Pheromonal Regulation and Sequence of the *Saccharomyces cerevisiae* SST2 Gene: a Model for Desensitization to Pheromone

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Strains of both haploid mating types containing *sst2* mutations are altered in response to pheromone; *MATa sst2* cells are supersensitive to α-factor, and *MATα sst2* cells are supersensitive to a-factor. This phenotype suggests that *SST2* encodes a component of the pheromone response pathway that is common to both mating types. We have cloned the *SST2* gene by isolation of multicopy plasmids that complement the *sst2*-1 mutation. One such plasmid contained a 4.5-kilobase *HindIII* fragment that was able to complement the *sst2*-1 mutation in high or low copy number, integrated at the *SST2* locus, and resulted in an *sst2* phenotype when disrupted, indicating that this fragment contained the *SST2* gene. We identified the functional region of the complementing DNA fragment by transposon mutagenesis. Sequencing of this fragment identified an open reading frame encoding 698 amino acids at a position that correlated well with the functional region. Expression of an Sst2-β-galactosidase fusion was haploid specific and induced by exposure to pheromone. We discuss a model in which induction of the *SST2* product results in inhibition of a component of the pheromone response pathway, resulting in desensitization to pheromone.

Each of the haploid mating types of the yeast *Saccharomyces cerevisiae* secretes a peptide pheromone; *MATα* cells secrete α-factor, and *MATα* cells secrete α-factor (reviewed in references 31 and 57). The pheromones induce several responses in cells of the opposite mating type, including arrest in the G1 phase of the cell cycle, cell wall changes, and morphological changes often called shmooing. A number of mating-type-specific gene products are induced by exposure to pheromone (3, 30, 47, 51, 56). α-Factor increases the level of expression of α-agglutinin in *MATα* cells, and α-factor increases the level of expression of α-agglutinin, a-factor, and barrier (a function involved in the degradation of α-factor) in *MATα* cells. Also, the RNA level of a number of genes, including the α- and α-factor receptor genes, is stimulated by exposure to the opposite pheromone (16, 17, 38). Mutants defective in pheromone production or pheromone response are defective in mating (19, 26, 28, 32).

Response to pheromone requires the presence of mating-type-specific receptors. Recent evidence indicates that the *STE2* gene encodes the α-factor receptor (5, 21, 22, 38) and the *STE3* gene encodes the α-factor receptor (1, 15). The two receptors are not homologous but do have similar structural features, including seven putative transmembrane domains and a hydrophilic carboxy terminus. The initial steps of α-factor response in *MATα* cells have been characterized recently (9, 24). After exposure of cells to α-factor, there is a loss of surface receptors (termed downregulation), as indicated by a loss of α-factor-binding sites. Concomitant with the downregulation of receptor, α-factor is internalized and degraded. These steps do not require protein synthesis and are defective in endocytosis mutants, suggesting that they occur by receptor-mediated endocytosis. Following downregulation, the cells reaccumulate α-factor receptor; protein synthesis is required for reaccumulation. The induction of *STE2* expression by exposure to α-factor is likely to be important for reaccumulation.

After the initial period of response to pheromone, cells are able to recover from cell cycle arrest. In *MATα* cells, this recovery includes two components (36). The first component involves degradation of α-factor by the barrier activity (10, 29, 54). Mutations in the *BAR1/SST1* gene result in a defect in α-factor degradation and supersensitivity of *MATα* cells to α-factor (7, 8, 50). The second component can allow recovery even in the absence of α-factor degradation and involves desensitization or adaptation of the cells to α-factor (24). Desensitized cells have reaccumulated α-factor receptor, as indicated by the ability to bind α-factor, but are unable to respond to α-factor (24, 36). No function able to degrade α-factor has been detected in *MATα* cells, and therefore recovery from α-factor arrest may occur by the desensitization process alone.

Recently, Bender and Sprague (1) have shown that the α- and α-factor receptor-pheromone interactions are interchangeable. For example, a *MATα* strain which produces α-factor receptor rather than α-factor receptor responds to α-factor in a manner similar to the usual α-factor response. These results indicate that the pheromone response pathways in the two mating types converge at a point after the pheromone-receptor interaction. However, the intracellular steps of this common pathway have not been characterized. (An early report that adenylate cyclase was inhibited in *MATα* cells by exposure to α-factor [27] has not been repeatable [6].) A possible candidate for a gene encoding a component of the common response pathway is the *SST2* gene. *MATα sst2* cells are supersensitive to α-factor, and *MATα sst2* cells are supersensitive to a-factor (7, 8). The *sst2* defect cannot be corrected by the presence of *SST2* cells, indicating that the *SST2* function is intrinsic to the cell. *MATα sst2*-1 cells arrest in G1 after exposure to 2 orders of magnitude less α-factor than necessary to arrest *MATα* *SST2* cells. Although *MATα sst2*-1 cells are able to degrade α-factor, they are defective in recovery from pheromone arrest, suggesting that they may be defective in the desensitization process. The effect of *sst2* mutations in *MATα* cells is less well characterized, but seems to be similar with regard to α-factor response and recovery.

Because the *SST2* product (Sst2) may be a component of the pheromone response or recovery pathway, we cloned...
and sequenced the SST2 gene. Analysis of disruption mutants indicated that the supersensitive sst2-1 phenotype corresponds to the null phenotype. Analysis of an Sst2-β-galactosidase fusion protein showed that Sst2 was induced by exposure to pheromone and that Sst2 expression was haploid specific. Based on these results and the phenotype of the sst2 mutants, we have proposed a model for the role of Sst2 in desensitization to pheromone.

MATERIALS AND METHODS

Strains and plasmids. S. cerevisiae strains are listed in Table 1. Escherichia coli RR1 was used for plasmid analysis and subcloning. Plasmids used include the 2μm LEU2 plasmids YEp13 (4) and YEp351 (19), the URA3-containing centromere plasmid YCP50 (41), pBR322-MATa (39), and pHS66 (45, 46), which contains the E. coli kanamycin resistance gene and was used for transposon mutagenesis.

Media. The media used have been described previously (26), except that the media used for transposon mutagenesis were as described by Seifert et al. (45, 46), and X-Gal (isopropyl-β-D-thiogalactopyranoside) plates were as described by Ruby et al. (43).

Pheromone sensitivity and mating assays. Sensitivity to pheromone was assayed by spreading a thin lawn or streak of the cells to be tested on BBM plates (YEPD plates containing 0.003% methylene blue and buffered to pH 4.8) and spotting 2 μl of α-factor (100 or 10 μg/ml; Sigma Chemical Co.) or partially purified α-factor (15 U/ml; provided by P. Lipke) on the lawn or streak. After 1 and 2 days of growth, the plates were scored for the presence or absence of a clear area within the lawn or streak at the position of the pheromone spot. Mating was tested by a standard prototroph formation assay, as described previously (25).

Screening transformants for resistance to pheromone. Strain D89-4B was transformed with a yeast clone bank on YEp13 (39), and approximately 15,000 transformants were pooled in four independent batches. Each of the four pools was plated on 10 MAC plates (synthetic dropout plates lacking leucine buffered to pH 4.8) containing 5 nM α-factor at a density of about 2,500 cells per plate. α-Factor-resistant transformants were analyzed as described in the Results.

Determination of functional region. pb3 was partially cleaved with HindIII or ClaI, religated, and transfected into E. coli RR1. Transformants resulting from partial ClaI digestion were screened for complementation of the E. coli leuB mutation, and Leu+ transformants were tested further. Plasmid DNA was restriction mapped to determine which insert fragments were present and, in the case of the CIA deletions, for the presence of the 2.6-kilobase (kb) 2μm ClaI fragment. The 4.5-kb HindIII fragment was subcloned into HindIII-cleaved YEp351 and YCP50, and the 6.7-kb EcoRI-SalI fragment was subcloned into EcoRI-Sall-cleaved YCP50 (Fig. 1). Strains D89-4B and D89-8A were transformed with the resulting plasmids and tested for complementation of the sst2-1 mutation by pheromone spotting.

Mapping. To map the pb3 insert with respect to the URA4 gene, the ura3-52 gene of W303-1B was first replaced with the URA3 gene by transformation with a HindIII fragment containing the URA3 gene and selection for Ura+ transformants. A plasmid was derived from the CIA deletions described above that had lost the 2μm-containing ClaI fragment and a ClaI plasmid-insert junction fragment, but had
retained a unique HindIII site within the insert. The plasmid was targeted to integrate at its chromosomal site in W303-1B (Ura·) (40). This strain was crossed into strains XS209-11C and D89-4B, and tetrads were analyzed to map the insert with respect to the URA4 and SST2 genes.

Transposon mutagenesis and gene replacements. The 4.5-kb HindIII fragment containing the SST2 gene was subcloned into pHS66, and transposon mutagenesis with mini-Tn3 transposons was done in E. coli as described by Seifert et al. (45, 46), except that conjugations were done by replica plating. Three mini-Tn3 transposons were used. One contained the URA3 gene; the second contained both URA3 and a version of the E. coli lacZ gene lacking the first eight codons; and the third contained both the LEU2 gene and GAL1,10 promoter. The purpose of using the latter two transposons was to obtain fusions of SST2 coding sequence to lacZ or fusions that placed SST2 under the control of the GAL1,10 promoter. Plasmids arising from independent transposition events were restriction mapped to determine the position of the transposon. Genes replacements were done by cleaving the plasmids with XbaI and Clai (or Clai partial restriction), transforming them into strains W303, W303-1A, and W303-1B, and selecting Ura+ or Leu+ transformants (42). After initial gene replacements showed that the sst2 disruption strains were viable, subsequent replacements were done in the haploid strains.

DNA sequencing. The 4.5-kb HindIII fragment was subcloned into the M13 phage mp11 in both orientations, and the 1.7- and 2.8-kb Clai-HindIII fragments were subcloned into mp11 (33). Nested deletions in both orientations were made in the single-stranded phage DNA by the method of Dale et al. (12) and were sequenced by the dideoxy sequencing method (44).

β-Galactosidase assays. Plate assays were done by replica plating to X-gal plates with or without α-factor. For quantitative β-galactosidase assays, cells were grown overnight to an optical density at 600 nm (OD600) of approximately 1.00 in selective medium. For induction experiments, cultures were split, and 0.5 μM α-factor or 0.6 U of α-factor per ml was added to one tube. At various time points, 1 ml of sample was removed and assayed for β-galactosidase activity. Cells were spun down and suspended in 2 ml of Z buffer (35). One milliliter was used for an OD600 estimation of cell density. The remaining cells were assayed for O-nitrophenyl-β-D-galactoside-hydrolyzing activity as described by Miller (35). Units were calculated as follows: units = 1,000 × OD420/OD600, where t is the time in minutes.

Strain construction for pheromone induction assays. The MATα sst2::lacZ229[URA3] strain was crossed to W303-1B to produce a MATα/MATα SST2/sst2::lacZ229[URA3] diploid. This diploid was cotransformed with YEp13 and a HindIII fragment containing MATα, and Leu+ transformants were selected and screened for the ability to mate as cells. The resulting strain should be MATα/MATα SST2/sst2::lacZ229[URA3].

Construction of isogenic SST2, sst2-1, and sst2::URA3 strains. The sst2-1 gene in MATα strain D89-4B was replaced by transformation with fragments containing mini-Tn3-URA3 insertions into the 4.5-kb HindIII fragment (described above). We constructed sst2 null mutants by gene replacement with mini-Tn3 insertions, which gave rise to an sst2- phenotype in W303-1A and mapped to the region of the SST2 open reading frame. Isogenic SST2 strains were constructed by using mini-Tn3 insertions which gave rise to an sst2+ phenotype in W303-1A and mapped well outside of the SST2 open reading frame. In each case, Ura+ transformants were isolated and tested for pheromone sensitivity, and gene replacement was confirmed by Southern analysis (49).

RESULTS

Isolation of the SST2 gene. Our scheme for cloning the SST2 gene by complementation made use of the higher level of resistance of Sst2+ cells than of Sst2- cells to α-factor. At an α-factor concentration of 5 nM, colony formation by the MATα sst2-1 strain but not by the MATα SST2 strain was inhibited (Table 2). This concentration was therefore used to select for (or enrich for) MATα sst2-1 transformants containing the SST2 gene on a plasmid.

MATα and MATα leu2 sst2-1 strains (D89-4B and D89-8A, respectively) were constructed, and the MATα sst2-1 strain was transformed with a yeast clone bank on the multicopy plasmid YEp13 (39), as described in Materials and Methods. α-Factor-resistant colonies were isolated and tested for mating to eliminate two classes of false-positives that should be unable to mate. The first class would be transformants that contained the MATα locus and would be unable to respond to pheromone due to the presence of both MATα and MATα. The second class would arise due to the tendency of sst2 strains to produce sterile α-factor-resistant mutants (8). Of 134 α-factor-resistant transformants, 93 were able to mate as a cells.

These transformants were then tested for loss of the supersensitive phenotype of sst2 by a variation of the "halo" assay described in Materials and Methods. The transformants showed various levels of complementation of the sst2-1 mutation by this assay. Three transformants, A2-3, B3, and C3, showed a wild-type or close to wild-type phenotype and were analyzed further. This phenotype was plasmid dependent; Leu+ segregants were supersensitive. As a final test of the plasmid-borne nature of the Sst+ phenotype, DNA was isolated from these three transformants, and plasmid DNA purified in E. coli was used to transform MATα sst2-1 and MATα sst2-1/strains. The three plasmids (called pA2-3, pB3, and pC3) were able to complement the sst2-1 mutation in both MATα and MATα strains, as would be expected of a plasmid containing SST2.

Restriction mapping indicated that pA2-3 and pB3 (which had been isolated from independent transformations) were identical and that pC3 contained a nonoverlapping insert (confirmed by hybridization; data not shown). Further analysis of the SCG1 gene encoded by pC3 is presented elsewhere (12a). The restriction map of pB3 is shown in Fig. 1.

To determine whether pB3 contained the SST2 gene, an easily scorables marker (LEU2) was integrated into the genomic site homologous to the pB3 insert and mapped by methods described in Materials and Methods. The integratable plasmid was obtained by isolating a deletion derivative of pB3 that lacked the 2μm sequences of YEp13 but retained the LEU2 gene and a portion of the original insert. This plasmid was targeted to integrate (in a Ura+ derivative of strain W303-1B) at the genomic site homologous to the insert by
cleavage at a unique HindIII site within the insert sequences (40). Southern analysis indicated that the shift in restriction fragment size expected for such an integration had occurred (data not shown). This strain was crossed to strains carrying ura4 (XS209-11c) and sst2-1 (D89-4B), and tetrat analysis was done (Table 3). The integrated LEU2 marker mapped 24 centimorgans from URA4 on the right arm of chromosome XII, consistent with the previously determined map position for SST2 (37). All 15 tetrads derived from the SST2 [LEU2]sst2-1 strain showed a 2 Leu+ Sst+:2 Leu− Sst− segregation pattern (Table 3); therefore, the integrated LEU2 gene mapped to SST2, suggesting that pB3 contains the SST2 gene.

**Determination of functional region and construction of null mutants.** We defined the region of the pB3 insert required for complementation of sst2-1 by deletion analysis of pB3 and subcloning fragments of the pB3 insert to multicopy and centromere plasmids as described in Materials and Methods. As diagrammed in Fig. 1, a 4.5-kb HindIII fragment was sufficient for complementation on either a multicopy or a centromere plasmid. Transposon mutagenesis allowed further definition of the functional region as well as construction of disruption mutants containing a large insert within the SST2 functional region (45, 46). We isolated and restriction mapped mini-Tn3 insertions into the 4.5-kb HindIII SST2 fragment as described in Materials and Methods. Three different mini-Tn3’s were used; one contained the URA3 gene, a second contained the URA3 gene plus the E. coli lacZ gene lacking the first eight codons to allow isolation of SST2-β-galactosidase fusions, and the third contained the LEU2 gene and GAL1,10 promoter.

Gene replacements were made with fragments containing mini-Tn3’s at positions throughout the 4.5-kb HindIII fragment and tested for SST2 function. Inserts at either end of the 4.5-kb HindIII fragment did not affect SST2 function, but inserts extending over an internal region of approximately 2.2 kb resulted in a phenotype similar to that of the original sst2-1 strain (Fig. 1). Because inserts throughout most of this region resulted in the same phenotype, we conclude that these disruptions represent null mutations. Some of the insertions towards the left end of this region resulted in an intermediate phenotype. These inserts were intermingled with inserts which gave a complete Sst2+ phenotype. Such a pattern might be expected for insertions into the promoter region. One insert resulted in an Sst2− phenotype and also showed β-galactosidase activity after exposure to pheromone (sst2::lacZ29[URA3]), as described further below. The orientation of this insert indicated that the SST2 gene was expressed from left to right, consistent with the suggestion that the promoter is towards the left end of the functional region.

Several transposon insertions were used to make gene replacements in the MATα sst2-1 strain (D89-4B) to produce isogenic SST2 and sst2::URA3 (null mutant) strains as described in Materials and Methods. The phenotype of the null mutants was similar to the phenotype of the original sst2-1 mutant. The labeled SST2 fragment hybridized only to itself in Southern analysis of total yeast DNA, even under low-stringency hybridization conditions, indicating that the SST2 gene is unique (data not shown).

**Induction of SST2 by pheromone.** The MATα sst2:: lacZ29[URA3] fusion strain showed no β-galactosidase activity on X-gal plates in the absence of α-factor. A dark blue color was seen on X-gal plates containing 0.5 μM α-factor, a concentration of α-factor inhibitory to growth of the sst2::lacZ29[URA3] strain, indicating that expression of SST2 was induced by pheromone. β-Galactosidase assays showed undetectable expression in an αα diploid, baseline basal expression in MATα and MATα haploids, and approximately 50-fold induction after exposure to the opposite pheromone (Table 4). Because the sst2::lacZ29[URA3] replacement resulted in an Sst2− phenotype, these experiments were investigating induction by pheromone in a strain that was abnormal in pheromone response. To investigate regulation of the Sst2-LacZ fusion in an a Sst2− strain, a MATα/MATα SST2/ssst2::lacZ29[URA3] strain was constructed as described in Materials and Methods. The basal level of β-galactosidase activity was about fivefold lower in the MATα/MATα SST2/ssst2::lacZ strain (Sst2+) than in the MATα sst2::lacZ strain (Sst2−). After exposure to α-factor, both the Sst2+ and Sst2− strains showed 50- to 70-fold induction of β-galactosidase activity (Fig. 2). The effect of pheromone on RNA levels is being tested to further define the level at which induction occurs.

**Sequencing of SST2.** A set of nested deletions in both orientations was constructed in the M13 phage mp11 containing the 4.5-kb HindIII fragment (Fig. 1), as described in Materials and Methods, and sequenced by the dyeode terminator method. Both strands of most of the 4.5-kb fragment were sequenced. Only one strand was sequenced from regions −314 to −617 upstream of the putative initiation codon and 543 to 728 nucleotides downstream of the putative termination codon. There was an open reading frame encoding 698 amino acids extending from left to right, beginning with an ATG codon at nucleotide 1 and ending...
with a TAA codon at nucleotide 2095 (Fig. 3). There were several in-frame termination codons just upstream of this ATG codon, so this was the first possible initiation codon. This ATG codon had an A at position -3 but did not have a T at position +6, as do many initiation codons in S. cerevisiae (13). The next in-frame ATG codon was at nucleotide 226 and contained neither an A at position -3 nor a T at position +6. The orientations of the open reading frame and the β-galactosidase gene fusion obtained by transposon mutagenesis were the same. The Sst2-β-galactosidase fusion contained the first 225 codons of the SST2 open reading frame followed by 16 codons from transposon sequences, fused to the ninth codons of β-galactosidase. No other long open reading frames were present within the functional region defined by transposon mutagenesis. No splicing consensus sequences were present in this region (55), indicating that the SST2 gene does not contain an intron. We feel confident that the 698-amino-acid open reading frame corresponds to the SST2 gene product, based on the close correlation between the positions of the functional region and the open reading frame and on the similarity in the phenotype of mutants with transposon insertions in this region to the original sst2 mutants.

The presumptive Sst2 protein was highly hydrophilic, containing 11% acidic, 16% basic, and 27% polar residues distributed throughout the protein sequence. A homology search of the Claverie protein database (11) showed no significant homology of the presumptive Sst2 protein to any other protein. The usage of preferred codons (2, 20) within the 698-amino-acid open reading frame was very low (data not shown). This low usage of preferred codons may reflect the correlation between the level of expression and the frequency of preferred codon usage (2, 20), given the low basal expression level of SST2 shown by β-galactosidase assays.

The 5' flanking region contained sequences with homology to TATA boxes at positions -162 (TATATTTA and -22 (TAAATTTA) relative to the presumptive start codon (Fig. 3). The latter sequence was too close to the ATG codon to be likely to be functional, assuming that our identification of the initiation codon is correct. The upstream region had an unusually high proportion of G residues in the coding strand for yeast genes; 25% of positions -1 to -100 were G. Several sequences similar to the consensus sequence implicated in negative a1a2 control in diploids (34) and in pheromonal induction in haploids (58, 59) were present in the upstream region. The region downstream of the termination codon at position 2095 contained sequences similar to sequences in the 3' flanking regions of other eucaryotic genes. There was an AATAAA sequence at position 2275. This sequence has been shown to be necessary for polyadenylation in higher eucaryotic systems but is not present in many yeast genes. The sequence TAG(14 nucleotides)TAGTGT was found beginning at nucleotide 2160. A similar sequence is present 3' of many yeast genes and has been implicated in transcription termination in S. cerevisiae (61).

DISCUSSION

Structure and regulation of SST2. We have isolated and sequenced a 4.5-kb HindIII fragment that is able to complement the sst2-1 mutation (7, 8) in both MATα and MATα cells (on either multicopy or centromere plasmids), integrates at the SST2 locus, and gives rise to an Sst2+ phenotype when disrupted within a region containing a 698-amino-acid open reading frame. These results indicate that the open reading frame corresponds to the SST2 gene product. The putative Sst2 protein is highly hydrophilic, containing 17% charged and 27% polar residues. No clearly significant homology was seen between the putative Sst2 protein and any sequence in a protein sequence database (11).

Sst2 was expressed at a low basal level in MATα and MATα cells but was not expressed in MATα/MATα cells (Table 4); therefore, it is a haploid-specific gene product. In α and α cells, exposure to pheromone resulted in about a 50-fold induction of Sst2 levels, as determined by β-galactosidase assays of an sst2::lacZ fusion. In MATα cells, Sst2 was induced by α-factor, and in MATα cells it was induced by α-factor. This induction of Sst2 is likely to be important in its role in the pheromone response pathway, as described below.

Sst2+ (α/α) and Sst2- (α) strains showed a difference in Sst2-β-galactosidase expression (Fig. 2). Both the basal and induced levels were 5- to 10-fold lower in an SST2/sst2::lacZ diploid (Sst2+) than in an isogenic sst2::lacZ haploid (Sst2-). Part of this difference may be due to the fact that sst2::lacZ expression represented only one-half of the total SST2 expression in the heterozygous diploid; however, the remaining severalfold difference probably represents an actual difference between the two strains.

Recent results indicate that one sst2 phenotype, called self-shmooining, is due to a low level of the opposite mating type pheromone in the culture medium, most likely due to rare heterothallic switches (24). Our basal measurements of Sst2-β-galactosidase activity in α strains reflected the level of Sst2 expression in the presence of this very low level of α-factor; therefore, our observed basal β-galactosidase levels may differ from the level of expression in the complete absence of α-factor. Similar to our results with Sst2, a higher basal level of expression of the pheromone-inducible sigma element in an Sst2- strain than in an Sst2+ strain has been reported (59). In this case, the elevated basal level in the Sst2- strain was reported to be due to the low level of endogenous pheromone. After addition of exogenous α-factor, the low level of endogenous α-factor would be insignificant; thus, our measurements of the induced level of β-galactosidase
activity reflect a real difference between the Sst2+ and Sst2− strains.

The interpretation of our observed difference in Sst2 expression between Sst2+ and Sst2− strains depends on how our measured basal level of β-galactosidase activity compares with the level of expression in the absence of the low level of the opposite mating type pheromone. One possibility is that the actual basal level of expression in the complete absence of α-factor is similar in Sst2+ and Sst2− strains. In this case, our observed difference in basal expression would indicate that the low level of endogenous α-factor is sufficient for induction of Sst2 in the Sst2− but not the Sst2+ strain. The difference in induced expression levels in comparison with identical basal expression levels, therefore, would indicate that the magnitude of induction of Sst2 is greater in the Sst2+ than in the Sst2− strain. An alternative possibility is that the basal level of Sst2 expression in the complete absence of α-factor is similar to our observed basal measurements, i.e., that the basal expression level is severalfold higher in Sst2+ than in Sst2− strains. In this case, the level of induction by pheromone is similar in the two strains, but Sst2 expression is severalfold higher in Sst2+ than in Sst2− strains in both the presence and absence of pheromone. For both of these possibilities (which probably represent the most extreme interpretations), there is a difference in Sst2 expression in Sst2− and Sst2+ strains, indicating that Sst2 regulates its own expression.

We believe that this “autoregulation” of Sst2 expression is indirect. The induction of Sst2 by pheromone indicates that it can be added to the list of genes and gene products that are induced by pheromone (3, 16, 17, 30, 32, 38, 47, 51, 56, 59), most of which are involved in mating or pheromone response or both. This induction is only one of several responses to pheromone. Because Sst2 is likely to play an important role in the modulation and desensitization of the pheromone response pathway, as discussed below, an Sst2− strain is likely to be altered in several aspects of pheromone response, including the expression of pheromone-inducible gene products. In Sst2− strains, the altered expression of pheromone-inducible gene products that are involved in the response may account for the supersensitivity associated with sst2 mutants.

Model for the role of SST2 in pheromone response and recovery. The normal course of α-factor response involves binding of the pheromone to its receptor, internalization of both pheromone and receptor (downregulation), and reaccumulation of receptor on the cell surface (9, 24). Following reaccumulation of α-factor receptor, MATa cells are able to bind α-factor but do not respond to α-factor (24, 36). This desensitization indicates that the pheromone-receptor interaction has been uncoupled from the intracellular pheromone response pathway. The phenotypes associated with sst2 mutations suggest that Sst2 is involved in desensitization (7, 8, 24, 36).

A model for the role of Sst2 in the response pathway must account for two phenotypes associated with sst2 mutants, the ability to respond to very low levels of pheromone (hypersensitivity) and the defect in recovery from pheromone arrest (7, 8). We propose that Sst2 acts to desensitize cells to pheromone by interfering with the activity of a component of the machinery that is involved in producing the response to pheromone. The sst2 defect in recovery from pheromone arrest is easily explained by this model. In a wild-type strain, exposure to pheromone results in a significant increase in the level of Sst2, inhibiting pheromone response even after reaccumulation of cell surface receptors. In an sst2 strain, the response cannot be inhibited, leading to a defect in desensitization and recovery from pheromone arrest. To account for the hypersensitivity of sst2 cells to pheromone, we propose that the low basal level of functional Sst2 present in wild-type strains in the absence of pheromone modulates the intensity of the response. This modulation could not occur in sst2 cells, resulting in hypersensitivity to pheromone. Our observation (unpublished results) that overexpression of Sst2 in a wild-type strain, due to the presence of SST2 on a multicopy plasmid, resulted in some inhibition of pheromone response is consistent with this model.

Before hypothesizing on the specific step at which Sst2 may act, we will describe our current picture of the pheromone response pathway based on analogy to other signal transduction pathways. Two lines of evidence suggest that the mechanism of pheromone response is similar to vertebrate signal transduction systems that are mediated by guanine nucleotide-binding (G) proteins (14, 52, 53). First, the structure of the a- and α-factor receptors is quite similar to the structure of receptors that are involved in these pathways, including rhodopsin, the β-adrenergic receptor, and the muscarinic-cholinergic receptor. Second, we have recently shown that the sst2-suppressing gene SCG1 encodes a homolog to the α subunit of G proteins (12a). In G protein-mediated signal transduction pathways, the activation of a receptor by an extracellular agonist leads to a change in activity of an intracellular effector protein, with a G protein acting as an intermediary. The activated effector then mediates the various responses associated with the pathway.

Where in the pheromone response pathway might Sst2 act? One possibility is that Sst2 acts at the receptor to prevent the first step in signal transduction. Such a role would be similar to the role of arrestin (also called S antigen or 48-kilodalton protein) in desensitization of the vertebrate phototransduction system (48, 52, 60). In the phototransduction system, activation of rhodopsin by a photon of light leads to phosphorylation of a cytoplasmic region of rhodopsin by rhodopsin-kinase, followed by the interaction of arrestin with phosphorylated rhodopsin. The rhodopsin-arrestin association prevents rhodopsin from interacting with transducin, the G protein involved in this system, preventing activation of the effector. The action of arrestin therefore results in desensitization by uncoupling activation of rhodopsin from activation of the effector. Because there is

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FIG. 3. SST2 sequence. The DNA sequence of 4,393 base pairs of the 4,482-base-pair HindIII fragment and the putative SST2 open reading frame are shown. The sequence of the last 89 base pairs is not shown due to ambiguities in the sequence and the distance from the functional region. In the 5′- and 3′-flanking regions, putative TATA boxes and termination and polyadenylation sequences are underlined. Sequences similar to the consensus sequence for negative regulation by α1 or α2 (TCRTGTNNLNANNNTACATCA; R: A or G; L: A or T [34]) were found at positions −788, −556, and −459 and are overlined. Sequences similar to the proposed consensus sequence for induction by pheromone (ATGAAAACA [58]) were found at positions −919, −905, −819, −794, −750, −741, −685, −600, −548, −535, −519, −486, −443, −418, −364, −338, −327, −306, −305, −265, −246, −164, −144, and −20. The position of the fusion in sst2::lacZ9 (URA3) is indicated by an arrow.
no homology between Sst2 and protein kinases, Sst2 is unlikely to act in phosphorylation of the pheromone receptors; however, Sst2 could act at the pheromone receptors to prevent further steps of the pathway in a manner similar to the mechanism of arrestin action.

An alternative site of Sst2 action is the effector. The effector in the pheromone response pathway has not been determined but is proposed to elicit the various aspects of pheromone response, including cell cycle arrest, morphological changes, cell wall changes, and induction of genes or gene products involved in mating (12a). In this model, inhibition of effector activity by interaction with Sst2 would result in desensitization to pheromone.

After removal of pheromone, desensitized cells must be able to return to the pheromone-responsive state. A return to the basal level of Sst2 expression might be adequate to allow resensitization to pheromone. Alternatively, modification of Sst2, eliminating its ability to participate in the proposed interaction with the effector or the receptor, might also be required. A possible modification is phosphorylation or dephosphorylation of Sst2, which contains a high level (18%) of serine and threonine residues. Additional genetic and biochemical analysis will further elucidate the mechanism of Sst2 action.

ACKNOWLEDGMENTS

We thank Kelly Tatchell and Steve Seifert for plasmids and Ayame Konishi, Lynn Miyazaki, and Vicki Cilley for technical assistance. We also thank Kelly Tatchell, Lee Hartwell, Breck Byers, Jamie Konopka, John Hill, Peter Lipke, and Shari Caplan for useful discussions during the course of this work and for comments on the manuscript.

This research was supported by grant ACS MV-310 from the American Cancer Society.

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