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Physiological Characterization of *Saccharomyces cerevisiae* Mutants Supersensitive to G1 Arrest by a Factor and α Factor Pheromones

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*Saccharomyces cerevisiae MAT***a** cells carrying mutations in either *sst1* or *sst2* are supersensitive to the G1 arrest induced by α factor pheromone. When *sst1* mutants were mixed with normal *SST*$^+$ cells, the entire population recovered together from α factor arrest, suggesting that *SST*$^+$ cells helped *sst1* mutants to recover. Complementation tests and linkage analysis showed that *sst1* and *bar1*, a mutation which eliminates the ability of *MAT***a** cells to act as a "barrier" to the diffusion of α factor, were lesions in the same genes. These findings suggest that *sst1* mutants are defective in recovery from α factor arrest because they are unable to degrade the pheromone. In contrast, recovery of *sst2* mutants was not potentiated by the presence of *SST*$^+$ cells in mixing experiments. When either normal *MAT***a** cells or mutant cells carrying defects in *sst1* or *sst2* were exposed to α factor for 1 h and then washed free of the pheromone, the *sst2* cells subsequently remained arrested in the absence of α factor for a much longer time than *SST*$^+$ or *sst1* cells. These observations suggest that the defect in *sst2* mutants is intrinsic to the cell and is involved in the mechanism of α factor action at some step after the initial interaction of the pheromone with the cell. The presence of an *sst2* mutation appears to cause a growth debility, since repeated serial subculture of haploid *sst2-1* strains led to the accumulation of faster-growing revertants that were pheromone resistant and were mating defective ("sterile").

Haploid cells of the yeast *Saccharomyces cerevisiae* secrete diffusible oligopeptide pheromones that act specifically on cells of the opposite mating type to arrest them in the G1 stage of the cell cycle (13, 14a, 15, 18).

The α factor is the tridecapeptide pheromone secreted by *MAT*α cells, and **a** factor is the undecapeptide pheromone secreted by *MAT***a** cells. Reversal of α factor arrest can be effected by the removal of α factor (2) and also occurs spontaneously, the time of recovery being dependent on the concentration of α factor added (3, 19). Proteolytic cleavage of α factor has been shown to be involved in the recovery of *MAT***a** cells from α factor arrest (5, 8, 14).

In the preceding paper (4), we described the isolation of mutants in *MAT***a** strains that are supersensitive (Sst$^-$) to G1 arrest by α factor. These mutations define two genes, *sst1* and *sst2*. The *sst1* mutation is *MAT***a** specific because it does not make *MAT*α cells supersensitive to **a** factor. In contrast, the *sst2* mutation is not specific to cell type because it also makes *MAT*α cells supersensitive to **a** factor.

In this paper, we describe the consequences of treating a mixture of Sst$^+$ and Sst$^-$ cells with α factor. If the Sst$^-$ phenotype is caused by failure to degrade α factor, the Sst$^+$ cells growing in the mixture might be able to compensate for this defect. In agreement with this expectation, we found that *SST*$^+$ cells can help *sst1* cells recover from α factor arrest. In contrast, the *sst2* mutants seem to have an internal defect since we found that *SST*$^+$ cells were unable to help *sst2* cells recover from α factor arrest.

Mutations called *bar1*, which have a phenotype consistent with an inability to destroy α factor, were recently isolated by Sprague and Herskowitz (J. Mol. Biol., in press). Unlike normal *MAT***a** cells, such mutants do not act as a barrier to the diffusion of α factor on an agar slab (11). We show here that *sst1* and *bar1* are mutations in the same gene; this supports the hypothesis that *sst1* mutants are supersensitive because they fail to inactivate α factor.

MATERIALS AND METHODS

Yeast strains. RC618 (*MAT***a** *SST*$^+$), RC622 (*MAT*α *SST*$^+$), RC629 (*MAT***a** *sst1-2*), RC631 (*MAT***a** *sst2-1*), RC639 (*MAT***a** *sst2-3*), RC687 (*MAT***a** *sst2-4*), RC757 (*MAT*α *sst2-1*), RC844 (*MAT*α *sst1-2*), RC870 (*MAT***a** *sst2-4 SUP*^{oc}), RC884 (*MAT*α *sst2-3*), and X2180-1B (*MAT*α) were described in the preceding paper (4). Other strains are listed in Table 1.

Media and techniques. All of the media and many of the techniques used in this study were described in the preceding paper (4).

Barrier test. The method of Sprague and Herskowitz (in press) was used for the barrier test. Parallel streaks of a *MAT***a** strain to be tested and of X2180-1B *MAT*α were made on a YEPD master plate. The master plate was incubated overnight at 30°C before replicating to a pH 3.5 YEPD plate freshly spread with 5×10^5 cells of RC629 *MAT***a** *sst1-2*. The pH 3.5 plate was incubated at 30°C for 1 to 2 days to allow the lawn of RC629 to grow to confluence. Since α factor is diffusible, RC629 cells in the vicinity of the X2180-1B streak fail to grow, resulting in a clearing or "halo." If the perimeter of the halo stopped at the edge of the *MAT***a** streak, the tested strain was scored as Bar^+. If the perimeter of the halo intersected or extended beyond the *MAT***a** streak, the tested strain was scored as Bar^-.

Identification of sterile revertants. A plate containing about 100 colonies to be tested and a plate containing a lawn of a tester strain of opposite mating type and with complementary auxotrophic markers were replicated in turn to a single YEPD plate. This new plate was incubated overnight at 30°C to allow mating and then was replicated to a synthetic medium plate to detect the formation of prototrophic diploids formed by mating between mating-proficient colonies and the tester lawn. Colonies on the original YEPD plate which did not form prototrophic diploids on the synthetic medium plate were considered to be sterile.

Efficiency of mating. The filter-mating assay of Hartwell (9) was used with minor modifications to measure the efficiency of mating of *sst* mutants. Cells were grown to exponential phase in YM-1 at 30°C. Cells (2×10^6) of each mating type were collected on membrane filters (Gelman Sciences, Inc.; GA-6, 25 mm, 0.45-μm pore size) which were then placed on YEPD plates and incubated at 30°C. After 6 h, the cells on the filter were suspended in sterile water, sonicated, diluted, and spread onto synthetic medium plates to select for the prototrophic diploids formed by mating. *MAT***a** strains were mated with *MAT*α tester strain RC63; *MAT*α strains were mated with *MAT***a** tester strain RC64. The efficiency of mating was defined as the number of prototrophs formed in a test mating/number of prototrophs formed in a control mating, where RC618 and RC622 were used as *MAT***a** *SST*$^+$ and *MAT*α *SST*$^+$ controls, respectively. With the aforementioned tester strains, RC618 and RC622 gave rise to an average number (± standard deviation) of $8.41 \pm 0.12 \times 10^6$ and $8.25 \pm 0.17 \times 10^6$ prototrophs, respectively.

RESULTS

sst1* and *bar1* are the same gene.** The preceding paper (4) described the isolation of mutations, in the *sst1* gene, which confer supersensitivity to α factor and map close to *his6* on chromosome IX. Using a different screening procedure, Sprague and Herskowitz (in press) have isolated a mutation, called *bar1-1*, that has a similar phenotype and map position. Normal *MATa** strains are Bar^+ because they can form a barrier to the diffusion of α factor on an agar slab (11). In contrast, *MAT***a** strains carrying the *bar1-1* mutation have a Bar^- phenotype. Examination of the barrier phenotype in our *sst* mutants revealed that all *sst1* mutants were Bar^- and all *sst2* mutants were Bar^+.

To determine whether *sst1* and *bar1* are in the same complementation group, *sst1/bar1* and *sst2/bar1* diploids were constructed. A *MAT***a**/*MAT***a** *sst1-2/bar1-1* diploid was Sst^- and Bar^-; this lack of complementation indicated that *bar1* and *sst1* are the same gene. A *MAT***a**/*MAT***a** *sst2-1/bar1-1* diploid was Sst^+ and Bar^+; this was expected if *sst2* and *bar1* are not the same locus and was anticipated from the fact that *sst2* and *sst1* are defects in different genes (4).

Three alleles of *sst1* differ in sensitivity to α factor. The relative sensitivity of *bar1-1*, *sst1-1*, and *sst1-2* to α factor are shown in Fig. 1. The

TABLE 1. Yeast strains

Strain	Genotype	Origin
Wild-type strains		
174-7-2	*MAT*α *ura1 trp1*	L. Hartwell
RC63	*MAT*α *thr4 tyr1*	This study
RC64	*MAT***a** *thr4 tyr1*	This study
RC347	*MAT***a** *ade1 ura1*	H242-7-3 × 174-7-2
RC620	*MAT***a** *rme ura1 leu2 met1 can1 cyh2*	Cross ×25 (4)
sst strains		
G130-D2-18b	*MAT***a** *bar1-1 ade2 ura3 met1 leu1 can1 cyh2 rme*	G. Sprague
H242-7-3	*MAT***a** *sst1-1 ade1 ura1*	L. Hartwell
RC346	*MAT***a** *sst1-1 ura1 trp1*	H242-7-3 × 174-7-2
RC664	*MAT***a** *sst1-2 rme ade2 his6 met1 can1 cyh2*	Cross ×39 (4)
RC687	*MAT***a** *sst2-4 rme ade2-1 ura1 his6 met1 can1 cyh2 GAL*	(4)
RC759	*MAT*α *sst2-1 rme ade2 his6 met1 can1 cyh2*	Cross ×40 (4)
RC821	*MAT***a** *sst2-1 rme ade2 his6 met1 can1 cyh2*	Cross ×49 (4)
RC840	*MAT*α *sst1-2 rme his6 met1 leu1 trp5 can1 cyh2*	Cross ×39 (4)
RC884	*MAT*α *sst2-3 rme ura1 his6 met1 leu1 trp5 can1 cyh2*	(4)
RC885	*MAT***a** *sst2-3 rme ade2 ura1 his6 met1 trp5 can1 cyh2*	Cross ×39 (4)
XMB4-12b	*MAT***a** *sst1-1 ilv3 ura1 arg9 his4*	L. Blair

strains can be ranked in the order of their sensitivity to α factor: *bar1-1* > *sst1-2* > *sst1-1* > wild type. Thus, of these three mutations, *bar1-1* appears to be the tightest and *sst1-1* appears to be the leakiest. It is interesting to note that these three alleles originated in different labs (4; Sprague and Herskowitz, in press; B. R. Reid and L. H. Hartwell, personal communication).

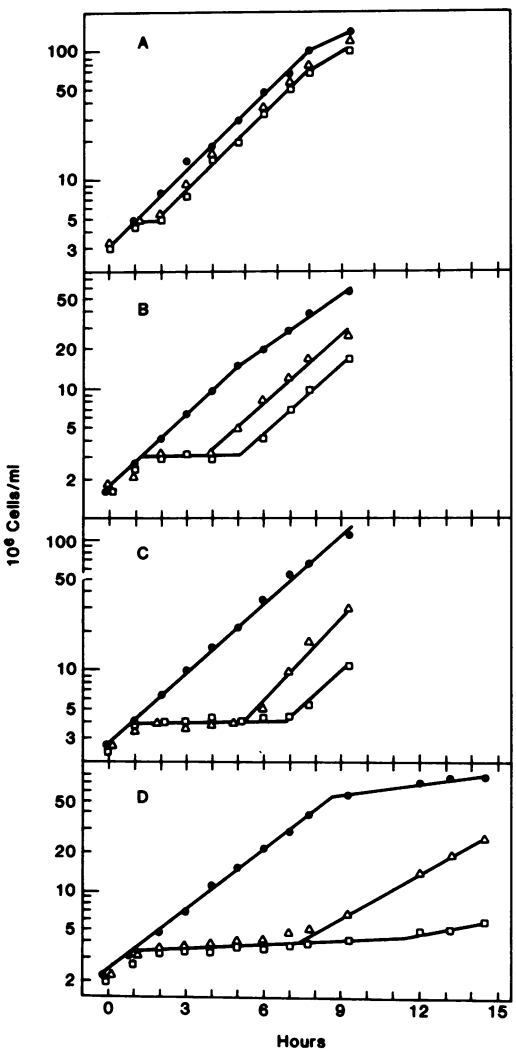

FIG. 1. Relative sensitivities of *SST*$^+$, *sst1-1*, *sst1-2*, and *bar1-1* to α factor. (A) RC618 (*MATa SST*$^+$), (B) XMB4-12b (*MATa sst1-1*), (C) RC629 (*MATa sst1-2*), and (D) G130-D2-18b (*MATa bar1-1*) were grown in YM-1, at pH 3.5 and 30°C to 2.5 × 10^6 cells per ml and divided into three flasks. Symbols: α factor was added to two of the flasks at final α factor dilutions of 1:10,000 (△) and 1:5,000 (□); no α factor added (●). At intervals, samples were removed for the determination of cell concentration with a Coulter Counter.

SST$^+$ cells can help *sst1* cells to recover from α factor arrest. Sst$^-$ cells take longer to recover from α factor arrest than wild-type cells (4). What happens if a mixture of wild-type cells and *sst* cells is treated with α factor? Such a mixing experiment is shown in Fig. 2. Figure 2C shows the effect when a mixture composed of 90% wild type and 10% *sst1-1* was treated with α factor. Figures 2A and B show the effect of α factor on each strain alone. Both strains in the mixture (Fig. 2C) showed the same pattern of recovery, which is intermediate to those shown in Fig. 2A and B. The reciprocal mixture is shown in Fig. 2D, where 90% of the cells were *sst1-1* and 10% of the cells were wild type. Here again the response was an intermediate one, but was delayed relative to the results in Fig. 2C. The validity of the recovery in Fig. 2D, which is based on a single time point at 10 h, was corroborated by an analogous experiment in which we observed the recovery of a mixture of *SST*$^+$ and *sst1-2* over the course of three time points (data not shown). These results show that wild-type cells can help *sst1* cells to recover from α factor arrest.

SST$^+$ cells cannot help *sst2* cells recover from α factor arrest. The mixing experiment was repeated with *sst2-1*. Figures 3A and B show the effect of treating each strain alone with α factor. Figure 3C shows the effect of α factor on a mixture composed of 90% wild type and 10% *sst2-1* cells. The wild-type cells recovered normally, but the *sst2-1* cells remained arrested. Although the data for *sst2-1* in Fig. 3C show variability, which we attribute to the low colony counts (1 to 14 pink colonies per plate) inherent in the experimental design, the same failure to recover in the presence of 90% *SST*$^+$ cells is suggested by a repetition of the experiment in Fig. 3C with the same strains and with a strain carrying the *sst2-3* allele (data not shown). A more dramatic demonstration is evident in Fig. 3D where the 10% minority of wild-type cells recovered even though the 90% majority of *sst2-1* cells remained arrested.

In one final experiment of this type, we found that *sst2-1* cells could help *sst1-2* cells recover from α factor arrest, but that *sst1-2* cells could not help *sst2-1* cells recover (data not shown).

Response of *sst* mutants to a pulse of α factor. The α factor activity disappears from the culture medium as *MATa* cells recover from α factor arrest (3). Other studies (5, 8, 14) demonstrate that this loss of activity is due, at least in part, to proteolytic cleavage of α factor. Thus, in the experiment shown in Fig. 3, the *sst2-1* cells remain arrested even though the wild-type cells in the mixture have presumably destroyed all of the α factor. This result implies that the continuous exposure to α factor needed to maintain G1

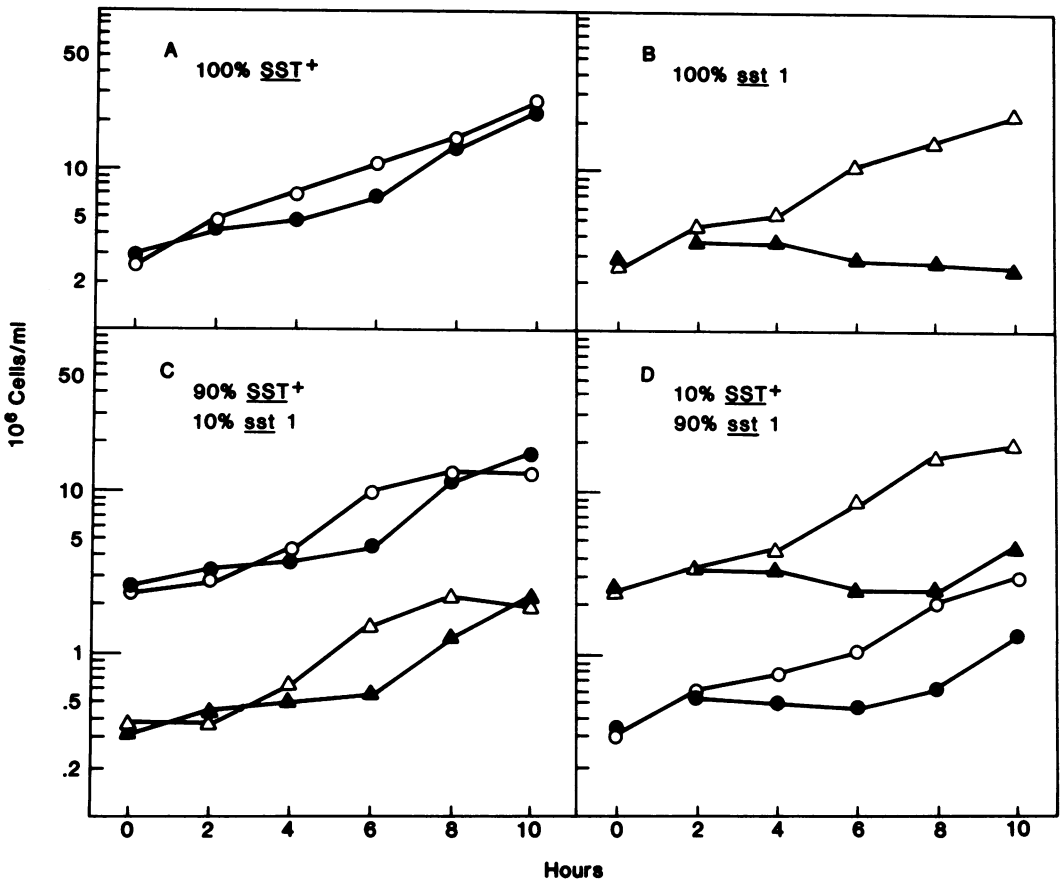

FIG. 2. Recovery of a mixed culture of *SST*$^+$ and *sst1-1* cells after treatment with α factor. Log-phase cultures of RC346 (*MAT***a** *sst1-1*) and RC347 (*MAT***a** *SST*$^+$ *ade1*) growing in synthetic medium at pH 3.5 and 23°C, were mixed in the proportions shown and divided into two flasks; α factor was added to one flask. Both flasks were then shaken at 23°C for 10 h. At 2-h intervals, samples were removed from each flask, sonicated (to break up clumps), diluted, and spread on YEPD plates for viable count. The colonies of RC346 *ADE*$^+$ and RC347 *ade1* could be distinguished on the same plate because Ade$^+$ colonies are white, but Ade$^-$ colonies are pink. In each panel, the growth curve as determined by viable count is plotted. Symbols: *SST*$^+$ untreated (○); *SST*$^+$ treated with α factor at a dilution of 1:10,000 (●); *sst1-1* untreated (△); *sst1-1* treated with α factor at a dilution of 1:10,000 (▲).

arrest of *SST*$^+$ cells is not necessary for *sst2-1* cells. This idea was tested directly by briefly exposing cells to α factor and examining their response.

In Fig. 4, wild-type, *sst1-2*, *sst2-1*, and *sst2-3* cells were "pulsed" with α factor for 1 h. As a control, α factor was not removed from a portion of the cultures. Figure 4A shows that, when the pheromone was not removed, the *SST*$^+$ cells were arrested as unbudded cells for only 1 h. When the α factor was washed out, the *SST*$^+$ cells were minimally affected by the previous 1-h exposure to the pheromone. In contrast, *sst1-2* cells were supersensitive to the continued presence of α factor and remained unbudded for 5 h (Fig. 4B). Strikingly, after the 1-h pulse, the *sst1* mutant did not remain arrested any longer than the normal cell control, suggesting that the washing procedure had effectively removed residual pheromone (Fig. 4B). The *sst2-1* and *sst2-3* mutants were particularly supersensitive to α factor (Fig. 4C and D) and did not recover from α factor arrest for the 8-h duration of the experiment if the α factor was not removed. Most importantly, after a 1-h pulse with α factor, the *sst2-1* and *sst2-3* cells remained arrested as unbudded cells for several hours (Fig. 4C and D), unlike either *SST*$^+$ or *sst1* cells. The leakiness of the *sst2-3* mutation (4) may account for its faster recovery relative to *sst2-1*.

These results show that a short pulse of α factor is sufficient to arrest *sst2* cells, and con-

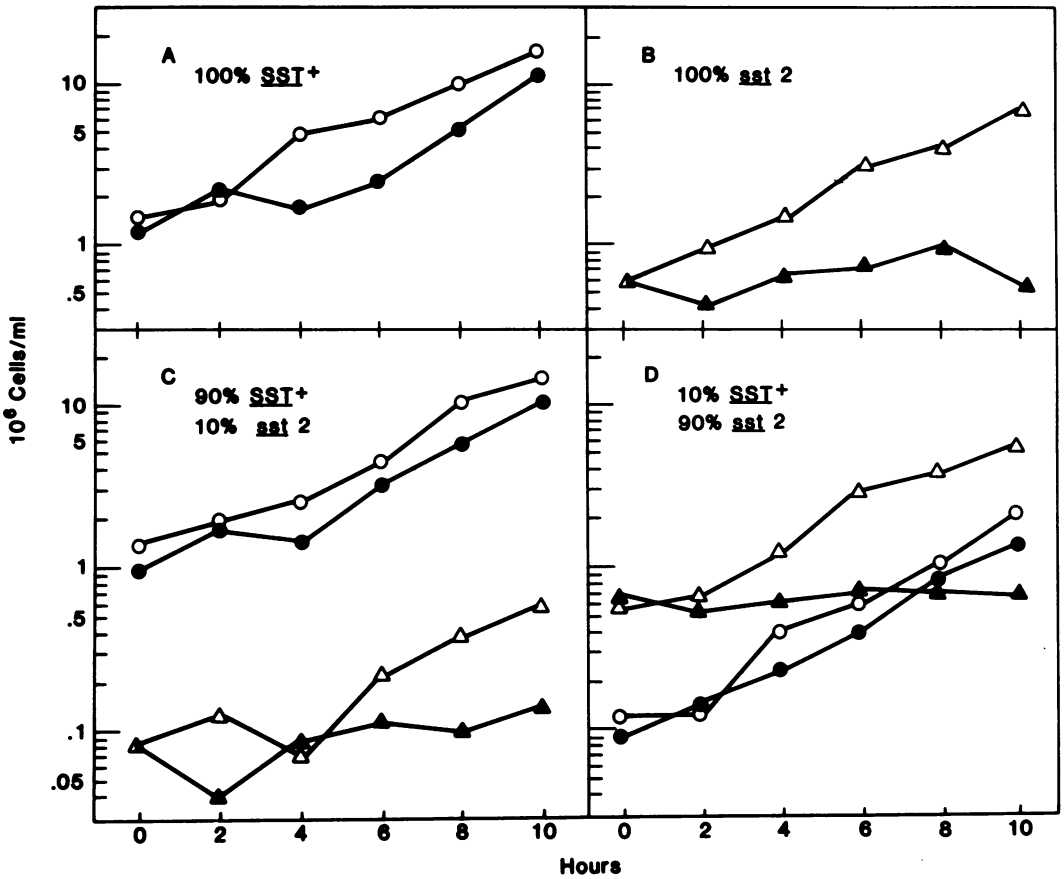

FIG. 3. Recovery of a mixed culture of *SST*$^+$ and *sst2-1* cells after treatment with α factor. Log-phase cultures of RC620 (*MATa SST*$^+$) and RC631 (*MATa sst2-1 ade2*) growing in YM-1 at pH 3.5 and 23°C were mixed and treated with α factor as described in Fig. 2. In each panel, the growth curve as determined by viable count was plotted. Symbols: *SST*$^+$ untreated (○); *SST*$^+$ treated with α factor at a dilution of 1:20,000 (●); *sst2-1* untreated (△); *sst2-1* treated with α factor at a dilution of 1:20,000 (▲).

tinued presence of α factor in the culture medium is not necessary for the maintenance of G1 arrest in *sst2* mutants.

***sst2-1* cultures accumulate sterile revertants.** Repeated serial subculture of *sst2-1*, but not *sst1-2* strains, led to the accumulation of revertants that were completely resistant to α factor, that were sterile, and that did not self-shmoo. The kinetics of accumulation of steriles is shown in Table 2 for a variety of *sst* strains. Since each serial transfer was diluted 10^{-5}, strains which accumulate steriles at a frequency of less than 10^{-5} would not be detected in our experiments. Note that no α factor was added to the culture medium in these experiments. Unlike *sst2-1*, *sst2-3* did not accumulate steriles. One difference between the strains that accumulated steriles (*sst2-1*) and those that did not (*sst1-2*, *sst2-3*) is that the strains that accumulated steriles also self-shmooed (4).

This property appears to be influenced by the *MAT* locus, since *MAT*α *sst2* strains accumulated steriles faster than *MATa sst2* strains (Table 2). We have also noticed that tetrads derived from the sporulation of MATa/MATα +/*sst2-1* diploids often yielded spores that grew very poorly. These small-colony-forming spores were invariably of the *MAT*α *sst2-1* genotype.

Mating efficiency of *sst* mutants. The mating efficiency of our *sst* mutants was quantitated using the filter-mating test of Hartwell (9). All *MATa sst1* strains mated with an efficiency of 0.03 to 0.24. In Fig. 1, we showed that the order of sensitivity of three alleles of *sst1* was *bar1-1* > *sst1-2* > *sst1-1*. It was interesting to note that the efficiency of mating of these three alleles paralleled their "leakiness." Thus, *sst1-1*, which is the leakiest of the three, mated best.

*MAT*α *sst1-2* mated normally, which was consistent with our observation in the preceding

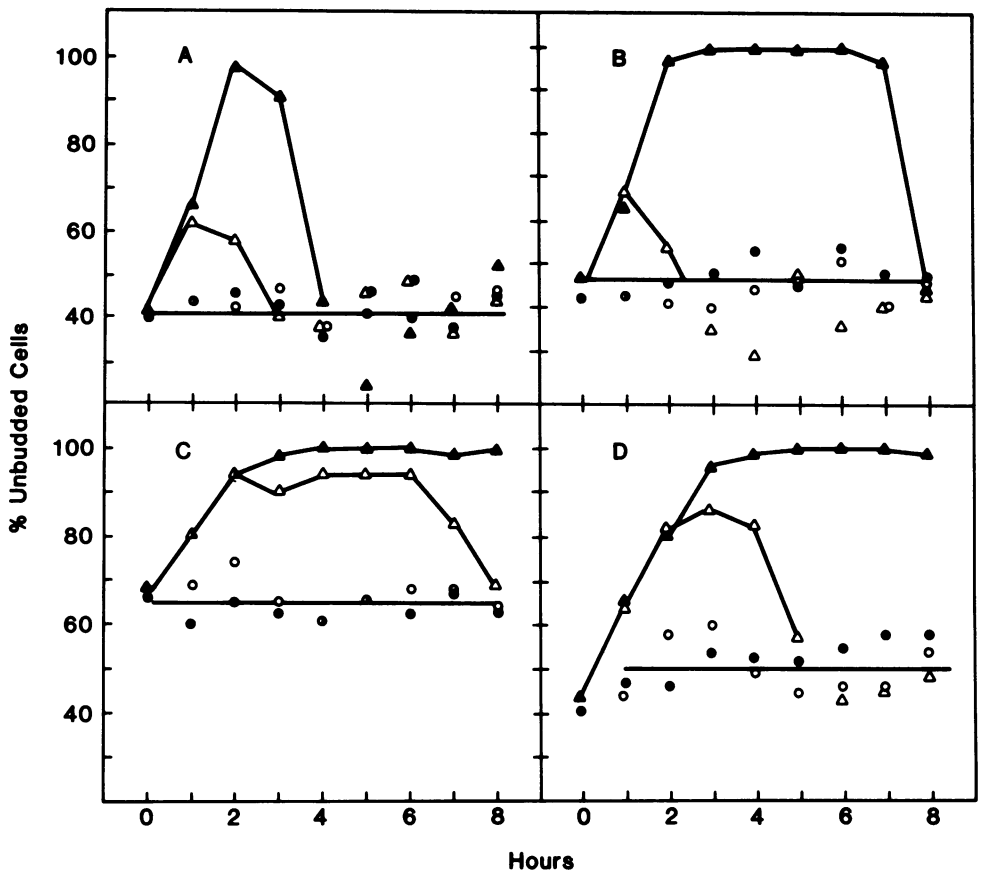

FIG. 4. Response of *SST*$^+$, *sst1-2*, *sst2-1*, and *sst2-3* to a "pulse" of α factor. (A) RC618 (*MATa SST*$^+$), (B) RC629 (*MATa sst1-2*), (C) RC631 (*MATa sst2-1*), and (D) RC639 (*MATa sst2-3*) were grown in YM-1 at pH 3.5 and 23°C to 2.5 × 10^6 cells per ml and divided into two cultures; α factor was added to one culture at a dilution of 1:5,000. After 1 h, portions of the control culture and of the treated culture were collected on membrane filters (Gelman GA-6, 25 mm, 0.45-μm pore size), washed three times with three volumes of fresh medium, and suspended in 1 volume of fresh medium. The percentage of unbudded cells was followed for 8 h. Symbols: Untreated cells (●); untreated cells washed after 1 h (○); α factor-treated cells (▲); α factor-treated cells washed after 1 h (△).

paper (4) that *sst1* is *MATa* specific (that is, *MATα sst1* cells are not supersensitive to a factor). Both *MATa sst2-1* and *MATα sst2-1* mated poorly—at the level of 0.03 to 0.11. This fact was also consistent with our previous observation that *sst2* affects both mating types (4).

Further correlations were found between the leakiness of a mutation and the efficiency of mating. *MATa sst2-3*, which is a leaky allele of *sst2*, mated at an efficiency of 0.91 compared to 0.11 for *sst2-1*. The *sst2-4 ochre* mutant mated with an efficiency of 0.16, but after suppression of the supersensitivity by an *ochre* suppressor (4) the mating efficiency was restored to a level of 0.67.

Production of mating factor. The streak-gap test was used to measure the production of mating factor by *sst* mutants. The three *sst* mutations (*1-2*, *2-1*, *2-3*) tested produced normal amounts of α factor in *MATα* strains and normal amounts of a factor in *MATa* strains. Two mutations (*1-2* and *2-1*) were tested for the production of the wrong mating factor. *MATa sst* did not make detectable amounts of α factor and *MATα sst* did not make detectable amounts of a factor.

DISCUSSION

The nature of *sst1*. Mutations in the *sst1* gene make *MATa* cells supersensitive to α factor, but have no detectable effect in *MATα* cells (4). Complementation and linkage tests indicated that the *sst1* gene (4) was identical to the *bar1* gene (Sprague and Herskowitz, in press). Like the *bar1-1* mutant, our *sst1* mutants were unable to act as a barrier to the diffusion of α factor on an agar slab (11).

Considerable evidence now exists for the spe-

cific proteolytic inactivation of α factor by *MAT***a** cells. Previously we showed that α factor activity disappears from the culture medium when *MAT***a** cells recover from α factor arrest (3). Using radiolabeled α factor, several groups (5, 8, 14) have shown that this loss of α factor activity is due to degradation rather than uptake into the cell. This degradative activity was cell associated, although, depending on the growth medium and conditions, degradative activity was sometimes found in the culture medium. It should be noted that the barrier effect is due in part to a diffusible product (11). Protease inhibitors which block the degradation of labeled α factor potentiate the biological activity of α factor (5, 8). Furthermore, Ciejek and Thorner (personal communication) have demonstrated that *MAT***a** *sst1* (or *bar1*) strains degrade α factor at a much reduced rate compared to wild-type *MAT***a** strains. These biochemical observations, together with the Bar$^-$ phenotype of the *sst1* mutants, suggest that the supersensitivity of *sst1* mutants is due to a defect in a diffusible or cell-bound protease that degrades α factor.

The results of our mixing experiment (Fig. 2) in which *SST*$^+$ cells were shown to help *sst1* cells recover from α factor arrest are also consistent with such a model and support the notion that the defect in *sst1* is external to the cell. In contrast, Hartwell (9) found that mutations in eight *ste* genes, isolated for their resistance to α factor, produced intrinsic or internal defects in mating. The mating defect in these *ste* mutants could not be corrected by the inclusion of *STE*$^+$ helper cells in the same culture. Since, as we have shown, the defect in *sst2* appears to be internal to the cell, the *sst1* mutation remains the only known mutation involved in the response to α factor that is correctable by extracellular complementation.

The nature of *sst2*. Unlike the *sst1* gene, the *sst2* gene is nonspecific in that *MAT***a** *sst2* strains are supersensitive to α factor and *MAT*α *sst2* strains are supersensitive to **a** factor (4).

In contrast to the results obtained with *sst1*, the mixing experiment (Fig. 3) shows that *SST*$^+$ cells could not help *sst2* cells recover from α factor arrest. Thus, even though an *SST*$^+$/*sst2* *MAT***a**/*MAT***a** diploid is Sst$^+$, indicating that the wild-type *SST*$^+$ function is *trans*-dominant to *sst2* inside the cell, our mixing experiment shows that the *sst2* defect is *cis*-dominant outside the cell. The *sst2* defect cannot be corrected from outside the cell, and we conclude that the defect in *sst2* mutants is internal. The concept of an internal defect is reinforced by the results of the pulse experiment (Fig. 4) in which we found that continuous exposure to α factor was not needed to induce cell cycle arrest by α factor in *sst2* mutants. This implies that the onset of G1 arrest in *sst2* mutants is irreversibly triggered by the initial exposure to α factor.

An assumption of the pulse experiment is that the filtration and wash procedure effectively removes α factor from the medium. This might not be true if the *sst2* mutation coded for a receptor with a higher affinity for α factor. Although we cannot rule out this possibility, a high affinity receptor for α factor would be hard to reconcile with the nonspecific nature of the *sst2* mutation, since one would not expect a single receptor to bind both mating factors.

The *sst2* mutation demonstrates that a function common to both *MAT***a** and *MAT*α is involved in mediating the response to mating factor. This is not unreasonable since both mating factors have similar effects. Furthermore, there is precedent from the *ste* mutations (9, 12, 13) that functions common to *MAT***a** and *MAT*α cells may be involved in mating.

Sterile revertants of *sst2*. The accumulation of sterile revertants in subcultures of *sst2-1* strains (Table 2) suggests that *sst2-1* cells have a growth disadvantage relative to Sst$^+$ cells. One possible explanation is that the sterile revertants do not waste energy forming shmoos. This is supported by the observation that *sst2-3*, the non-self-shmooing mutant, accumulated few sterile revertants (Table 2) compared to *sst2-1*. In addition, no sterile revertants were found in cultures of *sst1-2* which does not self-shmoo.

Table 2 also shows that *MAT*α *sst2-1* strains accumulated steriles faster than *MAT***a** *sst2-1* strains. Why the *sst2-1* mutation would be more deleterious in a *MAT*α strain than in a *MAT***a** strain is not presently understood.

Mating efficiency of *sst* mutants. With the exception of *sst2-3*, the *sst* mutants mated with an efficiency of 0.03 to 0.24 in the filter assay. In addition, there was a correlation between the leakiness of three *sst1* alleles (Fig. 1) and their efficiency of mating which may be useful in quantitating the supersensitivity of a given mutant.

This decreased mating efficiency may be an artifact of our mating method. In the filter mating procedure, 2×10^6 cells of each mating type are mixed and incubated on membrane filters for 6 h at 30°C before resuspension and plating on selective plates. If any division occurs before cells adhere and fuse, a differential increase in the number of one parent over the other is likely to affect the absolute level of mating. Such a differential increase is expected, since the *sst* strain is supersensitive to G1 arrest by the hormone produced by the *SST*$^+$ tester strain of the opposite mating type. Indeed, Sena et al. (16) have found that an excess of α factor inhibits mating. This possibility could be tested by measuring the efficiency of cell-cell matings

TABLE 2. Percentage of steriles after subcultures[a]

Strain	Genotype	27[b,c]	43[b,d]	59[b,d]	75[b,d]	91[b,d]
RC618	*MAT***a** *SST*$^+$	<0.2				<0.3
RC629	*MAT***a** *sst1-2*	<0.4				<0.3
RC664	*MAT***a** *sst1-2*	<0.4				<0.4
RC631	*MAT***a** *sst2-1*	<0.7	3	0.8	4	26
RC821	*MAT***a** *sst2-1*	<0.7	3	7	11	21
RC639	*MAT***a** *sst2-3*	<0.6				<0.4
RC885	*MAT***a** *sst2-3*	<0.3				<0.4
RC622	*MAT*α *SST*$^+$	<0.2				<0.4
RC844	*MAT*α *sst1-2*	<0.4				<0.4
RC757	*MAT*α *sst2-1*	<0.6	<1.6	10	40	70
RC759	*MAT*α *sst2-1*	<0.9	84	96	100	100
RC884	*MAT*α *sst2-3*	<0.6				0.5

[a] Each strain was serially subcultured for the indicated number of generations; then 50 to 400 colonies were tested for the ability to mate as described in the text.

[b] Approximate generations of subculture.

[c] An isolated colony was inoculated into 5 ml of YM-1 and grown at 30°C to stationary phase (1×10^8 to 2×10^8 cells per ml).

[d] The preceding culture was diluted 10^{-5} into fresh YM-1 and grown at 30°C to stationary phase.

or by testing the effect of exogenously added α factor in control matings.

It should be noted, however, that the mating-defective *ste* mutants isolated by others (9, 12, 13) display reductions in mating efficiency of 10^{-4} to 10^{-6}. Therefore, our *sst* mutants are only weakly mating deficient in comparison.

Regulation of *sst1* and *sst2*. Since *sst1* and *bar1* are the same gene, the method by which Sprague and Herskowitz (in press) isolated the *bar1* mutant is clearly relevant for understanding how *sst1* is regulated. The *bar1* mutation was isolated because it allowed the detectable production of α factor by a *matα2* strain (*matα2* is a mutation in the *α2* gene of the *MAT*α mating type locus). According to the *α1*-*α2* model for the regulation of **a**- and α-specific mating functions by the mating type locus (10), *α2* codes for a negative regulator of **a**-specific functions such as *BAR1*. Thus, *matα2* strains make α factor, but also destroy it, since the *BAR*$^+$ function is not turned off. The isolation of a mutation which allows *matα2* mutants to secrete α factor and which causes **a** cells to be Bar$^-$ supports this view and suggests that *SST1*/*BAR1* function is normally not expressed in *MAT*α strains due to the action of the *MATα2* gene product.

The absence of *sst2* function in *MAT***a** or *MAT*α cells makes them supersensitive to mating factor. However, the absence of *sst2* function in a *MAT***a**/*MAT*α diploid does not make the cell sensitive to mating factor. This could be explained by two possibilities: (i) a *MAT***a**/*MAT*α diploid normally does not have any *sst2* product present (because its synthesis is specifically repressed); (ii) *sst2* product is present, but cannot function in the cytoplasm of a *MAT***a**/*MAT*α cell (i.e., the nonmating state of a *MAT***a**/*MAT*α diploid is epistatic to the *sst2* function). For example *sst2* function may depend on the presence of receptors for α factor, but if the receptors are not made in a *MAT***a**/*MAT*α diploid, the presence of *sst2* is irrelevant.

Mechanism of self-shmooing. *sst2* mutants may self-shmoo because they are constitutive for functions which are normally induced by mating factor. Since α factor also induces sexual agglutination in *MAT***a** cells (1, 6, 7, 17), it should be possible to test *sst2 MAT***a** cells for constitutive expression of agglutinability. Unfortunately, RC618, the wild-type parent of our *sst* mutants is not very inducible for agglutination (data not shown). It will be necessary to cross the *sst2* mutations into a background with high inducibility before this question can be answered.

Another explanation for self-shmooing is that *sst2* mutants might make the wrong mating factor. This possibility was examined by looking for the production of α factor by *MAT***a** *sst2* cells and for the production of **a** factor by *MAT*α *sst2* cells. We did not detect secretion of the wrong mating factor. However, if the wrong mating factor functioned without being excreted into the medium, we would not have detected it.

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