell that produces α factor reduces the ability to mate with **a** cells by only 5-fold (compare *matα2 bar1 ste6 ste2* with *matα2 bar1 ste6*). This difference could indicate that the **a**-factor receptor has a greater capacity to interact with the next component in the pheromone response pathway (G protein?) and therefore sequesters that component from the α-factor receptor. A second possible explanation is that the two pheromone-receptor interactions may generate intracellular signals that are quantitatively different. In this case, adaptation to an **a**-factor-generated signal may confer adaptation to an α-factor-generated signal, but adaptation to an α-factor-generated signal may still allow some response to **a** factor. Whatever the detailed explanation for the two comparisons discussed here, it is apparent that the products of the **a**- and α-specific gene sets do not make equivalent contributions to the physiology of *matα2* mutants.

In conclusion, the phenotypes of *matα2* mutants—both the inability to respond to pheromone and the inability to mate—are the consequence of an autocrine response to **a** factor to which the cells adapt. The simultaneous expression of α factor and its receptor does not contribute significantly to the *matα2* phenotype for two reasons: BAR1 activity greatly reduces the amount of α factor that is actually secreted, and the **a**-factor receptor interferes with the action of the α-factor receptor.

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