STE16, a New Gene Required for Pheromone Production by a Cells of *Saccharomyces cerevisiae*

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ABSTRACT

Genes required for mating by **a** and α cells of *Saccharomyces cerevisiae* (*STE*, "sterile," genes) encode products such as peptide pheromones, pheromone receptors, and proteins responsible for pheromone processing. **a**-specific *STE* genes are those required for mating by **a** cells but not by α cells. To identify new **a**-specific *STE* genes, we have employed a novel strategy that enabled us to determine if a *ste* mutant defective in mating as **a** is also defective in mating as α without the need to do crosses. This technique involved a strain (K12-14b) of genotype *mat***a***1 HML*α *HMR*α *sir3ts*, which mates as **a** at 25° and as α at 34°. We screened over 40,000 mutagenized colonies derived from K12-14b and obtained 28 **a**-specific *ste* mutants. These strains contained mutations in three known **a**-specific genes—*STE2*, *STE6* and *STE14*—and in a new gene, *STE16*. *ste16* mutants are defective in the production of the pheromone, **a**-factor, and exhibit slow growth. Based on the distribution of **a**-specific *ste* mutants described here, we infer that we have identified most if not all nonessential genes that can give rise to **a**-specific mating defects.

THE **a** and α cells of yeast mate with each other to form **a**/α diploids in a multistep conjugation process that requires the function of gene products unique to each haploid cell type. In particular, each cell type produces a characteristic oligopeptide pheromone, **a**-factor by **a** cells and α-factor by α cells, that induces cell cycle arrest in the opposite cell type. Yeast cells also produce cell-surface agglutinins that are specific to **a** and α cells (reviewed by HERSKOWITZ 1987).

The two natural alleles of the yeast mating type locus, *MAT***a** and *MAT*α, determine which of the haploid cell types is exhibited: *MAT***a** confers the **a** cell type, and *MAT*α the α cell type (STRATHERN, HICKS and HERSKOWITZ 1981). In addition to *MAT*, several of the genes necessary for mating have been identified through the isolation of mutants defective in mating (*ste*, "sterile," mutants) (MACKAY and MANNEY 1974a; HARTWELL 1980). The *ste* mutants have been isolated by several different methods. Some have been found in selections for failure to mate (MACKAY and MANNEY 1974a) or for failure to respond to α-factor (HARTWELL 1980; WHITEWAY and SZOSTAK 1985). Some have been found in screens for defects in mating, assayed as failure to form prototrophs (RINE 1979), failure of *HO* strains to form **a**/α diploids by mating between siblings (BLAIR 1979; OSHIMA and TAKANO 1980), or failure to produce α-factor (FIELDS and HERSKOWITZ 1985). Other *ste* mutants have been found as additional phenotypes of mutants identified for other reasons, such as defective in production of killer toxin (LEIBOWITZ and WICKNER 1976) or ability to take up thymidine (WICKNER 1974).

Introduction of the *ste* mutations into cells of opposite mating type by crosses made it possible to determine whether the particular gene is needed for mating by one or both cell types (MACKAY and MANNEY 1974b). The *STE* genes so identified are of three types: (1) the nonspecific *STE* genes are required for mating by both **a** and α cells; (2) the **a**-specific *STE* genes are required for mating by **a** cells but not by α cells; and (3) the α-specific genes are needed for mating by α cells but not by **a** cells. There are seven nonspecific *STE* genes (*STE4*, *STE5*, *STE7*, *STE11*, *STE12*, *STE15* and *STE17*); four **a**-specific *STE* genes (*STE2*, *STE6*, *STE14* and *ARD1*); and four α-specific *STE* genes (*STE3*, *STE13*, *KEX2* and *TUP1*). The roles of several of these genes in cell specialization and the mating process are now known. *STE2* and *STE3* appear to be components of the receptors to α-factor and **a**-factor, respectively (JENNESS, BURKHOLDER and HARTWELL 1983; BURKHOLDER and HARTWELL 1985; NAKAYAMA, MIYAJIMA and ARAI 1985; HAGEN, MCCAFFREY and SPRAGUE 1986). The α-specific *STE* genes, *KEX2* and *STE13*, code for processing enzymes involved in cleavage of the α-factor precursor (JULIUS *et al.* 1983, 1984).

Four **a**-specific *STE* genes have been previously identified: *STE2* (MACKAY and MANNEY 1974a,b; HARTWELL 1980), *STE6* (RINE 1979), *STE14* (BLAIR 1979), and recently *ARD1* (WHITEWAY and SZOSTAK

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1985). *STE2* and *ARD1* are both involved in response to α-factor: mutants defective in these genes were selected as resistant to α-factor. Mutants defective in *STE6* and *STE14*, in contrast, respond to α-factor normally but are defective in production of **a**-factor (BLAIR 1979; RINE 1979; CHAN *et al.* 1983).

In the present study, we focus on the question of what other genes are required for cells to exhibit the mating behavior of **a** cells. The most extensive prior isolation of *ste* mutants (HARTWELL 1980) had a strong bias associated with it: mutants were isolated for resistance to α-factor. Consequently, mutants defective in *STE6* and *STE14* were not obtained. We have used a nonselective procedure to identify **a**-specific *ste* mutants. A slow step in previous analyses of *ste* mutants had been the crosses that determine whether a mating defect is specific or nonspecific with respect to cell type. We circumvented this problem by using a special strain wherein mutants defective in mating as **a** cells may be directly tested for their ability to mate as α. We have thereby isolated 20 mutants defective in previously identified genes and at least two mutants defective in a new **a**-specific *STE* gene, which we call *STE16*.

MATERIALS AND METHODS

Strains, genetic methods, and media: Yeast strains are listed in Table 1. Crosses, sporulation, and tetrad dissection were performed as described previously (SPRAGUE and HERSKOWITZ 1981). Matings involving mating-deficient strains were performed by selection for prototrophy aided, in the case of mating-deficient mutants derived from strain K12-14b, by previous growth of mutant strains at 34° so that they would be phenotypically α cells. Yeast rich medium (YEPD) and minimal medium (SD) were prepared as described previously (HICKS and HERSKOWITZ 1976).

Assays of mating phenotype: Mating phenotype was assayed on plates as described (WILSON and HERSKOWITZ 1984) using strains 70, 227, and 6B78 for mating type lawns, strain RC757 for assaying **a**-factor, and strain XMB4-12b for assaying α-factor. Response of strain K91-3b to α-factor was assayed as follows: α-factor (Sigma, final concentration 5 μg/ml) was added to exponentially growing cultures in YEPD medium previously adjusted to pH 4.0. Three hours after addition of α-factor, treated cells and untreated controls were observed by light microscopy for the "shmoo" morphology indicative of normal response to cell cycle arrest by α-factor (DUNTZE, MACKAY and MANNEY 1970). The plate assay for Barrier activity of strain K91-3b was performed as described (SPRAGUE and HERSKOWITZ 1981).

Strain K12-14b: The genotype of K12-14b, the parent strain of the mutant hunt, is shown in Table 1. K12-14b carries a temperature-sensitive allele of *SIR3*, *sir3-8* (RINE 1979; HARTWELL 1980), denoted hereafter as *sir3ts*, that determines its mating phenotype at different temperatures as described in RESULTS. The mating behavior of K12-14b at temperatures between 25° and 34° is consistent with a progressive loss of activity of the *SIR3* gene product as temperature increases. The *rme1* mutation was necessary for the genetic analysis of the mutants, since *rme1* allows diploid cells lacking *MAT***a** information to sporulate (KASSIR and SIMCHEN 1976; RINE, SPRAGUE and HERSKOWITZ 1981). When crossed with other *rme1* strains, strain K12-14b exhibited normal spore viability.

Mutagenesis: Strain K12-14b was mutagenized by exposure to ethylmethane sulfonate (EMS; Eastman Kodak Co.) for 1 hr as described (OSHIMA and TAKANO 1980) and refrigerated for two days while the titer was determined. Cells were then plated for screening. To assure independent isolation of mutants, ten single colonies of K12-14b were treated independently as mutagenesis series A through J. The frequency of survivors was approximately 4%. The effectiveness of mutagenesis was estimated by screening mutation to canavanine resistance and mutation from red to white colony color. The parent colonies are red, due to the *ade2-1* (*ochre*) mutation, and a color change from red to white can arise by mutation at any of five other *ADE* loci (JONES and FINK 1981) or to an *ochre* suppressor. The frequency of mutation for a given locus estimated by these two methods was a maximum of 2%.

RESULTS

Mutant isolation strategy: Because the majority of the mating-deficient mutants identified in previous hunts were nonspecific *ste* mutants (MACKAY and MANNEY 1974a,b; HARTWELL 1980), we constructed a strain to distinguish rapidly between **a**-specific *ste* mutants and nonspecific *ste* mutants. The mating phenotype of strain K12-14b is controlled by the temperature at which the cells are grown: at low temperature (25°) the cells mate as **a** (Figure 1, panels A and B), and at high temperature (34°) the cells mate as α (Figure 1, panels C and D). (The small amount of mating as α observed at 25° indicates that the product synthesized from the *sir3ts* mutant gene is not completely functional even at permissive temperature.) Production of the **a**- and α-specific mating factors is likewise temperature dependent: at low temperature, cells produce **a**-factor (and not α-factor); at high temperature, they produce α-factor and not **a**-factor (Figure 1, panels B and D).

The temperature-dependent mating phenotypes of K12-14b are due to a temperature-sensitive allele of *SIR3*, *sir3ts* (RINE 1979; HARTWELL 1980). *SIR* genes repress transcription of the two silent copies of mating type information located at *HML* and *HMR* (reviewed by HERSKOWITZ and OSHIMA 1981); in strain K12-14b both silent loci carry α information. Furthermore strain K12-14b carries a recessive *mat***a** mutation. At low temperature, the α cassettes at *HML* and *HMR* are repressed; hence cells exhibit the mating phenotype of an **a** cell. At high temperature, however, the strain is *Sir*$^-$, so that *MAT*α information is expressed from *HML* and *HMR*. The *mat***a***1* mutation is recessive to *MAT*α information (KASSIR and SIMCHEN 1976) so, like *MAT*α*/mat***a***1* diploids, strain K12-14b exhibits an α phenotype at high temperature because the **a**1-α2 activity required to establish the **a**/α (nonmating) phenotype is not present. The mating behavior of K12-14b at high temperature is precisely the same as the

TABLE 1

Strains and crosses

Name	Genotype	Source[a]
6B78	*MAT***a** *ade5 his5 ura4 met4 met13*	L. BLAIR
70	*MATα thr3-10*	F. SHERMAN
189	*MAT***a** *cry1 ste2-1 his2 ade2-1 lys1-1 trp5-18 gal2 can1*	T. MANNEY
227	*MAT***a** *lys1-1 cry1*	J. HICKS
381G	*MATα cry1 ade2-1 his4-580 lys2 trp1 tyr1 SUP4-3*	L. HARTWELL
763	*MAT***a** *ste6-3* (RSA3 allele) *ade6 lys2 arg4*	J. RINE
1369	*MATα leu2-3 leu2-112 ura3-52 trp1 his3 his4*	R. JENSEN
HR125-5d	*MAT***a** isogenic with 1369	R. JENSEN
HR129-2d	*MAT***a** *ste14-1 cry1 leu2-3 leu2-112 ade5 canR cyhR*	R. JENSEN
HR129-5d	*MATα cry1 ste14-1 leu2-3 leu2-112 ura4 met his3* and/or *his5*	R. JENSEN
JM153A-6c	*MAT***a** *cry1 rme1 lys2 ura3*	J. MARGOLSKEE
K12-14b	*mat***a***1 HMLα HMRα sir3-8 rme1 leu2-3 leu2-112 ura3-52 ade2 lys2 trp1 his4 his3*	
K39-3b	*MAT***a** *ste6-21* (RSA21 allele) *leu2-3 leu2-112 his4 ade6 lys2 can1*	
K39B-12c	*MAT***a** *ste6-21 arg4 his4 leu2-3 leu2-112 lys2*	
K43-4c	*MAT***a** *cry1 ste16-1*[b] *leu2-3 leu2-112 ura3 trp1 tyr1 his3 his4 met*	
K48-4b	*MATα ste14-2*[c] *ura3-52 ade2-1 leu2-3 leu2-112 his4 trp1 SUP4-3 (his3?)*	
K50-1a	*MAT***a** *cry1 ste16-1 trp1 ade2-1 lys2 ura3 tyr1 SUP4-3 his3* and/or *his4*	
K50-1b	*MAT***a** *cry1 ste16-1 ade2 leu2-3 leu2-112 lys2 tyr1 trp1 his4*	
K50-5c	*MATα cry1 ste16-1 ade2 leu2-3 leu2-112 tyr1 trp1 his4 SUP4-3*	
K85-1b	*MAT***a** *leu2-3 leu2-112 tyr1 lys2*	
K87-3c	*MAT***a** *cry1 ade2 lys2 tyr1 leu2-3 leu2-112 met*	
K91-3b	*MAT***a** *cry1 ste16-1 ura3-52 leu2-3 leu2-112 lys2 tyr1 trp1 his4*	
RC757	*MATα sst2-1 met1 his6 can1 cyh2*	R. CHAN
XMB4-12b	*MAT***a** *sst1-1 ilv3 arg9 ura1 [KIL*$^+$*]*	L. BLAIR

Crosses	Parents
K43	I11 mutant × JM153A-6c
K50	K43-4c × 381G
K52	K50-1a × K48-4b
K85	K50-5c × 763
K87	K50-1b × HR129-5d
K89	K87-3c × 1369
K91	K50-1b × 1369
K93	K85-1b × 1369
K95	K91-3b × I14 mutant

[a] Strains constructed for this work unless otherwise noted.
[b] Allele from strain I11.
[c] *mex2* allele of OSHIMA and TAKANO (1980).

*mat***a***1 HMLα HMRα sir1* strain described by RINE *et al.* (1979).

Because we could observe both **a** and α mating behavior by growth at two different temperatures, we easily screened out nonspecific *ste* mutants, which are defective in mating at both temperatures. Furthermore, the ability of our **a**-specific *ste* mutants to mate as α at high temperature facilitated complementation analysis.

Mutant screening: Mutagenized cells were plated on YEPD and grown at low temperature (25°). Over 40,000 resulting single colonies were replica plated to two YEPD plates and grown overnight, one plate at 25° and the other at 34°. The colonies grown at 25° were tested for their ability to mate as **a** by replica plating to a lawn of α cells (strain 70) on minimal medium (SD plates). Mating yields prototrophic diploids that grow on minimal medium. Colonies unable to mate as **a** were detected by the absence of such prototrophic diploids. Likewise, the colonies pregrown at 34° were tested for their ability to mate as α by replica plating to a lawn of **a** cells (strain 227). The 72 putative **a**-specific *ste* mutants—those that failed to mate as **a** (tested at low temperature) but did mate as α (tested at high temperature)—were examined further (Table 2).

Nonconditional mutations in any of the four *SIR* genes (RINE 1979; IVY, KLAR and HICKS 1986) would result in the same phenotype as the putative **a**-specific *ste* mutations. They can be easily distinguished from **a**-specific *ste* mutants because *HMLα HMRα mat***a***1 sir* strains (identified because they fail to mate as **a** at low temperature) are expected to mate as α at low temperature. Another class of "mutants" expected to mate as α at both temperatures would result from a mating type switch from *mat***a***1* to *MATα*. We found that 39 of the 72 mutants (55%) mated as α at 25° and 34°; the 39 strains were assumed to be either *sir* mutants

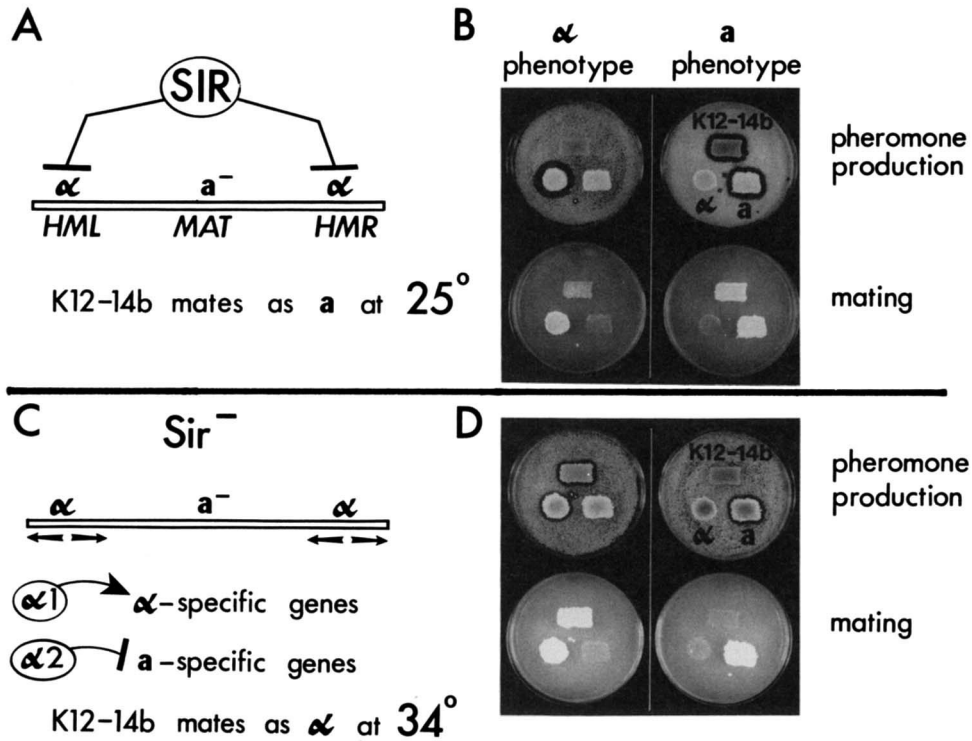

FIGURE 1.—Temperature-dependent mating by strain K12-14b (*mata1 HMLα HMRα sir3ts*). Panels A and B illustrate the phenotype of K12-14b at low temperature. Panel A, at 25°, *SIR* is functional and represses transcription at the silent α cassettes (*HML* and *HMR*). As shown in Panel B, the strain has the phenotype of an **a** cell: it mates efficiently as **a**) (and less efficiently as α) and produces **a**-factor but not α-factor. Panels C and D illustrate the effects of high temperature. Panel C, at 34° *SIR* is inactive and α information is expressed from the *HML* and *HMR* cassettes. The α1 and α2 proteins encoded by each cassette stimulate (arrowhead) and repress (line ending in bar) transcription of sets of genes required to exhibit each cell type as indicated. Thus, as shown in Panel D, at 34° K12-14b exhibits properties of an α cell: it mates only as α and produces only α-factor. The hollow bar represents chromosome *III*. Panels B and D, the upper patch on each plate is strain K12-14b, the patch labeled **a** is a mating-proficient **a** control (*MAT***a** *SIR* strain HR125-5d), and the patch labeled α is a mating-proficient α control (*MATα SIR* strain 1369). The three strains were grown overnight on YEPD and then replica plated to two separate YEPD plates: one was grown at 25° and the other at 34°. These plates were then tested (at 25° or at 34°) for mating ability (assayed as formation of prototrophs that grow on minimal medium) and pheromone production (assayed as clear zones [haloes] around a colony) by replica plating to the appropriate tester lawns (see MATERIALS AND METHODS).

TABLE 2

Initial mutant screening[a]

Mutagenesis series	Potential **a**-specific mutants[b]	*sir* mutants[c]	*thr* mutants	Others
A	10	7	0	3
B	13	7	0	6
C	7	2	1	4
D	14	9	0	5
E	7	3	1	3
F	3	2	0	1
G	5	3	1	1
H	3	2	0	1
I	4	1	0	3
J	6	3	2	1
Totals	72	39	5	28

[a] Approximately 4000 colonies screened per mutagenesis series.
[b] Colonies unable to mate with an α lawn at 25° but still able to mate with an **a** lawn at 34°.
[c] Colonies that mates as α at both 25° and 34°.

or switches to *MATα* and were not studied further.

Because the α strain (70) that we used to test for mating ability as **a** is a *thr3* auxotroph, mutants defective in *thr3* gene function would be unable to form prototrophs in the mating test and falsely appear to be deficient in mating. Five of the putative **a**-specific *ste* mutants were unable to grow on medium lacking threonine and presumably contain defects in the *THR3* gene.

Complementation analysis with a-specific *STE* mutants: The 28 remaining mutants were assigned to complementation groups based on their failure to complement the mating defect of **a** strains carrying mutations in the **a**-specific *STE* genes, *STE2*, *STE6* and *STE14*. Mutants were grown at 34° so that they would be phenotypically α, then mixed with each of three *MAT***a** tester strains that carried a mutation in *ste2*, *ste6*, or *ste14* (strains 189, K39-3b or K39B-12c, and HR129-2d, respectively). The strains were mixed, allowed to grow and mate on rich plates for 5–12 hr at 25° or 30°, and replica plated to select for diploids with two possible genotypes as illustrated below for the *ste2* tester strain:

$$\frac{MAT\mathbf{a}}{mat\mathbf{a}}\ \frac{SIR3}{sir3}\ \frac{steX}{STEX}\ \frac{ste2}{STE2} \text{ or } \frac{MAT\mathbf{a}}{mat\mathbf{a}}\ \frac{SIR3}{sir3}\ \frac{ste2}{ste2}.$$

TABLE 3

Mutant a-factor phenotypes and complementation analysis[a]

Mutant	Relative a-factor production	Complementation with		
		ste2	*ste6*	*ste14*
A66	0.2	+	−	+
B28	0.2	NT	−	+
B60	0.3	+	−	+
B61	0.1	+	−	+
C1[b]	0.2	+	−	+
C2[b]	0.3	NT	−	+
C11	0.3	+	−	+
D11	0.2	+	−	+
E3	0.2	+	−	+
E5	0.2	+	−	+
F20	0.2	+	−	+
H7[b]	0.2	+	−	+
I2	0.3	+	−	+
B36	0	+	+	−
B39	0	+	+	−
C8	0	NT	+	−
J3[b]	0	+	+	−
B64	1.0	−	+	+
D7	1.0	−	+	+
D25[c]	1.0	−	+	+
I11	0.1	NT	+	+
I14[b]	0.1	+	+	+
A20[b]	1.0	NT	−	NT
D29[b]	0.3	NT	−	NT
D15	0.2	+	NT	+
E9	1.0	NT	NT	+
H5	1.0	NT	NT	NT
G6[b]	0.5	±	−	±

[a] a-factor production was determined by halo assay: the halo is the clear zone immediately surrounding a colony or patch (see legend to Figure 2); 1.0 indicates wild-type halo size; 0 indicates no halo; and a number such as 0.2, for example, indicates a halo size 20% that of wild type. NT indicates not tested. Temperature-sensitive for growth indicates that the mutant strain does not grow at 34° but does grow at lower temperatures. Complementation tester strains were 189 (*ste2-1*), K39-3b and K39B-12c (*ste6-21*), and HR129-2d (*ste14-1*). + indicates that the *MAT***a**/*mat***a** diploid formed by mating between the two *ste* strains (described in MATERIALS AND METHODS) was able to mate as **a**; − indicates that the diploid was unable to mate as **a**; ± indicates that the diploid mated poorly as **a**.

[b] Original mutant strain is temperature-sensitive for growth.

[c] Leaky mutant (strain mates at low frequency).

Because *MAT***a** and *SIR3* are dominant to the *mat***a***1* and *sir3ts* mutations in these strains, the mating ability of these strains is dependent on the ability of the *ste* mutations to complement. These diploid strains were all auxotrophic (homozygous for *ade2*, *leu2* or other markers; see Table 1). Consequently their ability to mate could be scored by the standard prototroph assay (see MATERIALS AND METHODS). If the mutant was defective in a *ste* gene different from that of the tester strain, then the **a**/**a**$^-$ diploid was able to mate as **a**. Mutants were assigned to a known complementation group (*STE2*, *STE6* or *STE14*) if they formed non-mating **a**/**a**$^-$ diploids with one and only one of the testers. Most of the mutants carried recessive *ste* mutations as demonstrated by complementation of the mating defects of two of the tester strains. The complementation data are presented in Table 3. Twenty mutants appear to contain defects in *STE2*, *STE6*, or *STE14*. The minimum number of independent isolates is two for *STE2*, eight for *STE6*, and three for *STE14* (Table 4). Mutant G6 failed to complement or only partially complemented all three *ste* tester strains and may therefore carry a dominant mutation. Complementation analysis of G6 and five other ill-mannered mutants is incomplete (A20, D15, D29, E9 and H5).

TABLE 4

Number of mutants obtained in each complementation group

Complementation group	Mutant names	Minimum no. of independent mutants
STE2	B64, D7, D25	2
STE6	A66, B28, B60, B61 C1, C2, C11, D11 E3, E5, F20, H7, I2	8
STE14	B36, B39, C8, J3	3
STE16	I11, I14	2[a]

[a] I11 and I14 mutant strains have different properties and are thus presumed to be independent (see RESULTS).

Identification of a new a-specific *STE* gene: As already noted, 20 of the 28 **a**-specific *ste* mutants were found to be mutations in *STE2*, *STE6* or *STE14*. However, mutants I11 and I14 complemented all three tester strains (see Table 3) and defined at least one new complementation group. We determined that mutants I11 and I14 belong in the same complementation group: strain K91-3b (*MAT***a** *ste*-I11) was crossed with the I14 mutant (*mat***a** *ste*-I14) to form diploid K95, which exhibited the same mating defect as the parent strains. Mutants I11 and I14 are derived from the same mutagenesis series and are therefore not necessarily independent mutations. We believe that they are independent mutations because they display two phenotypic differences. First, mutant I14 appears more deficient in **a**-factor production than mutant I11. Second, I14 cells are temperature-sensitive for growth (possibly due to a second, unrelated mutation). Due to difficulties in outcrossing the I14 mutant strain, all subsequent analysis of this new complementation group was carried out using derivatives of the I11 mutant.

Initial genetic analysis of the I11 mutation: We crossed mutant I11 with a *MAT***a** *rme1* strain (JM153A-6c) by selection for prototrophy on minimal medium to form diploid K43. Sporulation and dissection of K43 yielded segregant K43-4c, in which the *ste* mutation was separated from *mat***a** and *sir3ts*. (*MAT***a** segregants were identified by cosegregation of the tightly linked marker *cry1*). Subsequent outcrosses are listed in Table 1. Tetrad analysis of diploids K50 and K91 (*MAT***a** *ste* × *MATα STE*) (13 tetrads total)

TABLE 5

ste*-I11 is not an allele of *STE6* or *STE14

Cross	Description	No. of tetrads[a] 2 **a**: 2 α	1 **a**: 1 nm: 2 α	2 nm: 2 α
K85	α *ste*-I11 × **a** *ste6*	0	4	3
K87,K52	**a** *ste*-I11 × α *ste14*	0	7	4
K89[b]	**a** *STE* × α *STE*	8	0	0
K93[c]	**a** *STE* × α *STE*	7	0	0

[a] nm indicates nonmating colony.
[b] *MAT***a** *STE* parental strain is a mating-proficient segregant from cross K87.
[c] *MAT***a** *STE* parental strain is a mating-proficient segregant from cross K85.

showed that I11 carries a single mutation and that this mutation is not linked to the mating type locus: half of the *MAT***a** spores were mating-proficient and half were mating-defective. All α segregants were mating-proficient. The phenotype of the I11 mutation does not depend on the *mat***a** mutation because *MAT***a** *ste*-I11 segregants from *MAT***a**/*MAT*α diploids exhibit the **a**-specific mating defect of the original mutant.

If the I11 mutation indeed identifies a new locus required for mating by **a** cells, it should be genetically separable from (that is, not allelic with) the known **a**-specific *STE* genes. The crosses shown in Table 5 demonstrate that the I11 mutation is distinct from *STE6* and *STE14*, because each cross readily yielded mating-proficient **a** spores.

Because construction of the diploids in this analysis involved a rare mating—between *MAT*α *ste*-I11 and *MAT***a** *ste6* or between *MAT*α *ste*-I11 and *MAT***a** *ste14*—we considered the possibility that the mating-proficient **a** segregants from these diploids are not *MAT***a** *STE* but rather are *MAT***a** *ste* and carry a suppressor of the mating defect. To test for the presence of a suppressor, we took mating-proficient **a** segregants from two diploids (K85 and K87), crossed them with α *STE* strain 1369 (to form diploids K89 and K93), and analyzed segregants (Table 5); no sterile **a** spores were found. These results demonstrate that an unlinked suppressor of the *ste* mutation was not present in diploids K85 and K87 and thus establish the validity of our allelism tests. Thus we conclude that the *ste*-I11 mutation is distinct from the *ste6* and *ste14* mutations.

Three lines of evidence show that the new complementation group is different from *STE2*: (1) the I14 mutation and *ste2* complement each other; (2) I11 mutants are defective in **a**-factor production and normal in their response to α-factor (described below), whereas *ste2* mutants are defective in their response to α-factor and normal in **a**-factor production (MACKAY and MANNEY 1974a); and (3) the restriction endonuclease maps of the cloned *STE16* (P. GARCIA and K. L. WILSON, data not shown) and *STE2* (BURKHOLDER and HARTWELL 1985) genes differ.

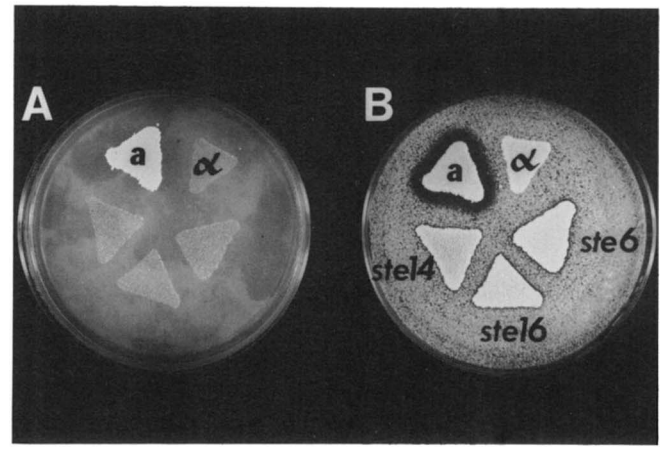

FIGURE 2.—Defective mating and **a**-factor production by *ste6*, *ste14* and *ste16* mutants. Panel A shows the mating defect of strains carrying mutations in *ste6* (strain K77), *ste14* (strain HR129-2d), and the newly identified *ste16* (strain K91-3b) (see panel B for labeling). Patches of the mutant strains and mating-proficient **a** strain HR125-5d ("**a**") and mating-proficient α strain 1369 ("α") were replica plated to a thin lawn of strain 70 (*MAT*α) on minimal medium. Mating results in prototrophs that grow as seen with the **a** control. Panel B shows defective **a**-factor production by the mutant strains, assayed by replica-plating onto a thin lawn of strain RC757 and incubating at 25° to slow growth of the lawn. Growth of strain RC757 is inhibited by **a**-factor, resulting in a clear zone or halo around the source of **a**-factor. The mutant strains produce a small but visible halo relative to the **a** control.

These data show that the I11 mutation defines a new gene required for mating proficiency in **a** cells, but not α cells. We designate the new locus *STE16*, the I11 allele *ste16-1*, and the I14 allele *ste16-2*.

MAT*a *ste16* cells are defective in a-factor production:** In addition to the inability to mate efficiently, *MATa** strains carrying the *ste16-1* mutation are defective in the biosynthesis of **a**-factor pheromone as shown in Figure 2. In segregants of crosses K50 and K91, the mating defect was associated with deficient **a**-factor production: all 14 sterile **a** segregants were **a**-factor deficient and all 12 fertile **a** segregants displayed wild-type **a**-factor activity. The *ste16-1* mutation may be leaky, since **a** strains carrying the mutation are able to mate at low frequency. *MAT***a** cells carrying the *ste16-1* mutation produce the barrier activity (SPRAGUE and HERSKOWITZ 1981; CHAN and OTTE 1982) and arrest normally in response to α-factor, and are thus not deficient in these two other **a**-specific functions (data not shown). Therefore the lack of mating seen in *ste16* cells may be specifically due to their defect in **a**-factor synthesis.

The *ste16* mutation confers a growth defect independent of mating type: Cells carrying the *ste16-1* mutation form small colonies (S. MICHAELIS, personal communication; POWERS *et al.* 1986). This defect is seen also in a longer doubling time: approximately 160 min in rich medium for strain K91-3b in compar-

ison to 100 min for a related, mating-proficient strain (HR125-5d) grown under the same conditions (data not shown). The slow growth of *ste16* cells is observed for haploid *MAT***a** and *MAT*α strains and *MAT***a**/*MAT*α diploids and cosegregates with the *ste16* allele through several outcrosses, suggesting that the *STE16* gene product is expressed and functions in all three cell types and not exclusively in **a** cells. This behavior further distinguishes it from *STE2* and *STE6*, which are transcriptionally repressed in α and **a**/α cells (WILSON and HERSKOWITZ 1984; MILLER, MACKAY and NASMYTH 1985).

DISCUSSION

We have isolated **a**-specific *STE* mutants in order to identify genes that are necessary for mating by **a** cells but not necessary for mating by α cells. Use of a parent strain that mates as **a** at low temperature and as α at high temperature has made it possible to identify 28 mutants with an **a**-specific mating defect. Of these, 22 have been placed into one of four complementation groups: previously known genes, *STE2*, *STE6* and *STE14*, and a newly identified gene, *STE16*.

Identification of a-specific *STE* genes: The most extensive previous hunt for *ste* mutants (HARTWELL 1980) had a strong selective bias against mutants that maintain response to α-factor. Our mutant hunt was extensive and nonselective—mutants were screened by a replica-plating procedure. We are thus able to glean some idea of the number of **a**-specific *STE* genes. We discuss first the types of mutants that we expected to be absent or under-represented in our mutant hunt. (1) Mutants defective in essential genes that are also required for mating by **a** cells would be under-represented. In this case, a simple gene inactivation would be lethal. Mutants in such genes would be found only if a mutation allowed the essential function to be performed but affected mating enough to exhibit a mutant phenotype. The *STE16* gene may be such a case since it appears to be essential: some alleles are temperature-sensitive lethals (POWERS *et al.* 1986). (2) Mutants defective in genes that are duplicated or functionally redundant would be absent or greatly under-represented. For example, both *MFa1* and *MFa2* encode a structural gene for **a**-factor. These genes, identified originally by hybridization of oligonucleotide probes designed to code for the **a**-factor oligopeptide (BRAKE *et al.* 1985), are functionally duplicated: mutants defective in either gene mate normally, whereas the double mutant has an **a**-specific defect in mating (S. MICHAELIS, personal communication). Another example of functional duplication is the existence of two α-factor genes (KURJAN 1985). (3) We expect that *ard1* mutants would be under-represented in our hunt. *ard1* mutants were isolated as resistant to α-factor and also exhibit reduced survival under different conditions (WHITEWAY and SZOSTAK 1985).

In contrast to these situations, the genes *STE2*, *STE6* and *STE14* are present in single copy and encode nonessential products. Based on our mutant hunt, we can estimate the probability that we have failed to isolate any single copy, nonessential *STE* genes. We exclude from this calculation mutants defective in the *STE16* gene since *STE16* appears to be essential for cell growth. Given 13 mutants known to be independently isolated that were observed to affect three genes (two for *STE2*, eight for *STE6*, and three for *STE14*) we can calculate the probability of failing to obtain mutants in a fourth gene of this type using the Poisson distribution: for an average number of independent mutants per gene (m) equal to 3.25, $p(0) = e^{-m} = 0.039$. We thus conclude that there is only a 3.9% probability that we have missed a fourth single copy, nonessential **a**-specific *STE* gene in our mutant hunt.

In addition to the **a**-specific *ste* mutants that we have isolated, we have also analyzed the two **a**-specific *ste* mutants isolated by OSHIMA and TAKANO (1980). The *mex1* mutation is a temperature-sensitive allele of *STE6*, and the *mex2* mutation is an allele of *STE14* (WILSON 1985). These observations support the view that most ordinary **a**-specific *STE* genes have been identified.

There are **a**-specific functions for which mutants have not yet been identified, most notably those defective in agglutination (HAGIYA, YOSHIDA and YANAGISHIMA 1977). We do not know whether genes coding for **a**-specific agglutinins are duplicated, whether such mutants are inviable, or even whether such mutants would be defective in mating.

We stress that our hunt for **a**-specific mutants identifies genes based on function. Our isolation procedure does not identify mutants in genes such as *BAR1*, which, though carrying out an **a**-specific function (degradation of α-factor; SPRAGUE and HERSKOWITZ 1981; CHAN and OTTE 1982), are not essential for mating.

Function of *STE16*: The *ste16* mutation confers a defect in **a**-factor production in **a** cells. Two other **a**-specific functions are unimpaired in *ste16* cells: response to α-factor and production of the *BAR1* activity. The *STE16* gene product, along with *STE6* and *STE14* (BLAIR 1979; RINE 1979; CHAN *et al.* 1983; HAGEN and SPRAGUE 1984), thus comprise a group of genes all required for some aspect of **a**-factor production. The observation that *ste16-1* results in slower growth in both **a** and α cells indicates that the *STE16* gene must be expressed in α cells. Thus, *STE16* is an **a**-specific *STE* gene but its expression is not limited to **a** cells. *STE16* is therefore distinct from at least two of the other **a**-specific *STE* genes, *STE2* and *STE6*,

which are not needed for mating by α cells and are not expressed in α cells (WILSON and HERSKOWITZ 1984; JOHNSON and HERSKOWITZ 1985; MILLER, MACKAY and NASMYTH 1985; WILSON and HERSKOWITZ 1986). The α-specific genes *STE13* and *KEX2* are other examples of genes that have a mating defect in only one cell type (α in this case) but which are expressed in all cell types (see JULIUS *et al.* 1983). The slow growth behavior of the *ste16* mutant furthermore suggests that *STE16* carries out an important cellular process. Recent studies lead to the hypothesis that *STE16* is involved in post-translational modification of **a**-factor (or its precursor) and *RAS* proteins (POWERS *et al.* 1986). It is specifically proposed that *STE16* might be responsible for addition of a palmitic acid moiety to a family of proteins, based on biochemical analysis of mutants carrying temperature-sensitive lethal alleles of *ste16* (POWERS *et al.* 1986). Perhaps one or more of our incompletely characterized **a**-specific *ste* mutants (Table 3) is defective in *STE16* or another gene with both essential and **a**-specific roles in yeast.

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