MOLECULAR AND CELLULAR BIOLOGY, Dec. 1988, p. 5410–5416
0270-7306/88/125410-07$02.00/0

Vol. 8, No. 12

Role of *SGP2*, a Suppressor of a *gpa1* Mutation, in the Mating-Factor Signaling Pathway of *Saccharomyces cerevisiae*

NAOKI NAKAYAMA,* KEN-ICHI ARAI, AND KUNIHIRO MATSUMOTO

Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, California 94304

Received 1 August 1988/Accepted 22 September 1988

Loss of function of *GPA1*, which encodes a guanine-nucleotide-binding protein, arrests the cell at the G1 phase and allows it to mate, suggesting that the *gpa1* mutation spontaneously exerts an intracellular signal that mimics the action of mating factor. We have cloned the *SGP2* gene, which was first identified as a secondary mutation that allowed a *gpa1*::*HIS3* mutant to grow and to show a non-cell-type-specific sterile phenotype. Disruption of *SGP2* confers temperature-sensitive growth and a-specific sterile phenotypes, characteristics similar to those conferred by the *dpr1* (*ram*) mutation, a suppressor of $RAS2^{Val-19}$. The following observations indicate that *SGP2* and *DPR1* are in fact identical. (i) The cloned *SGP2* complements both the temperature-sensitive growth and the a-specific sterility of the *dpr1* mutant and can be integrated into the chromosomal *DPR1* locus. (ii) The cloned *DPR1*, in turn, complements the ability of *sgp2* to suppress the lethality of *gpa1*::*HIS3*. (iii) The *dpr1* mutation suppresses the growth defect of *gpa1*::*HIS3*, and the *dpr1 gpa1*::*HIS3* strain shows a non-cell-type-specific sterile phenotype. (iv) *sgp2* is closely linked to the *dpr1* locus. The *DPR1* product has been shown to be responsible for processing and fatty acid acylation of a-factor and *RAS* proteins at their carboxyl termini. Therefore, the *SGP2* (*DPR1*) product may be involved in membrane localization of an essential component in the mating-factor signaling pathway.

The yeast mating factors **a** and α are peptide differentiation factors that initiate conjugation between haploid cell types **a** and α. Both factors arrest the cell cycle of target cells at the late G1 phase prior to onset of DNA replication, alter the mode of expression of a number of genes which are known to be essential for conjugation, and induce a morphological alteration (shmoo formation) (for a review, see reference 34). How these peptides trigger a negative growth signal that mediates their effects is an important question that may be applicable to other hormonal signaling systems.

Genetic and physiological analyses have revealed that *STE2* and *STE3* probably code for the α-factor receptor and **a**-factor receptor, respectively (13, 17, 19) and that both receptors appear to share common intracellular signal-transduction machinery (2, 26). The primary structures of the *STE2* and *STE3* products are proposed to contain seven transmembrane domains (6, 12, 25) and thus resemble β-adrenergic receptors (9), muscarinic acetylcholine receptors (18), and rhodopsins (27, 30). The parallel between these mammalian receptors and the yeast mating-factor receptors has been heightened by the discovery that, like the mammalian systems, the yeast signal-transduction system appears to require a guanine-nucleotide-binding protein (G-protein) encoded by *GPA1* (*SCG1*) (8, 21). The *GPA1* gene was initially isolated by cross-hybridization with a rat Gi cDNA (23). The phenotype of a *gpa1* disruption (*gpa1*::*HIS3*) strain resembles that of a haploid cell exposed to its responsive mating factor (8, 21). Also, a *MAT***a** *gpa1*::*HIS3* strain bypasses the requirement of α-factor receptor (*STE2*) for mating as well as for induction of pheromone-inducible gene expression (16, 21, 24). These results suggest that loss of the *GPA1* function in haploid cells exerts an intracellular signal which is equivalent to that generated through mating-factor–receptor interaction. This has led to the proposal that the *GPA1* product appears to be a signal transducer that couples the signal from a receptor to an effector in the mating-factor signaling system (21).

To identify other components involved in the mating-factor signaling pathway, attempts have been made to isolate an extragenic suppressor of *gpa1*::*HIS3*. The non-cell-type-specific *ste* mutations (13, 19), *ste4*, *ste5*, *ste7*, *ste11*, and *ste12*, were found to suppress the growth defect of *gpa1* (24). In addition, we have identified a gene different from these *STE* genes, designated *SGP2*, as a recessive mutation (*sgp2-1*) that suppresses the lethality of *gpa1*::*HIS3* (21). The *sgp2* mutant shows a non-cell-type-specific sterile phenotype in concert with *gpa1* yet expresses the major α-factor gene (*MFα1*) at the wild-type level (22).

In this report, we describe the molecular cloning and characterization of the *SGP2* gene and establish that *SGP2* is identical to *DPR1* (*RAM*), which was identified as a suppressor of $RAS2^{Val-19}$ (10, 31). A possible role of *DPR1* in mating-factor signal transduction is discussed.

MATERIALS AND METHODS

Yeast strains, media, and genetic manipulations. All yeast strains used are listed in Table 1. Yeast transformation was by the lithium acetate method (15). Other standard genetic methods were as described previously (28).

YPD medium contains 1% (wt/vol) yeast extract (Difco), 2% (wt/vol), Bacto-Peptone (Difco), and 2% (wt/vol) glucose. SSG medium contains 0.67% (wt/vol) yeast nitrogen base without amino acids (Difco), 0.2% (wt/vol) sucrose, and 5% (wt/vol) galactose. SD medium contains 0.67% (wt/vol) yeast nitrogen base without amino acids and 2% (wt/vol) glucose. SD Trp^- Ura^- and SSG Trp^- Ura^- are the latter two media containing 0.5% (wt/vol) Casamino Acids (Difco) and 50 μg of adenine per ml, respectively. SD Trp^- and SD Ura^- are SD Trp^- Ura^- medium containing 50 μg of uracil per ml and 50 μg of tryptophan per ml, respectively. Other required auxotrophic nutrients were supplemented at 50 μg/ml as needed. For plates, 2% (wt/vol) Bacto-Agar (Difco)

* Corresponding author.

TABLE 1. Yeast strains used

Strain	Genotype
AM242-1A	*MATα leu1 gal7-2*
AM242-1D	*MATa leu1 gal7-2*
DBY746	*MATα trp1 ura3 leu2 his3*
KMG2-2C	*MATα trp1 ura3 his3 lys2 ade8*
KMG2-2D	*MATa gpa1::HIS3 ura3 his3 lys2*
KMG2-12D	*MATα ura3 leu2 his3*
KMG4-7C	*MATa trp1 ura3 his3*
KMG4-7D	*MATα trp1 ura3 his3*
KMG4-8B	*MATα gpa1::HIS3 trp1 ura3 leu2 his3*
KMG4-8C	*MATa gpa1::HIS3 trp1 ura3 leu2 his3*
KMG9-1A	*MATa sgp2-1 gpa1::HIS3 trp1 ura3 his3 lys2 ade2*
KMG36	*MATa/MATα +/trp1 ura3/ura3 his3/his3 ade8/+*
KMG37	*MATa/MATα sgp2::URA3/+ +/trp1 ura3/ura3 his3/his3 ade8/+*
KMG37-2C	*MATα sgp2::URA3 trp1 ura3 his3*
KMG37-7A	*MATa sgp2::URA3 trp1 ura3 his3*
KMG37-7B	*MATa ura3 his3 ade8*
KMG37-7C	*MATα trp1 ura3 his3 ade8*
KMG37-7D	*MATα sgp2::URA3 ura3 his3*
KMG38	*MATa dpr1 SGP2-LEU2 trp1 ura3 leu2 his3*
KMG39-1C	*MATa dpr1 gpa1::HIS3 trp1 ura3 his3 lys2 ade8*
KMG39-8B	*MATα dpr1 gpa1::HIS3 trp1 ura3 leu2 his3 lys2 ade8*
KMY86-3B	*MATα dpr1 trp1 ura3 leu2*
KMY213-1D	*MATa dpr1 trp1 ura3 leu2 his3*
KMY213-2D	*MATα dpr1 trp1 ura3 leu2 his3 ade8*

was included. 5-Fluoro-orotic acid (FOA) plates were prepared by the method of Boeke et al. (4).

Plasmids and enzymes. Plasmid pG1301 is a YEp24-based plasmid (*URA3* as a selection marker) carrying the 4-kilobase *Xho*I-*Xba*I fragment containing the entire *GPA1* gene; pG1302 is a YCpN1-based plasmid (*TRP1* as a selection marker) carrying the same *GPA1* fragment as pG1301; pG1501 is a YCp-type plasmid (*URA3* as a selection marker) carrying the structural gene of *GPA1* fused with the promoter segment of *GAL1* such that *GPA1* is expressed only when galactose is used as a carbon source. Detailed procedures for construction of this plasmid were described previously (21). Plasmid YCpGPA102 is a YCpN1 derivative (*TRP1* as a selection marker) which carries the 5-kilobase *Xho*I fragment containing *GPA1* (24). YEpDPR1 containing a fragment on YEp24, which complements the temperature sensitivity of a *dpr1* mutant, was kindly provided by Fuyuhiko Tamanoi. Plasmid YCpSGP201 was constructed as follows. The *Sph*I fragment of YCpSGP2-4 was removed first to yield YCpSGP2-43, which contains the intact *SGP2* (see Fig. 2). The *Hin*dIII fragment containing *URA3* isolated from pURA3 was then inserted into the *Nco*I site of YCpSGP2-43. Plasmid pSGP202 was constructed by removing the *Eco*RI fragment, which contains *TRP1* and *ARS1*, from YCpSGP201. Plasmid YIpSGP203 was constructed as follows. The *Sph*I-*Sma*I fragment that carries the intact *SGP2* gene was subcloned in the *Sph*I-*Sma*I region of pUC18 (35) to yield pUSGP2-43. The *LEU2* fragment (*Xba*I-*Hin*dIII) from pLEU2 (24) was then introduced into the *Sma*I site of pUSGP2-43. Restriction enzymes and T4 DNA ligase were purchased from Boehringer Mannheim Biochemicals and from New England BioLabs.

Disruption and integration of *SGP2*. The *sgp2::URA3* mutation was introduced by the one-step gene-disruption method (32). KMG36 (*MATa/MATα ura3/ura3*) was transformed with the *Sph*I- and *Sma*I-digested plasmid pSGP202 DNA, and Ura$^+$ transformants were selected on an SD plate containing histidine and subjected to genomic Southern analysis to confirm the correct integration (33). The confirmed Ura$^+$ strain was subjected to sporulation and tetrad analysis.

The wild-type *SGP2* was integrated into a *dpr1* strain by transforming KMY213-1D (*MATa dpr1 leu2*) with YIpSGP203 DNA that had been linearized by *Xba*I (29). Leu$^+$ transformants were selected at 25°C on an SD plate containing tryptophan, uracil, and histidine. Integration of the plasmid was confirmed by genomic Southern analysis.

Mating assay. (i) Patch assay. Semiquantitative mating efficiency was measured as follows. All the strains were grown in a 96-well culture plate to saturation and transferred with a metal template to a YPD plate that had been streaked with a mating tester strain, AM242-1A (*MATα*) or AM242-1D (*MATa*). The plate was incubated for 24 h and then replicated onto another plate to select diploids in 48 h.

(ii) Quantitative assay. Quantitative mating efficiency was determined as described previously (21). In the case of strains harboring the *TRP1*-carrying plasmid (see Results and Table 3), cells were grown to mid-log phase at 25°C in SD Trp$^-$ medium. Then 10^6 cells from each culture were mixed with 10^6 cells of a tester strain, KMG2-2C (*MATα*), KMG4-7D (*MATα*), or KMG4-7C (*MATa*), collected on a nitrocellulose membrane filter (0.45-μm pore size; Millipore Corp.), placed on a YPD plate, and incubated at 25°C for 3 h to allow them to mate. The cells on each filter were suspended in 10 ml of water, diluted, and plated to select for the resulting diploids.

Other methods. Conditions for standard techniques such as restriction enzyme digestion and ligation of DNAs, isolation of DNA fragments from agarose gels, and *Escherichia coli* transformation were those used by Maniatis et al. (20). *E. coli* strains used were JM83 [Δ(*lac pro*) (φ*80lacZ* Δ*M15*)] and MC1061*recA* [*araD139* Δ(*ara leu*)*7697* Δ*lacX74 galU galK hsr strA recA*]. Nick translation was done by using a nick translation kit purchased from Amersham.

RESULTS

Isolation of the *SGP2* gene. The *sgp2* single mutation (*sgp2-1*) has no apparent phenotype other than negation of the mating-factor signal that has been constitutively induced by the *gpa1* defect (22). Therefore, we used a *gpa1 sgp2* double mutation strain, KMG9-1A (*MATa trp1 ura3 gpa1::HIS3 sgp2*), to clone the *SGP2* gene. Direct transformation of KMG9-1A with a genomic DNA library was expected to result in no positive clones since a *gpa1 sgp2* strain with an *SGP2*-containing plasmid must show the *gpa1* phenotype and would not grow. To prevent this selective elimination of *SGP2* clones, another plasmid, pG1501, in which expression of *GPA1* can be regulated by galactose, was introduced. Thus, all the cells transformed with a genomic library are expected to grow in the presence of galactose. Among them, *SGP2*-harboring clones should be selected as those which cannot grow on glucose, where expression of *GPA1* is repressed.

As summarized in Fig. 1, KMG9-1A carrying pG1501 was transformed with a YCp-based genomic library (70,000 initial Trp$^+$ Ura$^+$ transformants), collected, and replated on 96 SSG Trp$^-$ Ura$^-$ plates at 400 to 500 colonies per plate. Each plate was replicated onto an SD Trp$^-$ Ura$^-$ plate, incubated at 30°C for 24 h, replicated onto another SD Trp$^-$ Ura$^-$

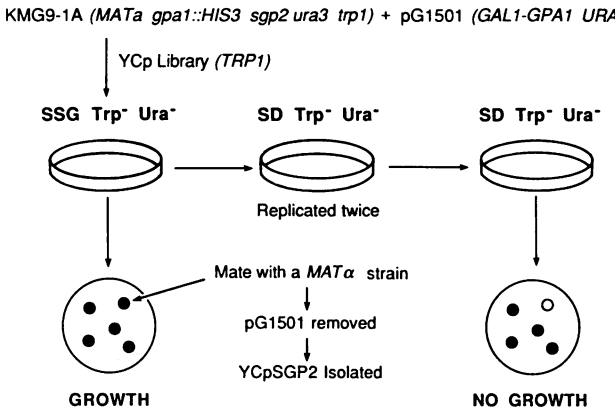

FIG. 1. Cloning strategy for *SGP2*. SSG Trp⁻ Ura⁻ and SD Trp⁻ Ura⁻ denote agar plates as described in Materials and Methods; a solid circle represents a growing colony, and an open circle represents a non-growing colony.

plate, and incubated further at 30°C for 24 to 48 h. Twenty-six colonies that did not grow on the second SD plates were picked from the SSG plates. Rescreening confirmed that two (clones 4 and 33) of them reproducibly ceased to grow and formed shmoos on an SD Trp⁻ Ura⁻ plate. Both were crossed with DBY746 (*MATα trp1 ura3*) on a YPD plate, Trp⁺ Ura⁺ diploids were selected, and pG1501 was removed. Then, the plasmids were recovered (YCpSGP2-4 and YCpSGP2-33). Retransformation of KMG9-1A carrying pG1501 by using either plasmid reproduced the growth-defective phenotype in the absence of galactose.

Restriction maps of both plasmids have revealed that they are from the same chromosomal locus (Fig. 2). Both YCpSGP2-4 and YCpSGP2-33 contain 7.5- to 8-kilobase inserts that largely overlap. Deletion analysis was performed with YCpSGP2-4 to estimate the minimum complementation

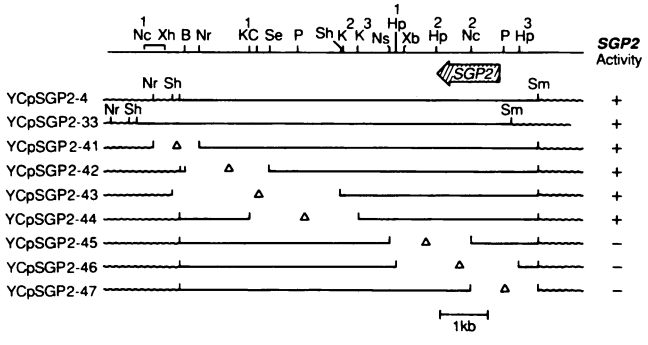

FIG. 2. Restriction map and deletion analysis of the *SGP2* region in the plasmid YCpSGP2-4. Structures of both original clones, YCpSGP2-4 and YCpSGP2-33, and the deletion derivative of YCpSGP2-4 are shown. The top line with restriction sites represents the *SGP2* locus on the chromosome. The arrow indicates the location and transcriptional direction of the *SGP2* (*DPR1*) structural gene (F. Tamanoi, personal communication). *Bam*HI (B), *Cla*I (C), *Hpa*I (Hp), *Kpn*I (K), *Nco*I (Nc), *Nru*I (Nr), *Nsi*I (Ns), *Pst*I (P), *Spe*I (Se), *Sph*I (Sh), *Sma*I (Sm), *Xba*I (Xb), and *Xho*I (Xh) restriction sites are drawn to physical scale. Restriction sites that appear more than once are numbered from left to right. The wavy line represents the vector (YCpN1) portion; the straight line represents the insert. A gap with a triangle indicates the site of deletion. The complementing ability of each clone is listed in the right column.

unit for *sgp2* (Fig. 2). As a result, the *Nsi*I-*Nco*I^2, *Nco*I^2-*Sma*I, and *Hpa*I^1-*Hpa*I^3 fragments were found to be indispensable. This indicates that the region including *Nco*I^2, which spans *Hpa*I^1 (or *Nsi*I) and *Hpa*I^3, is essential for the activity.

Construction and characterization of a strain harboring a disruption allele of *SGP2*. Since the *Nco*I^2 site was considered essential for *SGP2* activity, we inserted a *URA3* fragment into this site on YCpSGP2-43 to disrupt the *SGP2* activity. The resulting plasmid, YCpSGP201, was unable to complement *sgp2*. Then, a wild-type diploid strain, KMG36, was transformed with the *Sph*I-*Sma*I fragment from pSGP202 that contains the disrupted *SGP2* gene (Fig. 3). A transformant, KMG37, that harbored one copy of the *sgp2*::*URA3* allele (*SGP2*/*sgp2*::*URA3*) was sporulated and dissected at 25°C. Tetrads derived from 29 asci resulted in 2 fast-growing:2 slow-growing segregation at 25°C on YPD, whereas they showed 2 viable:2 inviable (2^+:2^-) segregation at 35°C (Fig. 4). The segregation pattern of the Ura phenotype in the 29 asci tested was 2^+:2^-, and all the Ura$^+$ segregants were temperature sensitive for growth (Fig. 4). Therefore, unlike the original allele of *sgp2* (*sgp2-1*), the *sgp2*::*URA3* single mutation conveys temperature sensitivity for growth.

On the other hand, the mating ability determined at 25°C resulted in significant deviations from a 4 fertile (2 **a** and 2 α): 0 sterile (4^+:0^-) segregation pattern expected for a normal *MAT***a**/*MAT*α diploid (Fig. 4). The proportion of 4^+:0^-, 3^+:1^-, and 2^+:2^- was 3:22:4 in the 29 asci examined, and it was always *MAT***a** Ura$^+$ segregants that were unable to mate (Fig. 4). This indicates that the *sgp2*::*URA3* mutation causes an **a**-specific sterile phenotype.

The *sgp2*::*URA3* mutation suppresses the *gpa1* phenotype. Since the *sgp2-1* and *sgp2*::*URA3* mutations give rise to different phenotypes, one might expect that *SGP2* encodes a multifunctional protein so that suppression of *gpa1* requires a specific allele of the *sgp2* mutation. We explored this possibility by determining whether *sgp2*::*URA3* suppresses the growth defect of *gpa1*::*HIS3* and whether the *sgp2*::*URA3 gpa1*::*HIS3* double mutant is a non-cell-type-specific sterile one. One segregant, KMG37-2C (*MAT*α *sgp2*::*URA3*) (2c in Fig. 4), was crossed with KMG4-8C (*MAT***a** *gpa1*::*HIS3*) carrying pG1302. Resulting diploids were sporulated and dissected at 25°C. Each segregant of all the tetrads was grown further in YPD medium and plated on a YPD plate to yield 200 to 300 colonies per plate. Then Trp⁻ cells were screened at 25°C by replicating them onto SD Trp⁻ plates. All the His⁻ segregants and His⁺ Ura⁺ segregants lost a Trp marker, namely the plasmid pG1302, and the His⁺ Ura⁺ Trp⁻ cells had a non-cell-type-specific sterile phenotype (data not shown). This indicates that disruption of *SGP2* also suppresses the growth defect of *gpa1*::*HIS3* so that the suppression is not specific to a certain point mutation of *SGP2*.

Cloned *SGP2* complements the *dpr1* phenotype, and cloned *DPR1* complements the *sgp2-1* phenotype. The temperature sensitivity for growth and **a**-specific sterility of the *sgp2*::*URA3* strain led us to compare its identity to a mutant of exactly the same phenotype, *dpr1* (*ram*) (10, 31). A *dpr1* strain, KMY213-1D (*MAT***a** *dpr1*), was transformed at 25°C with YCpSGP2-43 and with YCpN1 as a control. The values for mating efficiency, i.e., the numbers of diploids obtained by mating between KMY213-1D, carrying each plasmid, and a tester strain, KMG2-2C (*MAT*α), at 25°C as described in Materials and Methods, were 3.9×10^5 and 5, respectively. The *dpr1* strain carrying YCpN1 did not grow at 37°C,

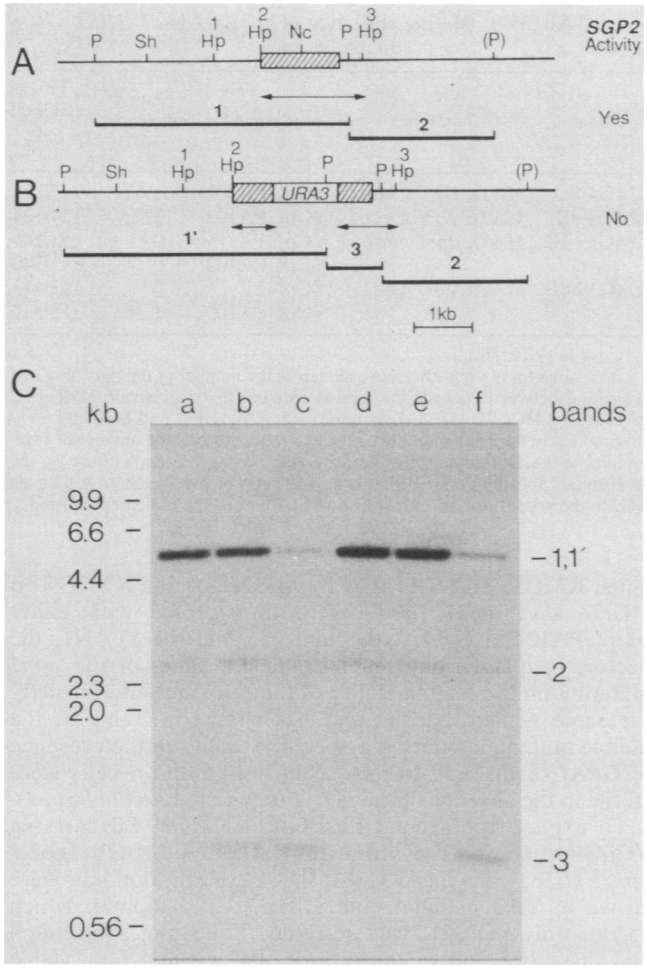

FIG. 3. Physical mapping and Southern blotting analysis of the disrupted *SGP2*. (A) Physical map of the *SGP2* region on the chromosome. (B) Physical map of the region with the disrupted *SGP2*. The hatched box represents the structural gene of *SGP2*. Restriction enzyme sites are drawn to physical scale as in Fig. 2. The open box indicates the *Hin*dIII fragment containing *URA3*, the arrow indicates the region hybridized with a probe (*Hpa*I fragment), and the bars indicate fragments visualized on the genomic Southern blot. In the right column, the complementing ability of (A) YCpSGP2-43 carrying the wild-type *SGP2* and of (B) YCpSGP201 carrying the disrupted *SGP2* is listed. (C) Genomic Southern blot with the *Hpa*I fragment containing the entire *SGP2* as a probe. The numbers on the right correspond to fragments shown in panels A and B. Chromosomal DNAs from KMG36 (lane a), KMG37 (lane b), KMG37-7A (lane c), KMG37-7B (lane d), KMG37-7C (lane e), and KMG37-7D (lane f) were digested with *Pst*I and separated by agarose gel electrophoresis. KMG37-7A, -7B, -7C, and -7D are the segregants 7a, 7b, 7c, and 7d shown in Fig. 4. kb, Kilobases.

whereas that carrying YCpSGP2-43 did. Thus, *SGP2* suppresses both the mating deficiency at 25°C and the growth defect at 37°C caused by *dpr1*. In addition, KMG9-1A (*gpa1*::*HIS3 sgp2-1*) was transformed both with YCpGPA 102 and with either YEpDPR1 or YEp24. Both transformants were grown in YPD media, plated onto YPD plates (200 to 300 colonies per plate), and transferred to several selection plates to distinguish cells with a different plasmid (Table 2). When YEpDPR1 was used, no Trp^- Ura^+ cells were obtained; that is, *DPR1* complements the *sgp2* mutation. These results suggest that *DPR1* and *SGP2* are functionally interchangeable.

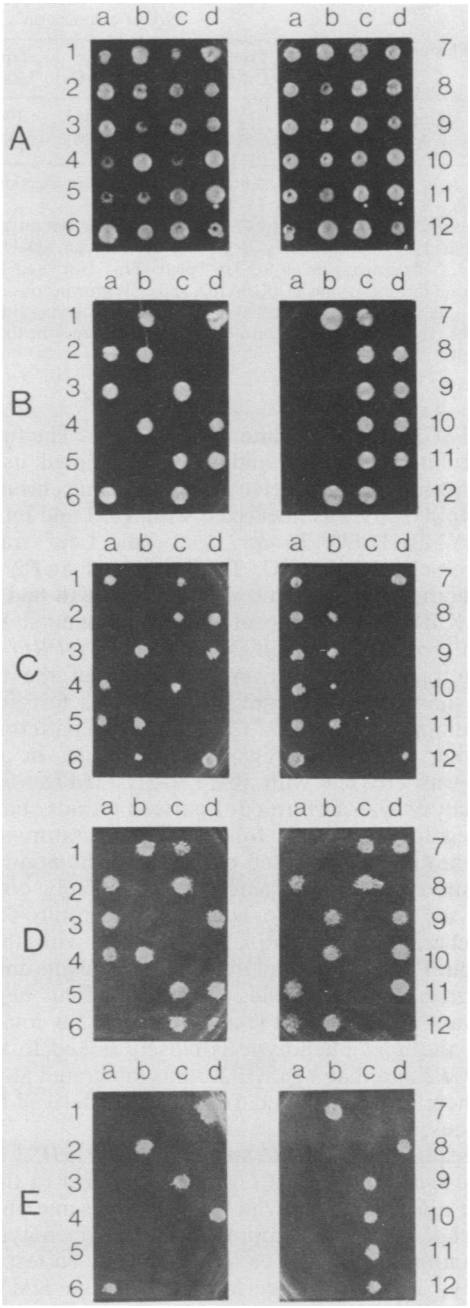

FIG. 4. Dissection of haploid progeny derived from *SGP2*-disrupted diploids. Diploid cells that had been manipulated as described in the legend to Fig. 3 were sporulated, and 29 tetrads were dissected. The spores were allowed to germinate on a YPD plate at 25°C and grown in YPD medium to saturation. Each segregant was then transferred to different plates by using a metallic stamp and grown on YPD at 25°C (A), YPD at 35°C (B), or SD Ura^- at 25°C (C) for 48 h. They were also transferred onto YPD plates that had been streaked with either AM242-1D (*MAT***a**) (D) or AM242-1A (*MAT*α) (E) and were incubated at 25°C for 24 h, and the plates were replica-plated to select diploids. Of the 29 tetrads, 12 are shown. The four spores from individual asci designated a, b, c, and d are aligned horizontally. The numbers indicate individual sets of four spores.

TABLE 2. Complementation of *sgp2* by YEpDPR1 in strain KMG9-1A (*gpa1*::*HIS3 sgp2-1 trp1 ura3*)

Plasmid[a]	No. of colonies on[b]:				
	Trp⁻ Ura⁻	Trp⁻ Ura⁺	Trp⁺ Ura⁻	Trp⁺ Ura⁺	YPD
YEp24 + YCpGPA102	21	30	45	176	272
YEpDPR1 + YCpGPA102	3	0	109	263	375

[a] YEp24 is a vector plasmid containing *URA3* as a selection marker. YEpDPR1 is a YEp24-based plasmid carrying *DPR1*.

[b] The presence of the Trp⁺ or Ura⁺ marker in cells grown on YPD plates was determined by replica-plating to an SD Trp⁻ or an SD Ura⁻ plate, respectively. Cells growing on an SD Trp⁻ plate (Trp⁺ Ura⁺ and Trp⁺ Ura⁻ cells) represent those carrying YCpGPA102, and cells growing on an SD Ura⁻ plate (Trp⁻ Ura⁺ and Trp⁺ Ura⁺ cells) represent those carrying either YEp24 or YEpDPR1. The colony number on a YPD plate represents the total number of viable cells.

Cloned *SGP2* integrates into the *dpr1* locus. The functional exchangeability of *SGP2* and *DPR1* prompted us to test whether both genes are derived from the same chromosomal locus. YIpSGP203 was linearized with *Xba*I and introduced into KMY213-1D (*MAT***a** *dpr1 leu2*), and Leu⁺ transformants were selected at 25°C. The integrated *SGP2* complemented both the temperature-sensitive growth and the sterility of KMY213-1D. One of the transformants, KMG38, was then crossed with KMG2-12D (*MAT*α *DPR1*) at 25°C. Eleven tetrads obtained from dissection of the resulting diploids showed 4⁺:0⁻ segregation patterns for growth on YPD medium at 35°C and 2⁺:2⁻ segregation patterns for the Leu marker. All the segregants were fertile. In addition, KMG38 was crossed with KMY86-3B (*MAT*α *dpr1*) and tetrad analysis was performed. Fourteen tetrads showed 2⁺:2⁻ segregation patterns for both temperature-sensitive growth on YPD and the Leu phenotype. The non-temperature-sensitive-growth characteristic completely cosegregated with the Leu⁺ marker, and the temperature-sensitive-growth characteristic completely crossed with the Leu⁻ phenotype. Furthermore, all the Leu⁺ segregants and *MAT*α Leu⁻ segregants were fertile, while *MAT***a** Leu⁻ segregants were always sterile. These results indicate that complementation of the *dpr1* phenotype is closely linked to the integrated *SGP2* and that YIpSGP203 has integrated at the *dpr1* locus. Therefore, the cloned *SGP2* gene is derived from the *DPR1* locus.

Dpr1* is allelic to *sgp2* and suppresses *gpa1*::*HIS3* in a way similar to *sgp2*.** If the *DPR1* gene is identical to the *SGP2* gene, the *dpr1* mutation, which causes the same phenotype as *sgp2*::*URA3*, should suppress the *gpa1* phenotypes, and both mutation loci should be tightly linked. To test the first possibility, a cross was performed between KMY213-2D (*MAT*α *dpr1*) and KMG2-2D (*MATa** *gpa1*::*HIS3*) carrying pG1301 and the doubly heterozygous diploids were sporulated and dissected at 25°C. Segregants from the tetrads that yielded four viable spores were transferred onto plates containing FOA at 25°C to eliminate pG1301 and onto a YPD plate at 35°C. The cross resulted in 1:8:3 being the ratio of 4⁺:0⁻, 3⁺:1⁻, and 2⁺:2⁻ asci for growth on an FOA plate at 25°C, while all the tetrads showed a 2⁺:2⁻ segregation pattern for growth on YPD medium at 35°C. All the temperature-sensitive and His⁺ segregants (*dpr1 gpa1*::*HIS3*) grew on YPD at 25°C in the absence of pG1301 and were defective in mating. These results indicate that *dpr1* suppresses the growth defect of *gpa1*::*HIS3* and causes non-cell-type-specific sterility under the *gpa1* background, as do *sgp2-1* and *sgp2*::*URA3*. Furthermore, among these double mutants, KMG39-1C (*MAT***a** *dpr1 gpa1*::*HIS3*) and KMG39-8B (*MAT*α *dpr1 gpa1*::*HIS3*) were transformed with either YCpGPA102, a *GPA1*-containing plasmid, or YCpN1, the vector, to quantitatively determine the effect of the *gpa1* mutation on the mating ability of the *dpr1* mutant in exactly the same genetic background. As shown in Table 3, the double mutants conferred **a**-specific sterility in the presence of *GPA1* (equivalent to *dpr1*), but both **a** and α cells were sterile in the absence of the *GPA1* (equivalent to *dpr1 gpa1*).

TABLE 3. Mating efficiency of the *dpr1 gpa1*::*HIS3* double mutant

Strain	Genotype[a]	Plasmid	Mating efficiency, 10^{5}[b]	Trp⁺ cell/total cell ratio[c]
KMG4-8C	*MAT***a** *DPR1 gpa1*	YCpGPA102	3.67	155/155
KMG4-8B	*MAT*α *DPR1 gpa1*	YCpGPA102	3.32	124/124
KMG39-1C	*MAT***a** *dpr1 gpa1*	YCpN1	0	53/149
		YCpGPA102	0	185/340
KMG39-8B	*MAT*α *dpr1 gpa1*	YCpN1	0	113/413
		YCpGPA102	1.36	115/337

[a] *gpa1* is *gpa1*::*HIS3*.

[b] The value for mating efficiency is given as the number of diploids obtained by mating between each of the strains listed with tester strain KMG4-7C (*MAT***a**) or KMG4-7D (*MAT*α), as described in Materials and Methods.

[c] Stability of the Trp⁺ marker is indicated as the ratio of the number of Trp⁺ colonies to that of colonies grown at 25°C (the permissive temperature for the *dpr1* mutant to grow) on a YPD plate. The ratio of Trp⁺ cells to total cells reflects the proportion of the population that harbor the indicated plasmid.

To explore the second possibility, KMG39-8B carrying YCpGPA102 was crossed with KMG9-1A (*MAT***a** *sgp2-1 gpa1*::*HIS3*) carrying pG1301. The resulting diploids were grown in YPD medium, and a Trp⁻ Ura⁺ diploid, which carries only pG1301, was selected. Following sporulation and dissection, all of the tetrads that yielded four viable spores were transferred to an FOA plate to select those that grew without *GPA1* at 25°C. For 18 asci tested, all resulted in a 4⁺:0⁻ segregation pattern for growth on FOA plates at 25°C. All the segregants devoid of pG1301 (His⁺ Ura⁻) also grew on YPD at 25°C and were sterile. This indicates that *dpr1* and *sgp2-1* are closely linked. In conclusion, all the observations support the idea that *SGP2* and *DPR1* are identical.

DISCUSSION

***Sgp2* is allelic to *dpr1*, which is defective in the posttranslational modification of a-factor and *RAS* proteins.** The *sgp2* mutation was isolated as a suppressor of *gpa1*::*HIS3* with the aim of identifying a downstream component of *GPA1*. Characterization of the cloned *SGP2* gene has revealed that disruption of *SGP2* (*sgp2*::*URA3*) gave rise to a phenotype different from that of the original point mutant (*sgp2-1*). The *sgp2-1* single mutation does not have any significant effect on cell growth or mating ability (22), while disruption of *SGP2* confers **a**-specific sterile and temperature-sensitive growth phenotypes (Fig. 4). Since these are identical to phenotypes of the *dpr1* (*ram*) mutant (10, 31), it is possible that *SGP2* and *DPR1* are identical. A series of genetic experiments presented in this report indicate that *SGP2* and *DPR1* are identical. This conclusion has been further supported by the fact that the restriction map for the *DPR1* gene, determined by nucleotide sequencing (F. Tamanoi, personal communication), is identical to that of the *SGP2* locus (data not shown). This has led us to conclude that *SGP2* is *DPR1*.

Interestingly, the *sgp2::URA3* mutant is also temperature sensitive for growth. Since the *URA3* fragment was inserted into the middle of the *SGP2* (*DPR1*) structural gene (F. Tamanoi, personal communication), the disrupted *SGP2* (*DPR1*) is likely to be a null allele. Hence, the temperature-sensitive growth of the *dpr1* mutant may be a consequence of the lack of *SGP2* (*DPR1*) activity rather than the temperature-sensitive activity of the *SGP2* (*DPR1*) product, although we cannot rule out that an aberrant product produced from the *sgp2::URA3* allele still possesses a temperature-sensitive *SGP2* activity.

Effect of the *DPR1* action on mating-factor signal transduction. The *dpr1* (*ram*) mutation was isolated as a secondary mutation that suppresses the heat sensitivity of a $RAS2^{Val-19}$ strain, and it confers temperature sensitivity for growth and **a**-specific sterility (10, 31). Biochemical characterization of the *RAS* protein in the *dpr1* mutant has revealed that processing and/or fatty acid acylation of the protein are defective (10, 31). Consequently, only a few mature *RAS* ($RAS2^{Val-19}$) molecules that can be localized on the membrane are present (10), which is believed to result in suppression of the $RAS2^{Val-19}$ phenotype. Also, the mutant is deficient in secreting **a**-factor (but not α-factor); therefore, it shows **a**-specific sterility (31, 36). Membrane localization is required for the mammalian *ras* proteins to elicit their biological functions. This is achieved by fatty acylation of the Cys residue nearest the carboxyl termini (for a review, see reference 1). This Cys residue resides in a sequence commonly found among the *ras* protein family, Cys-A-A-X, where A is any aliphatic amino acid and X represents no preference. This common sequence also exists in yeast *RAS* proteins (1, 31), and the Cys residue is essential for the membrane localization and in vivo function of *RAS2* (7). Similarly, the **a**-factor precursor peptide has this sequence (5), and the Cys residue was found to be at the carboxyl terminus and modified in the mature form (3). These observations suggest that the *DPR1* product regulates membrane localization of any proteins that possess the Cys-A-A-X sequence at their carboxyl termini through posttranslational modification.

What, then, is the role of *DPR1* in the mating-factor signaling pathway? One may ask why the *dpr1* mutation suppresses the growth defect of *gpa1*. In view of its effect on *RAS* proteins, *dpr1* may shut off the mating signal that has been constitutively generated by the *gpa1* mutation by reducing the amount of a crucial membrane component. Another question is why the *dpr1* (*ram*) single mutant can respond to mating factor (31) while the *dpr1 gpa1* double mutant cannot. It seems necessary to assume that the membrane localization of the crucial membrane component is dependent on *DPR1* function in the absence of *GPA1* but that the *DPR1* activity is no longer required when *GPA1* is present. What would be such a component? This component may (i) interact with the *GPA1* product, (ii) be unstable without *GPA1* so that its amount on the membrane, sufficient to introduce the signal, relies on *DPR1*, and (iii) be stabilized in the presence of *GPA1* with enough existing on the membrane to transmit a signal even without the *DPR1* function.

Possible targets of the *DPR1* action relevant to the mating-factor signaling pathway. One clue to understanding of the role of *DPR1* in the mating-factor signaling pathway may be the notion that the γ-subunit of transducin (a G-protein involved in visual transduction) terminates with a Cys-A-A-X(COOH) motif, as do *ras* proteins (14). Mammalian G-proteins are composed of three subunits, α, β, and γ; the γ-subunit (Gγ) is believed to act as a membrane anchor to specify the location of the soluble α-subunit (Gα) on the membrane (for a review, see reference 11). Therefore, one can imagine that membrane localization of G-proteins may be facilitated by fatty acid acylation. Thus, Gγ would suffice for the criteria mentioned previously. Considering both the structural similarity of yeast Gα (*GPA1*) and mammalian Gα and the ability of a mammalian Gsα to suppress the growth defect of *gpa1* (8; I. Miyajima and K. Matsumoto, unpublished data), it is reasonable to assume that there are β- and γ-subunits for the *GPA1* protein and that membrane localization of the γ-subunit might be affected by *DPR1*. Recently, *STE18* was identified as the yeast Gγ analog which possesses the Cys-A-A-X(COOH) motif and the *ste18* mutation was found to suppress the growth defect of *gpa1* (M. Whiteway, personal communication). This would agree with the view that the free β/γ subunits are responsible for stimulating the membrane effector in the *gpa1* mutant (8, 16). Alternatively, it is also possible that membrane localization of another signaling component that fulfills the criteria described before (which might be an effector or a membrane anchor for the effector) is controlled by *DPR1*. Whichever the case, the target(s) of *DPR1* in the mating-factor signaling pathway needs to be identified.

ACKNOWLEDGMENTS

We are particularly grateful to Fuyuhiko Tamanoi, University of Chicago, for providing the *DPR1* plasmid and to Malcolm Whiteway, NRC Biotechnology Research Institute, for communicating results prior to publication. We also thank Satoshi Nomoto for his help in genomic Southern hybridizations and Allan Waitz and Gerard Zurawski for critical readings of this manuscript.

LITERATURE CITED

1. **Barbacid, M.** 1987. *ras* genes. Annu. Rev. Biochem. **56**:779–827.
2. **Bender, A., and G. F. Sprague, Jr.** 1986. Yeast peptide pheromones, **a**-factor and α-factor, activate a common response mechanism in their target cells. Cell **47**:929–937.
3. **Betz, R., J. W. Crabb, H. E. Meyer, R. Wittig, and W. Duntze.** 1987. Amino acid sequences of **a**-factor mating peptides from *Saccharomyces cerevisiae*. J. Biol. Chem. **262**:546–548.
4. **Boeke, J. D., F. LaCroute, and G. F. Fink.** 1984. A positive selection for mutants lacking orotidine-5-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
5. **Brake, A. J., C. Brenner, R. Najarian, P. Laybourn, and J. Merryweather.** 1985. Structure of genes encoding precursors of the yeast peptide mating pheromone **a**-factor, p. 103–108. *In* M.-I. Gething (ed.), Current communications in molecular biology: protein transport and secretion. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Burkholder, A. C., and L. H. Hartwell.** 1985. The yeast α-factor receptor: structural properties deduced from the sequence of the *STE2* gene. Nucleic Acids Res. **13**:8463–8475.
7. **Deschenes, R. J., and J. R. Broach.** 1987. Fatty acylation is important but not essential for *Saccharomyces cerevisiae RAS* function. Mol. Cell. Biol. **7**:2344–2351.
8. **Dietzel, C., and J. Kurjan.** 1987. The yeast *SCG1* gene: a Gα-like protein implicated in the **a**- and α-factor response pathway. Cell **50**:1001–1010.
9. **Dixon, R. A. F., B. K. Kobilka, D. J. Strader, J. L. Benovic, H. G. Dohlman, T. Frielle, M. A. Bolanowski, C. D. Bennett, E. Rands, R. E. Diehl, R. A. Mumford, E. E. Slater, I. S. Sigal, M. G. Caron, R. J. Lefkowitz, and C. D. Strader.** 1986. Cloning the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. Nature (London) **321**:75–79.
10. **Fujiyama, A., K. Matsumoto, and F. Tamanoi.** 1987. A novel yeast mutant defective in the processing of *ras* proteins: assess-

ment of the effect of the mutation on processing steps. EMBO J. 6:223–228.
11. **Gilman, A. G.** 1987. G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. **56**:615–649.
12. **Hagen, D. C., G. McCaffrey, and G. F. Sprague, Jr.** 1986. Evidence the yeast *STE3* gene encodes a receptor for the peptide pheromone a factor: gene sequence and implications for the structure of the presumed receptor. Proc. Natl. Acad. Sci. USA **83**:1418–1422.
13. **Hartwell, L. H.** 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. J. Cell Biol. **85**:811–822.
14. **Hurley, J. B., H. K. W. Fong, D. B. Teplow, W. J. Dreyer, and M. I. Simon.** 1984. Isolation and characterization of a cDNA clone for the γ subunit of bovine retinal transducin. Proc. Natl. Acad. Sci. USA **81**:6948–6952.
15. **Itoh, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cation. J. Bacteriol. **153**:163–168.
16. **Jahng, K.-Y., J. Ferguson, and S. I. Reed.** 1988. Mutations in a gene encoding the α subunit of a *Saccharomyces cerevisiae* G protein indicate a role in mating pheromone signaling. Mol. Cell. Biol. **8**:2484–2493.
17. **Jenness, D. D., A. C. Burkholder, and L. H. Hartwell.** 1983. Binding of α-factor pheromone to yeast a cells: chemical and genetic evidence for an α-factor receptor. Cell **35**:521–529.
18. **Kubo, T., K. Fukuda, A. Mikami, A. Maeda, H. Takahashi, M. Mishina, T. Haga, K. Haga, A. Ichiyama, K. Kanagawa, M. Kojima, H. Matsuo, T. Hirose, and S. Numa.** 1986. Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature (London) **323**: 411–416.
19. **MacKay, V., and T. R. Manney.** 1974. Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae*. II. Genetic analysis of nonmating mutants. Genetics **76**:273–288.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Miyajima, I., M. Nakafuku, N. Nakayama, C. Brenner, A. Miyajima, K. Kaibuchi, K. Arai, Y. Kaziro, and K. Matsumoto.** 1987. *GPA1*, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. Cell **50**:1011–1019.
22. **Miyajima, I., N. Nakayama, M. Nakafuku, Y. Kaziro, K. Arai, and K. Matsumoto.** 1988. Suppressors of a *gpa1* mutation cause sterility in *Saccharomyces cerevisiae*. Genetics **119**:797–804.
23. **Nakafuku, M., H. Itoh, S. Nakamura, and Y. Kaziro.** 1987. Occurrence in *Saccharomyces cerevisiae* of a gene homologous to the cDNA coding for the α subunit of mammalian G proteins. Proc. Natl. Acad. Sci. USA **84**:2140–2144.
24. **Nakayama, N., Y. Kaziro, K. Arai, and K. Matsumoto.** 1988. Role of *STE* genes in the mating-factor signaling pathway mediated by *GPA1* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:3777–3783.
25. **Nakayama, N., A. Miyajima, and K. Arai.** 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. **4**:2643–2648.
26. **Nakayama, N., A. Miyajima, and K. Arai.** 1987. Common signal transduction system shared by *STE2* and *STE3* in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of *STE2*. EMBO J. **6**:249–254.
27. **Nathans, J., and D. S. Hogness.** 1983. Isolation, sequence analysis, and intron-exon arrangement of the gene encoding bovine rhodopsin. Cell **34**:807–814.
28. **Nogi, Y., K. Matsumoto, A. Toh-e, and Y. Oshima.** 1977. Interaction of super-repressible and dominant constitutive mutations for the synthesis of galactose pathway enzymes in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **152**:137–144.
29. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1983. Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101**:228–245.
30. **Ovchinnikov, Y. A.** 1982. Rhodopsin and bacteriorhodopsin: structure-function relationships. FEBS Lett. **148**:179–191.
31. **Powers, S., S. Michaelis, D. Broek, S. Santa Anna-A., J. Field, I. Herskowitz, and M. Wigler.** 1986. *RAM*, a gene of yeast required for a functional modification of *RAS* proteins and for production of mating pheromone a-factor. Cell **47**:413–422.
32. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
33. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.
34. **Sprague, G. F., Jr., L. L. Blair, and J. Thorner.** 1983. Cell interactions and regulation of cell type in the yeast. Annu. Rev. Microbiol. **37**:623–660.
35. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
36. **Wilson, K. L., and I. Herskowitz.** 1987. *STE16*, a new gene required for pheromone production by a cells of *Saccharomyces cerevisiae*. Genetics **155**:441–449.