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Saccharomyces cerevisiae Mutants Unresponsive to α-Factor Pheromone: α-Factor Binding and Extragenic Suppression

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Mutations in six genes that eliminate responsiveness of *Saccharomyces cerevisiae* a cells to α-factor were examined by assaying the binding of radioactively labeled α-factor to determine whether their lack of responsiveness was due to the absence of α-factor receptors. The *ste2* mutants, known to be defective in the structural gene for the receptor, were found to lack receptors when grown at the restrictive temperature; these mutations probably affect the assembly of active receptors. Mutations in *STE12* known to block *STE2* mRNA accumulation also resulted in an absence of receptors. Mutations in *STE4*, *5*, *7*, and *11* partially reduced the number of binding sites, but this reduction was not sufficient to explain the loss of responsiveness; the products of these genes appear to affect postreceptor steps of the response pathway. As a second method of distinguishing the roles of the vairous *STE* genes, we examined the sterile mutants for suppression. Mating of the *ste2-3* mutant was apparently limited by its sensitivity to α-factor, as its sterility was suppressed by mutation *sst2-1*, which leads to enhanced α-factor sensitivity. Sterility resulting from each of four *ste4* mutations was suppressed partially by mutation *sst2-1* or by mutation *bar1-1* when one of three other mutations (*ros1-1*, *ros2-1*, or *ros3-1*) was also present. Sterility of the *ste5-3* mutant was suppressed by mutation *ros1-1* but not by *sst2-1*. The *ste7*, *11*, and *12* mutations were not suppressed by *ros1* or *sst2*. Our working model is that *STE* genes control the response to α-factor at two distinct steps. Defects at one step (requiring the *STE2* gene) are suppressed (directly or indirectly) by mutation *sst2-1*, whereas defects at the other step (requiring the *STE5* gene) are suppressed by the *ros1-1* mutation. The *ste4* mutants are defective for both steps. Mutation *ros1-1* was found to be allelic to *cdc39-1*. Map positions for genes *STE2*, *STE12*, *ROS3*, and *FUR1* were determined.

Sexual conjugation in the yeast *Saccharomyces cerevisiae* requires the action of peptide pheromones (10, 17, 32). The α-factor pheromone is secreted by haploid cells of the α mating type (α cells) and acts on haploid cells of the **a** mating type (**a** cells). Similarly, **a** cells secrete **a**-factor pheromone that acts on α cells. Both of these celullar targets respond to pheromone by arresting division in the G1 phase of the cell cycle and increasing their agglutinability to cells of the opposite mating type. The **a** and α cells then fuse to form diploid cells that are unresponsive to either pheromone. In the absence of mating, **a** cells eventually recover from the effects of α-factor by inactivating the pheromone extracellularly (3, 6) and by reducing their sensitivity to active pheromone (21). The α-factor pheromone is a tridecapeptide that binds to specific saturable sites on **a** cells (14, 15); α cells are likely to contain analogous sites that bind the undecapeptide **a**-factor (8). α-Factor pheromone is actively internalized by responsive **a** cells (5, 16).

The responsiveness of haploid cells to pheromones has been the subject of numerous genetic analyses. A single genetic locus (*MAT*) determines the mating type of the cell (13). The *STE2* gene appears to encode the cell surface receptor for α-factor (14); the sequence of this gene corresponds to a polypeptide (431 residues) that may traverse the plasma membrane several times (1, 23). Mutations in the *STE3* gene render α cells unresponsive to **a**-factor (19); the sequence of this gene suggests it may encode an **a**-factor receptor (8, 23). Mutations in five other genes (*STE4*, *5*, *7*, *11*, and *12*) cause **a** and α cells to become unresponsive to pheromones (11, 19) and also lead to partial inhibition of other mating functions (**a**-factor production and α-factor destruction) that do not require pheromones for expression (11). Mutations in *STE12* are known to reduce the production of transcripts from several other genes required for mating (*STE2*, *STE3*, MF**a***1*, *MF**a***2*, *MF*α*1*, and *MF*α*2*) (7). Mutations in the *SST2* gene and the *BAR1* gene (also designated *SST1*) result in supersensitivity to pheromones (4, 30). Temperature-sensitive mutations (*cdc28*, *36*, *37*, and *39*) have been identified that arrest cell division at the same step in the cycle as that controlled by pheromones (12, 25). Mutations in genes *CDC36* and *CDC39* partially suppress the sterility of strains with the genotype *MAT***a** *ste5*, *MAT*α *ste5*, or *MAT*α *ste4* (27); mutations *cdc36* and *cdc39* may therefore affect the pheromone response pathway.

This paper concentrates on the roles that *STE* genes play in the response of **a** cells to α-factor. Some *STE* genes may control the synthesis of receptors, whereas others may control postreceptor steps in the response pathway. To test these possibilities, we examined the ability of *ste* mutants to accumulate α-factor receptor sites. In addition, these mutants were tested for suppression by mutation *sst2*. We reasoned that the *SST2* gene product and some *STE* gene products may have opposite functions on a common element of the α-factor response pathway (e.g., activation versus inactivation of the receptor); in double mutants (*ste sst2*), the defects in the two opposing functions may compensate for each other.

MATERIALS AND METHODS

Strains. Most of the *S. cerevisiae* strains used are isogenic or congenic to strain 381G (*MAT***a** *cry1 ade2 his4 lys2 trp1*

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tyr1 SUP4-3) (11). The exceptions are strains PT1 (*MAT***a** *hom3 ilv1 can1*) (27), PT2 (*MATα hom3 ilv1 can1*) (27), X2180-1A (*MAT***a** *SUC2 mal gal2 CUP1*), X2180-1B (*MAT***a** *SUC2 mal gal2 CUP1*), EMS63 (*MATα his2*) (11), 2908-18-1 (*MATα his6 leu1 sst2-1*), ST2 (*MAT***a** *cde28-4 ade1 aro met2 tyr1 cyh2*) (25), ST32 (*MAT***a** *cde36-1 his7 lys2 tyr1 cyh2*), ST33 (*MAT***a** *cde39-1 arg met2 tyr1 cyh2*) (25), and the strains containing *cdc4-1*, *cdc12-1*, *tub2-104*, *pet3*, *mak18*, and *ilv5-1*, which were used as markers for genetic mapping. Mutation *SUP4-3* is a temperature-sensitive amber suppressor (24); *sup4-87* is a nonsuppressing derivative of the *SUP4-3* allele. The *fur1* mutation caused resistance to 5-fluorouracil and failed to complement the *fur1-8* allele.

Culture media. Unless stated otherwise, liquid cultures were grown in YM-1 medium (14) supplemented with adenine (20 μg/ml). Inhibitor medium (14) was YM-1 containing 10 mM NaN_3, 10 mM KF, and 10 mM *p*-tosyl-L-arginine methyl ester. The minimal medium was yeast nitrogen base medium (without amino acids) (Difco Laboratories) supplemented with ammonium sulfate (1 mg/ml) as the nitrogen source and glucose (2%) as the carbon source; the minimal medium was supplemented with amino acids (50 μg/ml) or adenine (20 μg/ml) as needed. YEPD plates (22) were supplemented with adenine (20 μg/ml) and used as a rich solid medium. YEPG plates were the same as YEPD plates except that glycerol (3%) substituted for glucose as the carbon source. Low-pH plates were YEPD plates that had been adjusted to pH 3.5 with succinic acid (10 g/liter) and NaOH.

Genetic analysis. Standard genetic procedures were used for mating, diploid isolation, and tetrad analysis (22). The *ste* mutants used in this study were temperature sensitive for mating (11); therefore, crosses were performed at the permissive temperature (22°C), and *ste* mutants were detected among the segregants by the inability to mate at the restrictive temperature (34°C). In some crosses, segregants containing an *ste* mutation were fertile at 34°C due to the presence of a suppressor mutation (*sst2* or *ros*) or to the *MAT* specificity of *ste2* mutants. In these cases, the presence of an *ste* mutation was established by complementation tests (11) or subsequent outcrosses or because both parents contained the same *ste* allele.

Cross-stamp test for mating. A master plate was prepared by transferring patches of the strains to be tested to a YEPD plate that was then incubated overnight at 22°C. Replica-plate transfers were made to two plates that had been prewarmed to 22 or 34°C. Both plates received long thin inocula of strains PT1 and PT2 that intersected all of the patches. These two cross-stamp plates were incubated overnight at 22 and 34°C, respectively. Replica-plate transfers were made from the cross-stamp plates to unsupplemented minimal medium prewarmed to the same temperature. Both minimal plates were incubated overnight at 34°C. Strains were designated *MATα* if the patch showed growth where it intersected strain PT1 and *MAT***a** if the patch showed growth where it intersected strain PT2. Growth that resulted from mating at 22°C but not at 34°C indicated the presence of an unsuppressed *ste* mutation. Mating was also reduced detectably by mutations *sst2* and *bar1* (see Tables 4 and 5).

Cross-stamp test for supersensitivity. The following test for supersensitivity is a modification of the streak-gap procedure of Chan and Otte (4). A master plate was used to make a replica-plate transfer. The replica was then used immediately as a second master to make replica-plate transfers to two YEPD plates and to two low-pH plates. The final replicas contained patches of cells that were too sparse to be visible. One YEPD plate and one low-pH plate received a long thin inoculum of strain X2180-1A (*MAT***a**) that intersected the positions of all of the patches. The other YEPD plate and low-pH plate received an inoculum of strain X2180-1B (*MATα*). The four plates were incubated at either 22 or 34°C. Patches of **a** cells that were supersensitive to α-factor showed a zone of inhibition surrounding strain X2180-1B that was greater than that observed for the patches containing control **a** cells. Similarly, α cells that were supersensitive to **a**-factor showed a zone of inhibition surrounding strain X2180-1A that was greater than that observed for control α cells. The **a** cells affected by mutation *sst2* showed more inhibition on low-pH plates, whereas more inhibition on YEPD plates was apparent for α cells containing *sst2* and for **a** cells containing *bar1*. All of the *ste* mutations resulting in temperature-sensitive mating suppressed supersensitivity at 34°C but not at 22°C.

TABLE 1. Binding of α-factor to mutant cells

Strain[a]	Relevant genotype	Relative binding[b]	
		Binding sites	Dissociation constant
4277-70	*STE*$^+$	1.00	1.0
4279-14	*ste2-3*	<0.05[c]	—[d]
4266-50	*ste2-6*	<0.05[c]	—
4278-45	*ste4-3*	0.54	2.0
4390-131	*ste4-4 sup4-87*	0.50	1.7
4324-47	*ste4-6*	0.48	1.6
4270-28	*ste5-3*	0.39	1.2
5501-6	*ste5-6*	0.66	1.1
4368-67	*ste5-9 sup4-87*	0.42	1.0
4267-80	*ste7-2*	0.24	1.0
4335-9	*ste7-4*	0.29	0.9
4333-48	*ste11-1*	0.29	0.9
4269-19	*ste11-3*	0.35	1.4
4334-37	*ste12-1*	0.06[c]	—
4268-60	*ste12-2*	0.08[c]	—

[a] The congenic strains listed have been described previously (14). They contain the *ste* mutation indicated and genetic markers *MAT***a** *cry1 bar1-1 ade2 his4 lys2 trp1 tyr1 SUP4-3 cyh2*.

[b] Cultures of the strain indicated were grown at the restrictive temperature (34°C), treated with 10 mM NaN_3 and 10 mM KF, and tested for α-factor binding. The binding parameters were determined from Scatchard graphs as depicted in Fig. 1. The relative binding parameters are the number of binding sites per cell and the equilibrium dissociation constant of the mutant divided by the same parameter determined for the control strain (4277-7) in a parallel experiment.

[c] Number of binding sites occupied at the highest α-factor concentration used (0.2 μM).

[d] —, Binding was too low to permit the calculation of an equilibrium dissociation constant.

α-Factor-binding measurements. Cultures of the strains listed in Table 1 were grown at 34°C overnight to a final density between 10^6 and 10^7 cells per ml. NaN_3 (10 mM) and KF (10 mM) were added to the cultures 15 min before the cells were harvested by centrifugation. Cells were rinsed twice with inhibitor medium, and the cell concentration was determined as described previously (14). The binding reaction was initiated by mixing 0.1 ml of cells (5×10^8 cells per ml) with 0.1 ml of a dilution of ^{3}H-α-factor (13.2 Ci/mmol) in inhibitor medium. After 30 min at 22°C, a 0.18-ml sample was diluted to 2 ml with inhibitor medium (22°C), and the cells were collected on a presoaked filter (type HA, 0.45-μm pore size; Millipore Corp.). The filters were rinsed twice with 2 ml of inhibitor medium (22°C) for 0.4 to 0.5 min. The filters were then dried and counted in a liquid scintillation counter. The amount of nonspecific binding to α cells (strain 4345-32) was determined for each ^{3}H-α-factor dilution. Spe-

cific binding was calculated as the counts bound to the α cell filter (about 1% of the total radioactivity) substracted from the counts bound to the **a** cell filter. Quenching due to the cells on the filter was 45%. The free α-factor concentration was the concentration of α-factor bound to the cells substracted from the total α-factor concentration in the binding reaction mixture. The ^{3}H-α-factor was synthesized by Cambridge Research Biochemicals and is described elsewhere (15).

Quantitative mating experiments. The quantitative mating experiments were performed essentially as described by Hartwell (11). Cells were grown overnight at 22°C to a density between 10^6 and 10^7 cells per ml. For mating at 22°C, 2×10^6 **a** cells to be tested were mixed with 2×10^6 α cells (strain EMS63), collected on a type HA filter (0.45-μm pore size), and rinsed with 10 ml of YM-1 medium (22°C), and the filter was transferred to a YEPD plate, on which it was incubated at 22°C for 6 h. The cells were then suspended in 2 ml of ice-cold minimal medium (without ammonium sulfate or glucose), dispersed by mild sonication, diluted, and plated onto unsupplemented minimal medium. For mating at 34°C, the growing cultures were shifted to 34°C 2 hours before the **a** and α cells were mixed. The filters were rinsed with 34°C medium and incubated for 6 h on prewarmed YEPD plates at 34°C. The suspended cells (0°C) were plated onto prewarmed minimal medium (36°C) and incubated subsequently at 36°C; this was necessary to prevent some *ste* mutants from mating on the selective plates at high cell concentrations.

Isolation and characterization of *ros* mutants. An overnight culture of strain 3247-3-4 (*MAT***a** *cry1 ste4-3 sst2-1 ade6 his4 lys2 trp1 tyr1 SUP4-3*) was mutagenized with ethylmethane sulfonate to about 20% survival. The cells were cultured overnight (22°C) to give a density of 4×10^6 cells per ml, and 10^7 mutagenized **a** cells were then mixed with 10^7 α cells (strain 3244-6-1; *MATα cry1 ade2 his4 lys2 trp1 SUP4-3*) and collected on a type HA filter, and the filter was transferred to a YEPD plate (34°C). After overnight incubation (34°C), the filter was removed to 100 ml of liquid minimal medium supplemented with histidine, lysine, and tryptophan. After 2 days of incubation with shaking (36°C), 0.1 ml of the culture was diluted to 100 ml with the same medium and allowed to grow to saturation (36°C). The cells were then induced to sporulate, and tetrad analysis was performed on 36 asci. Thirteen asci yielded more than two fertile spores, suggesting the presence of a suppressor mutation. Furthermore, each of these asci contained one fertile spore of the **a** mating type that showed supersensitivity to α-factor at 22°C but not at 34°C, indicating the presence of a suppressed *ste4-3* mutation. Three of these fertile isolates which showed a conditional growth phenotype were chosen for further analysis. All three yielded sterile progeny when they were backcrossed to strain 3244-6-1 or 3227-2-3 Trp$^+$ (*MATα cry1 ade6 his4 lys2 try1 SUP4-3*). The genetic determinants specifying the conditional growth phenotype were then traced through at least three additional backcrosses to spontaneous derivatives of strain 381G. The three determinants, designated *ros1-1*, *ros2-1*, and *ros3-1*, showed 2:2 segregation for conditional growth phenotypes in nearly all asci. Mutations *ros1-1* and *ros2-1* resulted in slow growth at 38°C; mutation *ros3-1* led to slow growth at 22°C. None of these mutations affected growth at 30°C.

The *ros* mutants were tested for suppression by the temperature-sensitive amber suppressor mutation *SUP4-3* (24). Strain 3262-14-3 (*MATα cry1 ade6 his4 lys2 trp1 sup4-87*), which does not contain an active suppressor, was crossed to the temperature-sensitive suppressor strains listed in Table 3: 4239-2-4 (*ros1-1 SUP4-3*), 4321-4-4 (*ros2-1 SUP4-3*), and 4395-92 (*ros3-1 SUP4-3*). The progeny were tested for temperature-dependent growth and for suppression of amber mutations *his4* and *trp1*. For the first cross (*ROS1*$^+$ *sup4-87* × *ros1-1 SUP4-3*), 16 tetrads were analyzed; 42 spores resulted in viable colony formation, 2 spores failed to germinate, and the remaining 20 spores gave rise to microcolonies (30 to 50 cells each). All segregants terminating division at the microcolony stage were predicted to be of genotype *ros1-1 sup4-87* (assuming 2:2 segregation of both markers), and no *ros1-1 sup4-87* segregants were recovered among the viable colonies. This suggested that *ros1-1* was an amber mutation in an essential gene. In the second cross (*ROS2*$^+$ *sup4-87* × *ros2-1 SUP4-3*), all 80 spores of the 20 tetrads germinated to produce large colonies; however, 22 of these segregants proved to be inviable after subculturing. All members of the inviable class were predicted to be *ros2-1 sup4-87*; no *ros2-1 sup4-87* segregants were represented among the viable class. This suggested that *ros2-1* was also an amber mutation in an essential gene. For the third cross (*ROS3*$^+$ *sup4-87* × *ros3-1 SUP4-3*), all except two spores were viable among the 15 tetrads analyzed, and 16 *ros3-1 sup4-87* segregants were recovered. Therefore, *ros3-1* is apparently not an amber mutation. The residual growth of the *ros1-1 sup4-87* and *ros2-1 sup4-87* segregants was probably due to the presence of *ROS*$^+$ and *SUP4-3* gene products in the spores.

Selection of new supersensitive mutants. An overnight culture of strain 4211-12-2 (*MAT***a** *cry1 ros1-1 ste4-3 ade2 ade6 his4 lys2 trp1 SUP4-3*) was mutagenized with ethylmethane sulfonate to about 60% survival, diluted into nine tubes containing YM-1, and cultured overnight at 22°C to a density of about 10^7 cells per ml; 10^7 mutagenized **a** cells from each independent culture were mixed with 10^7 α cells (strain 3239-8: *MAT***a** *cry1 ade8 his4 lys2 trp1 tyr1 SUP4-3 cyh2*) collected on a type HA filter, and the filter was transferred to a YEPD plate (34°C). After 6 h at 34°C, the filters were transferred to 100 ml of minimal medium supplemented with histidine, lysine, and tryptophan. The resulting diploid cells were induced to sporulate, the asci were digested with glusulase, and the spores were spread on YEPD plates containing cycloheximide (10 μg/ml). For each independent isolation, 40 red cycloheximide-resistant colonies (*ade2 ADE6*$^+$ *ADE8*$^+$ *cyh2*) were transferred to YEPD plates, and the cross-stamp test was used to identify supersensitive clones. Two supersensitive mutants from each of the nine isolations were crossed to spontaneous derivatives of strain 381G to obtain *sst* strains with identical genetic markers (*MAT***a** *sst ade2 his4 lys2 trp1 tyr1 SUP4-3 cyh2*). Complementation analysis and dominance tests (4) were performed on each of these 18 strains. Strains 4213-67 (*MATα cry1 sst2-1 ade3 lys2 trp1 SUP4-3 can1 cyh2*), 4204-2 (*MATα cry1 bar1-1 ade3 lys2 trp1 SUP4-3 can1 cyh2*), and 3284-12 (*MATα cry1 ade3 lys2 trp1 SUP4-3 can1 cyh2*) were used as testers.

RESULTS

Binding of α-factor to *ste* mutants. Mutations *ste2*, *ste4*, *ste5*, *ste7*, *ste11*, and *ste12* cause **a** cells to lose responsiveness to α-factor (11, 19). The binding of radioactive α-factor to these *ste* mutants was examined to determine whether the unresponsive phenotype could be attributed to an absence or alteration of the receptors for α-factor. In our previous studies (14), temperature-sensitive *ste* mutants were grown at the permissive temperature (22°C) and then tested for the

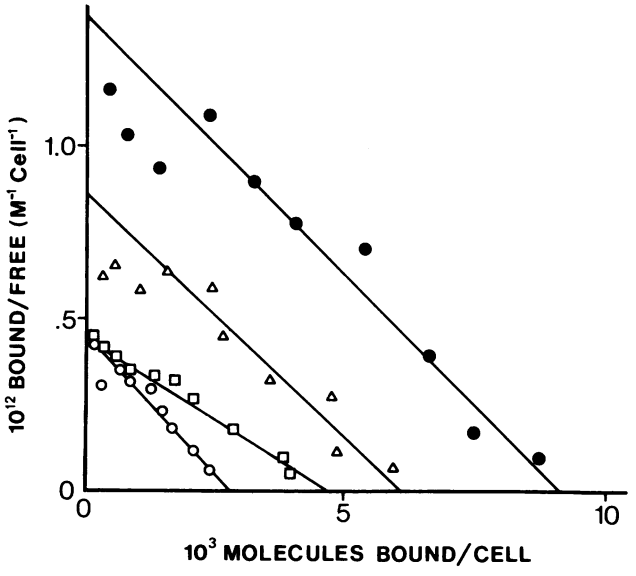

FIG. 1. Binding of α-factor to mutant **a** cells. The *ste* mutants were cultured for 10 to 12 generations at the restrictive temperature (34°C). Cells were inactivated with NaN_3 and KF and then tested for their ability to bind radioactive α-factor at 22°C. The results obtained with various concentrations of α-factor were plotted by the method of Scatchard (26). Bound/free indicates the number of α-factor molecules bound per cell divided by the free α-factor concentration. Significant binding of α-factor was observed for (○) strain 4333-48 (*ste11-1*), (□) strain 4278-45 (*ste4-3*), and (△) strain 5501-6 (*ste5-6*). However, fewer binding sites were detected for the mutants than for control strain 4277-7 (*STE*$^+$) (●). Full genotypes are indicated in Table 1.

presence of thermolabile α-factor binding sites. In this way, temperature-sensitive *ste2* mutants were found to produce a thermolabile binding activity, suggesting that the *STE2* gene encodes a structural component of the receptor. In the present study, we report the binding properties of *ste* mutants that were grown at the restrictive temperature (34°C). The three possible outcomes of these studies lead to the following conclusions about the control of the α-factor response by *STE* genes. (i) Mutants that fail to accumulate binding sites at 34°C are defective for either receptor function, stability, or synthesis. (ii) Mutants that retain binding sites when grown at 34°C harbor defects in functions that operate after α-factor binding has occurred. (iii) Mutants that alter the affinity for α-factor may be defective for receptor structure or enzymatic functions that modify the structure of receptors.

The *ste* mutants were grown for at least 10 generations at 34°C. The cells were then treated with poisons of energy metabolism (10 mM NaN_3 and 10 mM KF), washed, and incubated with radioactive α-factor (5×10^{-10} to 1×10^{-7} M). The amount of radioactivity bound to the cells at the various ligand concentrations was analyzed by the method of Scatchard (26) (Fig. 1). The results for strains 4333-48 (*ste11-1*) and 5501-6 (*ste5-6*) are described by lines parallel to the line representing the *STE*$^+$ control (strain 4277-7). These mutants appear to show wild-type affinity for α-factor. Binding to strain 4278-45 (*ste4-3*) was described by a line of a less negative slope, indicating that these cells may produce a receptor that binds α-factor with somewhat reduced affinity. All three mutants displayed fewer binding sites for α-factor than the control strain (4277-7), as indicated by the intercepts of the curves on the abscissa. Table 1 summarizes the binding properties of 14 *ste* mutants which represent six different complementation groups. Mutations in genes *STE2* and *STE12* (strains 4279-14, 4266-50, 4334-37, and 4268-60) led to dramatic reductions in α-factor binding, whereas mutations in genes *STE4*, *STE5*, *STE7*, and *STE11* (strains 4278-45, 4390-131, 4324-47, 4270-28, 5501-6, 4368-67, 4267-80, 4335-9, 4333-48, and 4269-19) led to only partial reductions in the number of binding sites (Table 1). The three *ste4* mutants (strains 4278-45, 4390-131, and 4324-47) appeared to show a small but consistent reduction in affinity for α-factor. Strains 4390-131 and 4368-67 carried amber-suppressible mutations *ste4-4* and *ste5-9* in a nonsuppressing genetic background (*sup4-87*); hence these mutants are likely to show a null phenotype.

Both of the *ste2* mutants (strains 4279-14 and 4266-50) were defective for binding when the cultures were grown under restrictive conditions. These results agree with our previous study, which indicated that receptors in *ste2* mutants are thermolabile. The *ste12* mutants failed to accumulate binding sites at the restrictive temperature (34°C) even though thermostable binding sites were produced under permissive conditions (22°C) (14). This result suggests that the *STE12* gene controls either the synthesis or the turnover of receptors. As *ste12* mutants accumulate very few transcripts from the *STE2* gene (7), it would appear that they are defective for receptor synthesis. Mutations in genes *STE4*, *STE5*, *STE7*, and *STE11* led to partial reductions in the number of α-factor binding sites. As these mutants are completely defective in their responses to α-factor (11) (cell division arrest and agglutinin induction), they are likely to have additional defects for steps of the response pathway that are executed after α-factor binds to its receptor. Mutants with lesions in genes *STE5*, *STE7*, or *STE11* showed equilibrium dissociation constants that were indistinguishable from that for the control strain. The *ste4* mutants showed a small but consistent increase in this parameter; this difference raises the possibility that the *STE4* product leads to structural modification of the receptor.

Genetic map positions. The assignment of genetic map positions is important for identifying allelisms between the *ste* mutations and other mutations that affect related cellular functions. Mutational sites have been mapped for only four of the six α-factor-unresponsive mutations discussed above (*ste4*, *ste5*, *ste7*, and *ste11*) (2, 27). We have determined that mutation *ste2* maps to chromosome VI, 33 centimorgans from *cdc4* and 29 centimorgans from *tub2* (Table 2). Mutation *ste12* was mapped to chromosome VIII, 11 centimorgans from *cdc12* and 27 centimorgans from *fur1*; in the course of mapping *ste12*, we also mapped mutation *fur1* to a position 6 centimorgans from *mak18* and 18 centimorgans from *cdc12* (Table 2). We also report *ste11-ilv5* linkage data which locate more precisely the position of the *STE11* gene on chromosome XII.

Suppression of *ste4* mutations. We wished to determine whether the available *ste* mutations could be suppressed by *sst2-1*. Because *sst2* confers supersensitivity (4), this test may identify mutants whose mating defects derive primarily from their failure to respond to α-factor. As a preliminary test, we crossed strain 2908-18-1 (*MATα sst2-1*) to each of seven temperature-sensitive sterile derivatives of strain 381G (*ste4-3*, *ste5-3*, *ste7-2*, *ste8-5*, *ste9-2*, *ste11-3*, and *ste12-2*). Some (but not all) of the resulting *MAT***a** *ste4-3 sst2-1* segregants showed a fertile phenotype at the restrictive temperature (34°C), whereas none of the *ste4-3 SST2*$^+$ segregants were fertile. The crosses involving the other *ste* mutants yielded no fertile segregants of genotype *ste sst2-1*.

These observations suggested that mutation *sst2-1* in conjunction with other genetic factors could suppress the mating defects imposed by mutation *ste4-3*.

When the sst2-1 mutation was first introduced into the genetic background that was common to the *ste* mutants (by backcrossing 10 times consecutively) and then crossed into the *ste4-3* strain, it did not suppress the sterility phenotype of *ste4-3* mutants. Quantitative mating analysis (Table 3) indicated that the mating abilities of segregant 3254-8-4 (*MAT***a** *ste4-3 sst2-1*) and sterile control strain 3273-20-3 (*MAT***a** *ste4-3*) were both reduced by a factor of 10^6 compared with the wild-type control strain 381G. This observation is consistent with the previous result indicating that a genetic factor in addition to mutation *sst2* is required for suppression of *ste4* mutations. This factor must have been present in strain 2908-1 but absent from strain 381G.

Isolation of *ros* mutants. We wished to identify the factor(s) which, in conjunction with mutation *sst2-1*, led to the suppression of *ste4-3* mutations. Haploid **a** cells of strain 3247-13-4 (*ste4-3 sst2-1*) which had been treated with a mutagen, ethylmethane sulfonate, were challenged to mate with α cells (strain 3244-6-1) at 34°C; the resulting diploid cells were induced to sporulate. Of the 36 asci analyzed, 13 contained more than two spores that were fertile at 34°C. This segregation pattern suggested the presence of a suppressor mutation. The presence of a suppressed *ste4-3* mutation in three fertile **a** cell isolates was then verified by crossing each to α cells (strain 3227-2-3 Trp^+ or 3244-6-1) and obtaining unsuppressed sterile progeny. The suppressor loci were designated *ros* (for relaxation of sterility). Two of these suppressors (*ros1-1* and *ros2-1*) were correlated with temperature-sensitive growth (38°C restrictive temperature), and one suppressor (*ros3-1*) was correlated with cold-sensitive growth (22°C restrictive temperature). The growth defects associated with all three *ros* mutations showed a 2:2 segregation pattern through five consecutive backcrosses to unmutagenized derivatives of strain 381G. The haploid progeny of the fifth backcross are unlikely to contain other mutations induced during mutagenesis which are unlinked to the determinant causing the growth defect. These strains were used for all subsequent constructions, and the presence of the *ros* mutation was monitored by the temperature-sensitive growth phenotype. All three mutations (*ros-1*, *ros2-1*, and *ros3-1*) were found to be unlinked (Table 2), and all proved to be recessive in that the heterozygous diploids (*ros*/$^+$) did not show temperature-sensitive growth. Mutations *ros1-1* and *ros2-1* proved to be amber suppressible; the temperature-sensitive growth phenotype was due to the presence of the temperature-sensitive amber suppressor mutation *SUP4-3* (see Materials and Methods).

The three *ros* mutants were crossed to strains containing mutations *sst2-1* and *ste4-3* to yield all combinations of *ros1-1*, *ros2-1*, and *ros3-1* with the *MAT*, *SST2*, and *STE4* loci. (Each possible combination was examined in at least four spore clones.) In all cases, the strains of genotype *MAT***a** *ste4-3 sst2-1 ros* showed appreciable mating with α cells at 34°C, whereas the *MAT***a** *ste4-3 ros* and the *MAT***a** *ste4-3 sst2-1* strains were sterile. Table 3 shows quantitative mating results for representative strains. Liquid cultures were grown at 22°C, and the cells were then challenged to mate with α cells at either 22 or 34°C. Control cells (strain 381G) formed a greater number of diploid cells at 34°C than

TABLE 2. Tetrad analysis

Chromosome[a]	Gene pair[b]	Ascus type[c] (no.)			Map distance[d] (centimorgans)	Linkage relationship[e]
		PD	NPD	T		
VI	*ste2-cdc4*	21	1	24	33	Direct
	ste2-tub2	25	1	20	29	Direct
	cdc4-tub2	13	2	31	50	Direct
XII	*ste11-ilv5*	29	0	1	2	Direct
VIII	*ste12-cdc12*	43	0	12	11	Direct
	ste12-fur1	57	0	69	28	Direct
	fur1-cdc12	65	0	37	18	Direct
	fur1-mak18	21	0	3	6	Direct
	fur1-pet3	12	0	5	15	Direct
III	*ros1-MAT*	33	14	146	70	Direct
V	*ros3-ura3*	37	0	4	5	Direct
	ros3-can1	12	1	25	43	Direct
	ros3-trp1	11	22	6	8	Centromere
	trp1-ura3	12	23	4	5	Centromere
	ura3-can1	11	2	25	53	Direct

[a] The chromosome-loss method of Wood (33) was used to make chromosomal assignments for mutations *ste2-3*, *ste11-3*, *ste12-2*, *fur1*, and *ros3-1*. Mutation *ros1-1* was assigned to the right arm of chromosome III by mitotic linkage to *cry1* and *MAT*.

[b] Strains containing the unmapped mutations were crossed to strains that contained known markers on the appropriate chromosome. Markers showing detectable linkage are listed in this column. Markers on the same chromosome were analyzed in a single cross for chromosomes V, VI, and XII. Segregation results from several crosses were combined for the analysis of chromosomes III and VIII.

[c] P, Parental ditype; NPD, nonparental ditype; T, tetratype. Only those asci showing 2:2 segregation for both markers were included.

[d] Determined by the X' approximation method of Ma and Mortimer (18).

[e] The genetic markers are linked to one another on the same chromosome (direct) or linked to centromeres on different chromosomes (centromere).

TABLE 3. Suppression of sterility in *ste4-3* mutants

Strain[a]	Relevant genotype[b]	Mating[c] (diploids formed/filter)	
		22°C	34°C
381G	Wild-type	3×10^6	2×10^7
3273-20-3	*ste4-3*	8×10^5	<10
3254-8-4	*ste4-3 sst2-1*	7×10^4	40
4239-2-4	*ros1-1*	2×10^6	1×10^7
4230-2-4	*ste4-3 ros1-1*	2×10^6	40
4241-6-4	*ste4-3 ros1-1 sst2-1*	1×10^5	2×10^5
4321-4-4	*ros2-1*	2×10^6	8×10^6
4322-10-2	*ste4-3 ros2-1*	1×10^6	20
4318-2-4	*ste4-3 ros2-1 sst2-1*	2×10^5	1×10^5
4395-9-2	*ros3-1*	1×10^6	1×10^7
4349-1-3	*ste4-3 ros3-1*	7×10^5	20
4349-7-4	*ste4-3 ros3-1 sst2-1*	3×10^5	8×10^3
5566-6-3	*cdc39-1*	7×10^6	2×10^7
5601-12-1	*ste4-3 cdc39-1*	9×10^5	300
5601-1-1	*ste4-3 cdc39-1 sst2-1*	1×10^5	4×10^5

[a] All strains listed are congenic to strain 381G.

[b] The strains listed also contain *MAT***a** *cry1 ade2-1 his4-580 lys2 trp1 tyr1 SUP4-3*.

[c] The strain indicated was cultured overnight at 22°C. A sample of the culture (2×10^6 **a** cells) was challenged to mate with an equal number of α cells (strain EMS63) at 22°C on a filter, or the culture was first shifted to 34°C and incubated for 2 h and then the **a** cells were challenged to mate with α cells at 34°C. After the 6-h mating period, diploid cells were detected by complementation of auxotrophic markers. Entries are the median values from at least four measurements. More diploids were sometimes recovered at 34°C than at 22°C because the doubling time of the diploid cells was shorter at the higher temperature.

at 22°C because of the shorter generation time at the higher temperature. The mutant strain 3273-20-3 (*MAT***a** *ste4-3*) lost mating ability rapidly after the culture had been shifted to 34°C; that is, the cells pregrown at 22°C were sterile when mated at 34°C. The *sst2-1* mutation in strain 3254-8-4 (*MAT***a** *ste4-3 sst2-1*) was unable to compensate for the sterility resulting from mutation *ste4-3*; similarly, mutations *ros1-1*, *ros2-1*, and *ros3-1* (strains 4230-2-4, 4322-10-2, and 4349-1-3) alone did not suppress sterility. In contrast, strains 4241-6-4 (*MAT***a** *ste4-3 sst2-1 ros1-1*), 4318-2-4 (*MAT***a** *ste4-3 sst2-1 ros2-1*), and 4349-7-4 (*MAT***a** *ste4-3 sst2-1 ros3-1*) retained high levels of mating after the cultures had been shifted to 34°C. Therefore, mutation *sst2-1* acts synergistically with any one of three other mutations (*ros-1*, *ros2-1*, or *ros3-1*) to suppress sterility in **a** cells containing the *ste4-3* mutation. Although the mating ability of the suppressed mutants was significant, the efficiency was less than that observed for wild-type **a** cells (strain 381G). Suppression of sterility in α cells was relatively weak and was not examined quantitatively.

Mutation *ros1-1* is allelic to mutation *cdc39-1*. Certain temperature-sensitive mutants harboring lesions in *CDC28*, *CDC36*, *CDC37*, or *CDC39* are defective in completing a specific step in the cell division cycle, start, when cultures are shifted from the permissive to the restrictive temperature (25). This is the same step that is controlled by α-factor. In addition, mutations *cdc36* and *cdc39* are known to suppress sterility in α cells containing the *ste4-3* mutation (27). Therefore, we considered the possibility that some of the *ROS* genes may be identical to these *CDC* genes. Mutations *ros1-1* and *cdc39-1* were shown to be noncomplementing by mating strain 4239-1-1 (*MATα ros1-1*) with strain ST33 (*MAT***a** *cdc39-1*) at 22°C to obtain diploid cells (*ros-1/cdc39-1*) that were able to form colonies at 22°C but not at 38°C; in the control cross (381G-2-1 × ST33), the resulting diploid cells ($^{+}$/*cdc39-1*) formed colonies at 38°C. Genes *ROS2* and *ROS3* are probably not identical to *CDC28*, *36*, or *39*. Temperature-sensitive mutant 4288-10-1 (*MATα ros2-1*) complemented temperature-sensitive defects in strains ST2 (*MAT***a** *cdc28-4*), ST32 (*MAT***a** *cdc36-16*), and ST33 (*MAT***a** *cdc39-1*); mutation *ros3-1* mapped to chromosome V (Table 2) and is therefore unlinked to mutations *cdc28-4*, *cdc36-16*, *cdc37-1*, and *cdc39-1*.

Mutation *cdc39-1* was similar to *ros1-1* in its ability to suppress *ste4-3* mutations. Mutation *cdc39-1* was introduced into our standard genetic background by backcrossing the mutant 10 times successively to derivatives of strain 381G. Inbred strains were then crossed (*ste4-3 cdc39-1* × *ste4-3 sst2-1*) to produce 5 segregants (*MAT***a** *ste4-3 cdc39-1*) that were uniformly sterile and 10 segregants (*MAT***a** *ste4-3 cdc39-1 sst2-1*) that were uniformly fertile. Quantitative mating results for two representative segregants (strains 5601-12-1 and 5601-1-1) are listed in Table 3. Partial suppression of *ste4-3* by *cdc39-1* in α cells was apparent, as previously noted by Shuster (27).

In our standard genetic background, neither *ros1-1* nor *cdc39-1* resulted in stage-specific arrest of cell division when the appropriate cultures were shifted to the restrictive temperature. Stage-specific arrest of *cdc39* mutants was shown previously to be dependent on the genetic configuration at the mating type locus (*MAT***a** or *MATα* but not *MAT***a**/*MATα*) (25, 27) and on the carbon source used in the culture medium (28). Therefore, although the *CDC39* gene product may be essential for the completion of the α-factor-controlled step of the cell cycle, its function is specific to the G1 phase of the mitotic cycle only in a defined genetic and nutritional context. Nevertheless, the fact that *cdc39* mutants can mimic α-factor-induced arrest of cell division and suppress pheromone-resistant mutations (*ste4*) suggests that the *CDC39* gene product affects post receptor steps in the α-factor response pathway, although its action may be indirect.

TABLE 4. Effects of mutations *ros1-1* and *sst2-1* on the mating ability of *ste* mutants[a]

Strain	Relevant genotype	Mating (diploids formed/filter)	
		22°C	34°C
381G	Wild type	2×10^6	1×10^7
3268-8-2	*sst2*	3×10^5	1×10^6
4239-2-4	*ros1*	2×10^6	1×10^7
4469-6-1	*sst2 ros1*	4×10^4	5×10^5
5544-1-4	*ste2-3*	1×10^6	1×10^3
5544-7-1	*ste2-3 sst2*	3×10^5	4×10^4
5544-5-4	*ste2-3 ros1*	1×10^6	2×10^3
5544-2-4	*ste2-3 sst2 ros1*	4×10^5	6×10^4
5554-8-1	*ste4-4* (amber)	4×10^4	10
4305-1-3	*ste4-4 sst2*	7×10^3	200
4305-4-1	*ste4-4 ros1*	3×10^4	30
4305-10-3	*ste4-4 sst2 ros1*	1×10^4	4×10^3
5525-3-3	*ste4-5*	1×10^6	20
5525-7-1	*ste4-5 sst2*	8×10^4	20
5525-4-4	*ste4-5 ros1*	2×10^6	20
5525-15-3	*ste4-5 sst2 ros1*	2×10^5	6×10^4
118B	*ste4-6*	1×10^6	10
4306-1-1	*ste4-6 sst2*	8×10^4	10
4306-2-3	*ste4-6 ros1*	1×10^6	10
4306-17-4	*ste4-6 sst2 ros1*	1×10^5	1×10^5
5528-4-1	*ste5-3*	2×10^6	10
5528-10-3	*ste5-3 sst2*	1×10^5	10
5528-2-3	*ste5-3 ros1*	2×10^6	3×10^4
5528-25-1	*ste5-3 sst2 ros1*	2×10^5	1×10^4
5559-3-2	*ste7-2*	1×10^6	10
5548-4-1	*ste7-2 sst2*	4×10^4	20
5559-11-2	*ste7-2 ros1*	1×10^6	20
5548-1-2	*ste7-2 sst2 ros1*	7×10^4	20
5540-2-4	*ste11-3*	2×10^6	10
5540-22-1	*ste11-3 sst2*	1×10^5	10
5540-4-2	*ste11-3 ros1*	2×10^6	10
5540-8-1	*ste11-3 sst2 ros1*	2×10^5	10
5527-3-4	*ste12-2*	1×10^6	10
5527-13-2	*ste12-2 sst2*	4×10^6	10
5527-24-3	*ste12-2 ros1*	1×10^6	10
5527-7-2	*ste12-2 sst2 ros1*	6×10^4	10

[a] See Table 3, footnotes *a*, *b*, and *c*.

Suppression of other *ste4* alleles by *ros1-1* and *sst2-1*. The *ste4-3* mutant appeared to be defective for at least two aspects of the mating process in that two mutations (*ros* and *sst2*) were required for its suppression. We wished to determine whether mutants carrying other *ste4* alleles (*ste4-4*, *ste4-5*, or *ste4-6*) also required both *ros* and *sst2* mutations for suppression. The effect of *ros1-1* and *sst2-1* on *ste4* alleles was tested by crossing inbred strains (*ste4 ros1-1* × *ste4 sst2-1*); mating proficiences for representative segregants are summarized in Table 4. Results obtained with the

TABLE 5. Effects of mutations *bar1-1* and *ros1-1* on the mating of *ste4-3* mutants[a]

Strain	Relevant genotype	Mating (diploids formed/filter)	
		22°C	34°C
381G	Wild type	3×10^6	2×10^7
4202-15-3	*bar1-1*	4×10^5	4×10^6
3273-20-3	*ste4-3*	2×10^6	10
4226-7-2	*ste4-3 bar1-1*	2×10^5	10
4230-2-4	*ste4-3 ros1*	3×10^6	30
4229-1-1	*ste4-3 bar1-1 ros1*	4×10^5	1×10^4

[a] See Table 3, footnotes *a*, *b*, and *c*.

three aditional *ste4* alleles were similar to the results obtained with *ste4-3* mutant (Table 3). Thus, all four alleles examined (*ste4-3*, *ste4-4*, *ste4-5*, and *ste4-6*) required both *sst2-1* and *ros1-1* for full suppression. The *ste4-4* allele is amber suppressible and leads to temperature-sensitive mating in the presence of the temperature-sensitive amber suppressor *SUP4-3* (11). For strains of genotype *ste4-4 SUP4-3*, a shift to the restrictive temperature presumably blocks the synthesis of new *STE4* gene product but is unlikely to affect the activity of the gene product already present. The *ste4-4* mutant (strain 5554-8-1) containing amber suppressor *SUP4-3* lost its mating ability rapidly when the culture was shifted from the permissive (22°C) to the restrictive (34°C) temperature (Table 4); thus, the active *STE4* gene product produced at the permissive temperature was unable to support mating in the absence of its continued synthesis.

Suppression of *ste2* and *ste5* mutations. Mutants that harbored lesions at other *STE* loci (*STE2*, *STE5*, *STE7*, *STE11*, or *STE12*) were examined for suppression by *sst2-1* and *ros1-1*. The appropriate mutant strains were constructed by crossing inbred strains (*ste sst2-1* × *ste ros1-1*); the mating proficiencies of representative segregants are listed in Table 4. Strain 5544-1-4 (*MAT***a** *ste2-3*) lost mating ability when the culture was shifted from the permissive (22°C) to the restrictive (34°C) temperature. This loss of mating ability was not as severe for strains containing mutation *sst2-1* (Table 4; compare strain 5544-7-1 with 5544-1-4 and strain 5544-2-4 with 5544-5-4); however, suppression of sterility by mutation *ros1-1* was not detected (Table 4; compare strain 5544-5-4 with 5544-1-4 and strain 5544-2-4 with 5544-7-1). In contrast, the mating defects resulting from mutation *ste5-3* were partially suppressed by mutation *ros1-1* (Table 4; compare strain 5528-2-3 with 5528-4-1 and strain 5528-25-1 with 5528-10-3) but not by mutation *sst2-1* (compare strain 5528-10-3 with 5528-4-1 and strain 5528-25-1 with 5528-2-3). The sterile phenotypes of the *ste7-2*, *ste11-3*, and *ste12-2* mutants were not suppressed by mutation *sst2-1* or *ros1-1*. Our observation that mutation *ros1-1* suppresses *ste5-3* mutants agrees with the observation of Shuster (27) that *cdc39-1* suppresses *ste5* mutants.

Suppression of the *ste4-3* mutant by mutation *bar1-1*. Wild-type **a** cells produce an activity that inactivates α-factor extracellularly (3, 6, 30). Cells that harbor mutation *bar1-1* are defective for this activity (5, 30); as a result, they show enhanced sensitivity to α-factor. We found that mutation *bar1-1* together with mutation *ros1-1* partially suppressed the sterility of the *ste4-3* mutant. As shown in Table 5, strains 4226-7-2 (*MAT***a** *ste4-3 bar1-1*) and 4230-2-4 (*MAT***a** *ste4-3 ros1-1*) showed no significant suppression of sterility compared with control strain 3273-20-3 (*MAT***a** *ste4-3*), whereas strain 4229-1-1 (*MAT***a** *ste4-3 bar1-1 ros1-1*) showed relatively high levels of mating at 34°C. Thus, the mating defect in *ste4* mutants that is suppressible by mutation *sst2-1* can also be suppressed by mutation *bar1-1*. This observation supports the view that mutation *sst2-1* suppresses the sterility of *ste4* mutants by enhancing the sensitivity of **a** cells to α-factor. The enhanced sensitivity provided by mutation *bar1-1*, however, was not sufficient to suppress the sterility of the *ste2-3* mutant (not shown); this observation probably reflects the fact that *bar1-1* exhibits a weaker supersensitivity phenotype than *sst2-1* (4).

Selection of *sst2* and *bar1* mutations as suppressors of sterility. Since strains of genotype *MAT***a** *ste4-3 ros1-1 sst2-1* mate nearly 10^4 times more frequently at 34°C than do strains of genotype *MAT***a** *ste4-3 ros1-1 SST*$^+$, it should be possible to select α-factor-supersensitive mutants as fertile revertants of the appropriate sterile mutant (*MAT***a** *ste4-3 ros1-1*). We wished to determine whether mutations in genes other than *SST2* or *BAR1* could result in supersensitivity to α-factor. A series of supersensitive mutants were selected (see Materials and Methods). Complementation analysis identified *sst2* mutations in isolates arising from each of the nine independent cultures; a *bar1* mutation was also identified in one isolate. No new complementation groups were identified. Therefore, if genes other than *SST2* control α-factor sensitivity, they do not mutate to supersensitivity as frequently or the resulting mutations do not suppress sterility as efficiently.

DISCUSSION

Biochemical and genetic studies of pheromone action in *S. cerevisiae* have focused principally on the regulation of **a** cell functions by α-factor. Specific binding of α-factor to **a** cells has been demonstrated (14, 15) and dose-response relationships have been described (20) for the various α-factor-mediated processes (cell division arrest, agglutination, and morphological alterations). Mutations which prevent **a** cells from responding to α-factor have been useful for dissecting the various elements of the response pathway. Mutants bearing lesions in each of eight *STE* loci have been selected by their inability to arrest cell division in response to α-factor (11, 19). All of these mutants also fail to produce cell surface agglutinins and fail to alter their cellular morphology when they are exposed to α-factor; hence, they are defective for regulatory elements common to all three α-factor-mediated processes (11). The *STE2* gene product is a structural component of the α-factor receptor (14); the molecular functions of the other *STE* gene products are unknown. Some of these gene products may affect the response pathway indirectly, since all of the *ste* mutants (except *ste2*) also show reduced levels of the mating functions that do not require α-factor for their expression (**a**-factor production and α-factor degradation) (11). Indeed, *MAT***a** *ste12* strains fail to accumulate normal levels of transcripts from at least three genes required for mating (*STE2*, *MFa1*, *and MFa2*) (7), and *MAT***a** *ste4* strains show reduced levels of the *STE2* transcript (9). It is possible that the pleiotropic phenotypes associated with the other *ste* mutations may also be the result of similar regulatory phenomena.

In this paper, we describe properties of the *ste* mutants that relate to the inability of **a** cells to respond to α-factor. We found that the mutants containing *ste2* and *ste12* failed to accumulate α-factor-binding sites when they were cultured at the restrictive temperature, whereas the other mutants (*ste4*, *ste5*, *ste7*, and *ste11*) accumulated significant numbers

of binding sites (50, 49, 26, and 32% of the wild-type value, respectively). The lack of binding sites in the *ste2* mutants was expected for cells carrying defects in the receptor structural gene. The partial reductions in the number of binding sites for the other mutants (*ste4*, *ste5*, *ste7*, and *ste11*) are not likely to account for the severe loss of α-factor responsiveness that has been observed for these mutants (11); therefore we propose that the *STE4*, *5*, *7*, and *11* genes also control postreceptor steps of the response pathway. Since *ste12* mutants do not accumulate *STE2* transcripts (7) or α-factor-binding sites, the *ste12* mutant is at least defective for receptor synthesis, and it may be defective for other postreceptor functions as well. The partial reduction in binding sites that we observed in the *ste4* mutants is consistent with the findings of Hartig et al. (9), who observed that the level of *STE2* transcripts was reduced partially (about 10-fold) in strains containing either the amber *ste4-4* allele (included in this study) or a deletion of the *STE4* gene. The differences between binding capacity and transcript level in the *ste4* mutants may reflect the differences in culture conditions and genetic background. The affinity of the α-factor-binding sites for α-factor in *ste5*, *ste7*, and *ste11* mutants was the same as that found in the STE^+ control strain; a small reduction in affinity was observed for each of the three *ste4* mutants examined. It is possible that the *STE4* gene controls structural features of the receptor and that receptors collect in a low-affinity (perhaps inactive) form when *STE4* gene products are absent.

Our second approach for analyzing *ste* mutants was to test the ability of mutation *sst2* to suppress the sterility phenotype. If mating in these mutants is limited by their sensitivity to α-factor, then a mutation which leads to enhanced sensitivity may compensate for the defect. Suppression of *ste4* mutants by mutation *sst2* required one of at least three additional mutations (*ros1*, *ros2*, or *ros3*). The *ste5* mutant was suppressed by mutation *ros1* but not by *sst2*. In contrast, the *ste2* mutant was suppressed by *sst2* but not by *ros1*.

The suppression results suggest that the *STE4* gene controls two independent steps in the mating process; defects in one step are suppressed by mutation *sst2* and defects in the other step are suppressed by *ros* mutations. The two steps can be inactivated separately, since the *ste2* mutant contained only an *sst2*-suppressible defect, whereas the *ste5* mutant contained only a *ros*-suppressible defect. Mutation *sst2* appears to operate in suppression by simply causing an increase in α-factor sensitivity, since the *bar1* mutation, which leads to inhibition of α-factor degradation, substituted for *sst2* in suppression of the *ste4* mutant. The *ros* mutations do not affect α-factor sensitivity but instead appear to elicit suppression at a postreceptor level, since the *ros1* mutation suppressed the *ste5* mutant, which accumulates significant numbers of receptor sites, and *ros1* is allelic to mutation *cdc39*, which leads to cell division arrest even in the absence of α-factor binding (25).

From our analysis, it is not possible to determine whether the *STE4* and *STE5* genes encode elements that are directly involved in the cellular response or whether they regulate the activity of these elements indirectly. Fields and Herskowitz (7) have recently suggested that many of the *STE* genes (such as *STE12*) may encode factors which regulate the transcription of other genes whose products are required for mating (such as *STE2* and *MFa1*). If the *STE4* and *STE5* gene products act indirectly as regulators of structural elements in the α-factor response pathway, then they apparently affect the different steps of the pathway to various extents, since defects in these two genes are compensated by different combinations of suppressor mutations.

Although other models are possible, we suggest a model for α-factor action in which the receptor plays a central role in mediating the various cellular responses. The *STE2* gene appears to encode the receptor. Mutation *sst2* may enhance sensitivity to α-factor and suppress temperature-sensitive defects in the receptor structure by blocking inactivation of the receptor. Documented examples of receptor inactivation include receptor-mediated endocytosis (downregulation) (31) and covalent modification of receptors on the cell surface (29). Recent evidence (16) indicates that mutation *sst2* does not block downregulation of α-factor receptors; hence, we suspect that the *SST2* gene controls modification of receptor structure. One function of the *STE4* gene may be to control modification of receptor structure, so that in *ste4* mutants the receptors accumulate in an inactive form. The reduced affinity of *ste4* mutants for α-factor may be symptomatic of this defect. Suppression of both *ste2* and *ste4* mutants by *sst2* corroborates the suggestion that the *STE4* gene product affects the α-factor receptor. The *STE5* gene and the second function of the *STE4* gene may control the generation of intracellular signals when α-factor-receptor complexes are present. The *ros* mutations may suppress defects in these functions by inhibiting intracellular processes that destroy these signals or by making the cell more sensitive to the signal. Consistent with this view is the observation that in the proper genetic and nutritional context (28), the *ros1* allele, *cdc39-1*, leads to stage-specific arrest of the mitotic cycle even when α-factor is absent (25).

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LITERATURE CITED

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