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# Stoichiometry of G Protein Subunits Affects the *Saccharomyces cerevisiae* Mating Pheromone Signal Transduction Pathway

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**The *Saccharomyces cerevisiae GPA1*, *STE4*, and *STE18* genes encode products homologous to mammalian G-protein α, β, and γ subunits, respectively. All three genes function in the transduction of the signal generated by mating pheromone in haploid cells. To characterize more completely the role of these genes in mating, we have conditionally overexpressed *GPA1*, *STE4*, and *STE18*, using the galactose-inducible *GAL1* promoter. Overexpression of *STE4* alone, or *STE4* together with *STE18*, generated a response in haploid cells suggestive of pheromone signal transduction: arrest in $G_1$ of the cell cycle, formation of cellular projections, and induction of the pheromone-inducible transcript *FUS1* 25- to 70-fold. High-level *STE18* expression alone had none of these effects, nor did overexpression of *STE4* in a *MAT*a/α diploid. However, *STE18* was essential for the response, since overexpression of *STE4* was unable to activate a response in a *ste18* null strain. *GPA1* hyperexpression suppressed the phenotype of *STE4* overexpression. In addition, cells that overexpressed *GPA1* were more resistant to pheromone and recovered more quickly from pheromone than did wild-type cells, which suggests that *GPA1* may function in an adaptation response to pheromone.**

G proteins function as molecular transducers of extracellular stimuli, coupling these stimuli to intracellular responses (reviewed in references 12 and 48). G-protein-mediated signal transduction has now been described in a number of eucaryotes as phylogenetically diverse as fungi and vertebrates. Elements from several of these systems have been biochemically characterized in some detail, and they appear to conserve many aspects of structure and function. In the best-understood pathways, in mammalian cells, G proteins transduce a signal generated by stimulation of a membrane receptor of the rhodopsin/β-adrenergic family (10, 36). These G proteins, consisting of α, β, and γ subunits, exist as inactive heterotrimers with GDP bound to the α subunits. Receptor stimulation leads to exchange of GDP for GTP, activating the G protein, which then dissociates into α and βγ subunits. Both α and βγ subunits are then capable of interacting with diverse intracellular effectors in different systems (reviewed in reference 37), including adenylate cyclase, cyclic GMP phosphodiesterase, and phospholipase $A_2$. Hydrolysis of GTP to GDP inactivates the α subunit, which then reassociates with free βγ to return to the inactive αβγ configuration.

Haploid cells of the yeast *Saccharomyces cerevisiae* respond to peptide mating pheromones released by cells of the opposite mating type through a G protein-mediated pathway. Pheromone-activated cells arrest in the $G_1$ interval of the cell cycle, induce a number of specific transcripts, and undergo morphological and physiological changes preparative to mating (reviewed in references 7 and 49). These responses are dependent on genes encoding proteins homologous to mammalian α (*GPA1* or *SCG1*), β (*STE4*), and γ (*STE18*) G protein subunits (8, 16, 32, 52). Genetic data suggest that the transducer of the signal in the mating pathway is $G_{\beta\gamma}$, whereas the α subunit has a negative regulatory role. For example, deletion of either *STE4* or *STE18* leads to an inability to activate the response (52), whereas deletion of *GPA1* causes constitutive activation of the pathway (8, 16, 29). Furthermore, *ste4* and *ste18* loss-of-function mutations are epistatic to *gpa1* loss-of-function mutations (33; Malcolm Whiteway, personal communication), suggesting that $G_{\beta\gamma}$ functions at a point downstream of $G_\alpha$ as a positive signal transducer. In addition, expression of rat $G_{s\alpha}$ is able to rescue the constitutive signal phenotype of *gpa1* deletions, although these cells are unable to respond to pheromone (8). This phenotype is probably due to the ability of $G_{s\alpha}$ to bind $G_{\beta\gamma}$ in an inactive trimer but inability to be activated by receptor-pheromone binding. Finally, a dominant *STE4* allele has been isolated that is constitutively activated for the pheromone response (2).

To gain a more complete understanding of the function of each G protein subunit in regulating the response to mating pheromone as well as to elucidate how the different subunits interact, we have conditionally overexpressed *GPA1*, *STE4*, and *STE18* in a variety of genetic backgrounds. The results of these experiments provide strong support for the notion that $G_{\beta\gamma}$ mediates the mating signal in *S. cerevisiae*. We also provide evidence that $G_\alpha$ itself plays a role in an adaptive response to pheromone.

## MATERIALS AND METHODS

**Yeast strains and plasmids.** All yeast strains used in these experiments are derived from strain 15Dau (*MAT***a** *ade1 his2 leu2-3,112 trp1 Δura3*), which is congenic to strain BF264-15D described previously (40). The *MAT***a**/α diploid strain JH-O44, used to assay the growth of diploid cells overexpressing *STE4* (see Fig. 2C), was made by expression of the *HO* gene from the *GAL1* promoter in strain 15Dau. Expression of *HO* from this plasmid causes cells to switch mating type (43). Cells that switched and mated with other cells were isolated as diploid zygotes. *sst2* mutants were generated by transformation of 15Dau cells with an integrating plasmid containing an internal fragment of the *SST2* gene (kindly provided by William Courchesne). Digesting this plasmid with the restriction enzyme *Sst*II, followed by transformation of yeast cells (15), generated a tandemly deleted *SST2* gene in the yeast genome.

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Constructions for conditional overexpression of yeast G protein subunits were in plasmid YCpGAL2, a centromere plasmid derived from YCpGAL1p::CDC28 (27), from which the coding sequences of *CDC28* were deleted, but leaving sequences 3′ to *CDC28*, presumably required for transcriptional termination and polyadenylation. YCpGAL2 contains both the *LEU2* and *URA3* markers. Cloning coding regions of genes into a unique *Bam*HI site places them under the transcriptional regulation of the yeast *GAL1* promoter (18).

The *STE4* and *STE18* genes were tailored to have *Bam*HI sites at their 5′ and 3′ termini by amplification of sequences contained in a yeast YEp13 genomic library (35). Amplification was carried out by a modification of the polymerase chain reaction (44), using oligonucleotide primers (Genetic Designs, Inc., Houston, Tex.) that incorporate a *Bam*HI site at their 5′ ends. *Bam*HI fragments containing only the coding regions of *STE4* and *STE18* (52) were then cloned directly into the *Bam*HI site of YCpGAL2. To verify that polymerase chain reaction-amplified sequences represent true clones of *STE4* and *STE18*, these constructs were tested for the ability to complement null mutations of *ste4* and *ste18* on galactose medium. Each construct rescues the mating defect of its cognate mutant.

*GPA1* was cloned as a 1.9-kilobase *Eco*RI fragment into pT7T3 19U (Pharmacia Inc., Piscataway, N.J.). A *Bam*HI site was inserted nine nucleotides 5′ to the ATG of the *GPA1* gene by site-directed mutagenesis of the wild-type sequence (8, 32). The entire *GPA1* gene could then be excised from PT7T3 19U as a *Bam*HI fragment and inserted into the *Bam*HI site of YCpGAL2. YCpGAL STE4 is YCpGAL2 with *STE4* inserted into the *Bam*HI site. YCpGAL STE18 has *STE18* at the *Bam*HI site, and YCpGAL GPA1 has *GPA1* inserted into the *Bam*HI site. To express two plasmids containing different G protein subunits simultaneously, the *LEU2* or *URA3* marker was inactivated separately in each. This was done by cutting at a unique *Nco*I site (*URA3*) or a *Bst*EII site (*LEU2*) in the coding regions of these genes, filling in the overhang with the large fragment of DNA polymerase, and religating the plasmid. This generates a frameshift of -1 in *URA3* and -2 in *LEU2*.

A *ste18* disruption was generated by inserting the *LEU2* *Pst*I fragment from plasmid YEp13 (3) into a unique *Nsi*I site in *STE18* (52) and then replacing the chromosomal copy of *STE18* with the disrupted copy (42).

**Galactose induction of G protein subunit genes.** All media were based on synthetic complete SD medium (46). For a neutral noninducing medium, sucrose (2%) rather than glucose was used as the carbon source. For induction of G-protein subunit genes, galactose (2%) was substituted for glucose. To maintain various plasmids, either leucine or uracil or both were omitted from the medium. Strains to be induced were grown to mid-log phase in noninducing sucrose medium at 30°C, pelleted in a centrifuge, washed once in inducing galactose medium, and resuspended in inducing medium. Growth was continued at 30°C.

For *FUS1* induction assays, samples of total RNA were prepared from cells grown in noninducing medium for 2.5 h after transfer to inducing medium. Total RNA was prepared as previously described (6). A 10-μg sample of RNA was loaded onto 1% denaturing formaldehyde gels, run at 3 V/cm for 2 h, and blotted to GeneScreen Plus nylon membranes (DuPont NEN Research Products, Boston, Mass.). Hybridization to a probe specific for the *FUS1* transcript (26, 50) was carried out for 16 h at 42°C in 50% formamide–1% sodium dodecyl sulfate–1 M NaCl–10% dextran sulfate. Quantitation of the RNA sample was verified by stripping the *FUS1* probe from the membrane and rehybridizing with a probe that recognizes the *HIS3* and *DED1* transcripts (47). These transcripts are not affected by galactose induction. Autoradiography was on Kodak XAR-5 film with one intensifying screen (Cronex Lightning-Plus; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Autoradiographs were quantified by using an Ultrascan XL laser densitometer (LKB Instruments, Inc., Rockville, Md.).

**α-Factor sensitivity and recovery assays.** For the growth inhibition or halo assays shown in Fig. 4, strains to be assayed were grown overnight in galactose inducing medium. Leucine was omitted from the medium for strains carrying YCpGAL GPA1 to maintain plasmids. A total of $10^5$ cells were mixed in melted galactose agar (galactose inducing medium with 2% Bacto-Agar [Difco Laboratories, Detroit, Mich.]) and poured onto plates of the same medium. Synthetic α-factor (a gift from Richard Houghton) was then spotted on the surface of the solidified top agar at various concentrations in 3 μl of sterile water. Two sets of plates for each were analyzed. Plates were incubated at 30°C for 2 days.

**Other methods.** Cell growth was monitored on a hemacytometer (Reichert). Photomicrographs of yeast cells were taken by using an ×100 objective with a Zeiss Axiophot photomicroscope fitted with differential interference contrast (Nomarski) optics.

## RESULTS

**Overexpression of *STE4* leads to a pheromonelike response.** The yeast *STE4* gene encodes a product homologous to the $\beta_1$ and $\beta_2$ subunits of bovine and human transducin, whereas the *STE18* product shows less, but still significant, homology to the bovine γ transducin subunit (52). To obtain conditional, high-level expression of these genes in yeast cells, we placed each under the control of the yeast *GAL1* promoter (18). This promoter is transcriptionally inactive during growth on neutral carbon sources such as sucrose or raffinose. Transferring cultures grown on these media to galactose medium rapidly derepresses the *GAL1* promoter, leading to transcriptional induction up to 1,000 times the uninduced level.

The effects of high-level expression of the *STE4* and *STE18* genes on the morphology and growth of haploid yeast cells are shown in Fig. 1. Wild-type strains responding to mating pheromone arrest as unbudded cells in the $G_1$ phase of the cell cycle and form projections to assume distended, irregular shapes known as shmoos. Strain 15Dau (*MAT***a**) cells harboring either YCpGAL STE4 or YCpGAL STE4 and YCpGAL STE18 together acquired a similar phenotype after switching from a neutral carbon source (sucrose) to galactose medium (Fig. 1C, D, I, and J). These effects were apparent within 4 h after transfer to galactose medium. The photomicrographs in Fig. 1 were taken after 8 h of incubation in galactose medium, when projection formation had become pronounced. By this time, >99% of the cells in cultures overexpressing *STE4* or *STE4* and *STE18* together had arrested in $G_1$ and formed projections. The extent of the response was suggestive of cells exposed to high α-factor concentrations. We did not observe a significant difference in either the rate of induction or the extent of the response as measured by either the cell cycle arrest phenotype or projection formation in cells overexpressing *STE4* versus those overexpressing both *STE4* and *STE18*. That is, overexpression of $G_\beta$ alone was sufficient to fully activate a response that resembled the response of wild-type haploid cells to mating pheromone.

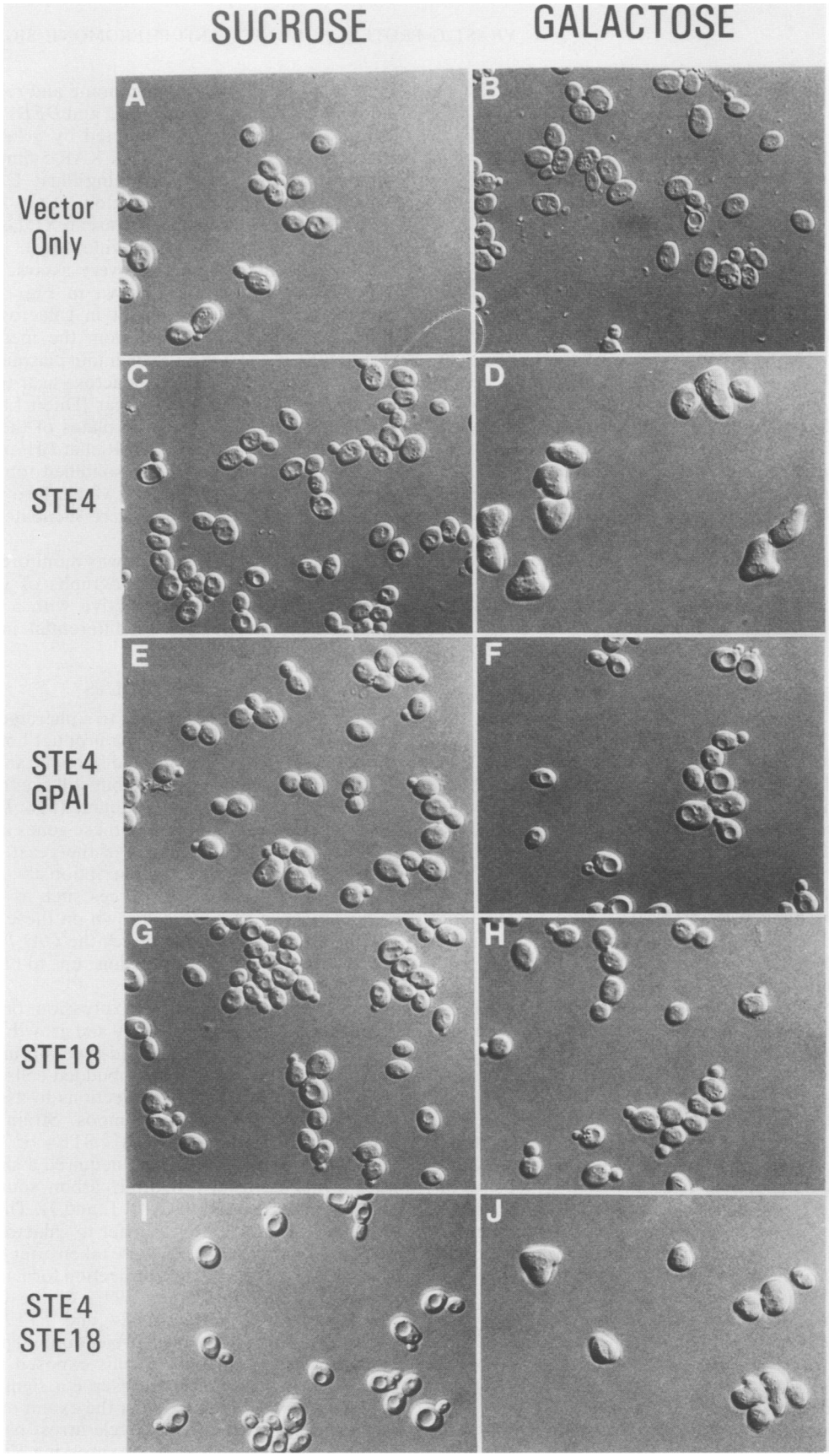


FIG. 1. Morphology of haploid yeast cells hyperexpressing G protein subunit genes. (Left) Cells carrying plasmids with various combinations of the *GPA1*, *STE4*, and *STE18* genes under the control of the *GAL1* promoter grown in noninducing sucrose medium. (Right) The same cells 8 h after transfer to inducing galactose medium. Photomicrographs of yeast cells were taken with a Zeiss Axiophot photomicroscope fitted with differential interference contrast (Nomarski) optics.

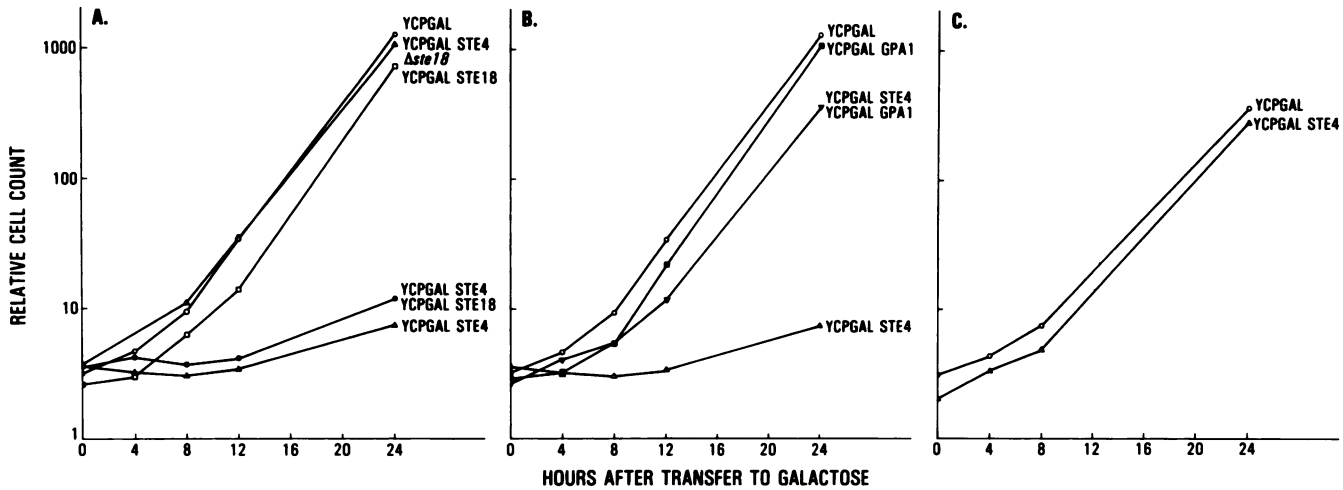


FIG. 2. Growth of yeast cells expressing G protein subunit genes. Genetic and plasmid configurations for each strain are indicated to the right of each curve. Cells were grown to mid-log phase in neutral sucrose medium and then transferred to inducing galactose medium. Samples were withdrawn at the indicated time points, and the cell density was determined. The ordinate is a logarithmic scale of arbitrary units. (A) Effects of *STE4* and *STE18* induction on the growth of haploid cells; (B) suppression of the growth defect of YCpGAL STE4 cells by concomitant overexpression of *GPA1*; (C) effects of *STE4* induction on the growth of a diploid *MAT***a**/α strain compared with growth of an isogenic diploid with a plasmid lacking the *STE4* insert.

Overexpression of *STE18* alone did not affect the growth or morphology of 15Dau cells. These cells continued to bud in galactose medium and did not assume an altered morphology (Fig. 1G and 1H). Similar results were obtained for wild-type control cells expressing plasmid YCpGAL with no insert (Fig. 1A and B). *MAT*α cells harboring YCpGAL STE4 also arrested in $G_1$ of the cell cycle and formed projections on galactose medium (not shown) with kinetics similar to those of the *MAT***a** cells depicted in Fig. 1.

The phenotypes of cells carrying *gpa1* and *ste4* mutations indicate opposing roles for these genes in mating, with *GPA1* having a negative regulatory function and *STE4* a positive role (8, 13, 16, 25, 29, 52). We therefore wished to determine whether overproduction of the putative $G_\alpha$ subunit encoded by *GPA1* could suppress the constitutive response of YCpGAL STE4 cells, which overproduce the $G_\beta$ subunit. To this end, we placed wild-type alleles of both *GPA1* and *STE4* under the control of the *GAL1* promoter on separate *CEN* plasmids and assayed cells carrying both plasmids for growth on galactose medium. These cells appeared normal, exhibiting none of the characteristic morphological alterations of cells constitutively overexpressing *STE4* (Fig. 1E and F). Cells appeared to bud normally and follow wild-type cell cycle growth and kinetics.

To analyze the effects of high-level expression of *STE4* more quantitatively, we monitored the growth of cells harboring YCpGAL STE4 alone or in combination with other plasmids. Figure 2 depicts the results of this analysis for a period of 24 h after transfer to galactose. 15Dau cells overexpressing either *STE4* or *STE4* and *STE18* together (Fig. 2A) executed a first-cycle arrest with 4 h of induction, followed by a partial resumption of cell division. Afterward, these cells grew very poorly on galactose, doubling only once during the 24-h period of observation. Cultures of these cells do eventually adapt to grow on galactose, although with a considerably longer doubling time (10 to 12 h) than for wild-type cells. In contrast to cells overexpressing *STE4*, cells expressing *STE18* alone under the control of the *GAL1* promoter grew at a rate indistinguishable from the wild-type rate, doubling eight times in 24 h.

If overexpression of *STE4* alone is capable of inducing a strong response in haploid cells to the same extent as hyperexpression of *STE4* and *STE18* together, it may be that *STE18* serves an accessory role in generating the signal, facilitating the interaction of the *STE4* product with a putative effector(s) although not essential for this interaction. If so, overexpression of *STE4* in a strain in which *STE18* has been mutationally eliminated may be capable of suppressing the sterile defect conferred by the loss of *STE18* function. However, we were unable to observe a response in YCp GAL STE4 cells disrupted for the chromosomal copy of the *STE18* gene (YCpGAL STE4 Δ*ste18*). This strain grew normally on galactose (Fig. 2A) and did not exhibit morphological alterations (not shown). In addition, it was unable to mate with a *MAT*α strain on either galactose or glucose medium and was growth insensitive to high concentrations of α-factor (not shown). These results argue that *STE18* plays an essential, rather than a merely accessory, role in the pheromone response pathway, and they suggest that in this system the β and γ subunits form a functional unit.

The morphology of cells overproducing both *GPA1* and *STE4* appeared normal (Fig. 1E and F), indicating that *GPA1* overproduction suppresses the constitutively activated phenotype of *STE4* overexpression. However, more quantitative growth analysis (Fig. 2B) indicated that these cells grew somewhat more slowly than did the wild type. Cells expressing both *GPA1* and *STE4* from the *GAL* promoter doubled approximately six times in 24 h, versus approximately eight times for cells expressing either YCpGAL with no insert or YCpGAL GPA1 alone. This corresponds to a doubling time significantly longer than for the wild type, about 4 h versus about 3 h. These growth curves suggest that overexpression of *GPA1* suppresses but does not entirely eliminate the pathway activation seen in cells constitutively expressing *STE4*.

The pheromone response is specific to haploid cells. Diploid *MAT***a**/α cells are insensitive to the mating pheromone of either haploid cell type because many genes encoding essential components of the response are transcribed only in haploid cells (14, 34). To determine whether consti-

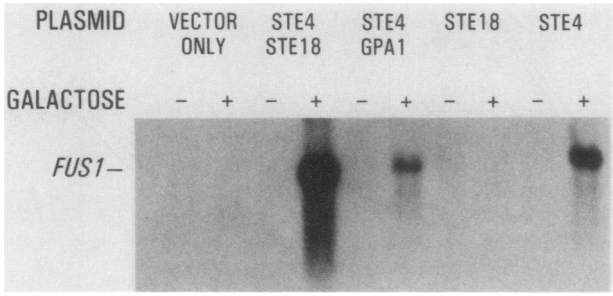


FIG. 3. Expression of *FUS1* mRNA in response to high-level expression of G protein subunit genes from the *GAL1* promoter. Blots of total RNA were prepared and hybridized to a *FUS1*-specific probe as described in Materials and Methods. Plasmid configurations and growth conditions (with [+] or without [−] galactose) are indicated above the lanes. Quantitation of samples was verified by stripping the *FUS1* probe from the blot and rehybridizing with a probe specific for *HIS3* and *DED1* (not shown).

tutive expression of *STE4*, which is not normally expressed in *MAT***a**/α diploids, is able to affect the growth of these cells, we analyzed the growth of *MAT***a**/α cells containing the YCpGAL STE4 plasmid in galactose medium (Fig. 2C). These cells grew normally on galactose relative to the same cells harboring the YCpGAL vector alone, doubling over seven times in 24 h. In addition, the morphology of diploid cells overexpressing *STE4* was identical to that of the control diploids (not shown), from which we conclude that overexpression of *STE4* is unable to activate a response signal in mating-type heterozygous cells.

***STE4* overexpression induces a pheromone-inducible transcript.** In wild-type haploid cells, treatment with pheromone induces the expression of a number of genes involved in mating (reviewed in reference 7), probably via a pheromone-responsive promoter element common to these genes (24, 51). One such inducible transcript is encoded by the *FUS1* gene (26, 50). The transcription of *FUS1*, which is involved in cell fusion of mating partners, increases more than 40-fold in response to pheromone. If high-level *STE4* expression is capable of mimicking pheromone stimulation, it should result in transcriptional induction of *FUS1*. We therefore assayed induction of the *FUS1* transcript by RNA blot analysis in response to overexpression of *STE4*, *STE18*, or both (Fig. 3).

All cells utilizing a neutral carbon source, sucrose, showed the same low basal level of *FUS1* transcription regardless of the plasmid they carried. However, 2.5 h after transfer to galactose, both the strain carrying YCpGAL STE4 and the strain carrying YCpGAL STE4 and YCpGAL STE18 together showed a dramatic increase in *FUS1* expression. *STE4* and *STE18* overexpression together induced *FUS1* mRNA levels about 70-fold, whereas expression of *STE4* alone increased *FUS1* message about 25-fold, as determined by densitometric analysis of the blot shown in Fig. 3. Interestingly, expression of both *STE4* and *STE18* from the *GAL* promoter yielded a slightly higher induction of *FUS1* (two- to threefold more) relative to values for cells expressing *STE4* alone. Overexpression of *STE18* alone did not activate *FUS1* transcription above control levels of cells containing plasmid with no insert. These results are consistent with the data for cell morphology and growth characteristics and indicate that overexpression of *STE4* alone in haploid cells is sufficient to activate the pheromone response pathway to a significant degree.

In cells overproducing both *GPA1* and *STE4*, *FUS1* expression is still induced approximately eightfold. The partial induction of *FUS1* observed here is consistent with the partial growth defect of the same cells (Fig. 2B). This level of induction is about three times less than that of cells overproducing *STE4* alone; those cells arrest growth and form projections. High-level *GPA1* expression thus acts to dampen the signal in these cells at some point upstream of transcriptional induction of pheromone-responsive genes. However, stimulation of downstream components of the pathway is not an all-or-nothing response; intermediate levels of activation are possible.

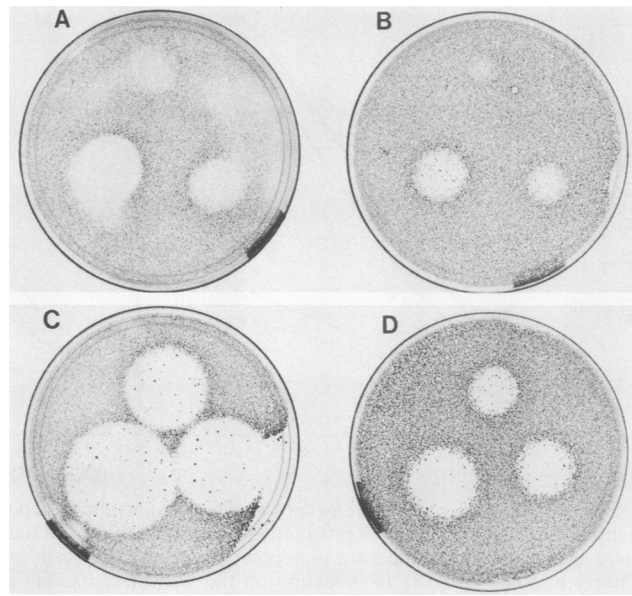


FIG. 4. Increased resistance to and recovery from pheromone effects for strains overexpressing *GPA1*. A total of $10^5$ cells from either wild-type *SST2* (A and B) or mutant *sst2* strains (C and D), either untransformed (A and C) or transformed with YCpGAL GPA1 (B and D), were mixed in inducing galactose top agar and plated on galactose plates as described in Materials and Methods. Synthetic α-factor was then spotted onto the surfaces of plates in sterile water in fivefold increments of absolute amount. For each plate, clockwise from the top α-factor spot, 240 ng, 1.2 μg, and 6 μg of α-factor were added. Plates were incubated at 30°C for 2 days.

**Constitutive expression of *GPA1* affects sensitivity to and recovery of cells from pheromone.** Wild-type yeast cells are able to adapt to the presence of pheromone and continue growth and division (31). To characterize more completely the role of *GPA1* in the mating-pheromone response, we analyzed the sensitivity and recovery of cells overproducing *GPA1* to pheromone-induced growth arrest. This was done by a growth inhibition zone or halo assay (Fig. 4). This technique generates a gradient of α-factor pheromone concentration from a single point of concentrated pheromone dotted onto the surface of a lawn of tester cells and allowed to diffuse through the agar medium. It therefore yields a quantitative assessment of the sensitivity or recovery of cells exposed to pheromone (19, 41). Wild-type cells of strain 15Dau were treated with increasing doses of synthetic α-factor, 240 ng, 1.2 μg, and 6 μg (Fig. 4A, clockwise from the top of the plate). An identical lawn of the same strain transformed with plasmid YCpGAL GPA1 was exposed to the same doses of α-factor (Fig. 4B). As can be seen, the cells overexpressing *GPA1* required about five times as much α-factor to generate a zone of growth inhibition equivalent in diameter to that produced by wild-type cells.

The halos corresponding to the two highest doses for wild-type cells were approximately the same size as those for the two lowest doses for YCpGAL GPA1 cells. At the lowest pheromone concentration, cells induced for *GPA1* did not form a pronounced halo. After 2 days of incubation at 30°C, the halos for YCpGAL GPA1 cells became turbid and filled in with growing cells, whereas the same-size halos in wild-type cells remained clear. This effect cannot be explained as merely a result of increased resistance to pheromone, since the renewed growth within the halos of YCpGAL GPA1 cells was qualitatively different from that of wild-type cells with the same initial level of resistance to lower pheromone concentrations. The results argue instead that cells overexpressing *GPA1* activate an adaptive response to pheromone.

This conclusion appeared to be validated by experiments on *sst2* mutants carrying YCpGAL GPA1. *sst2* mutants are significantly more sensitive than wild-type cells to pheromone and are unable to recover from pheromone-induced growth arrest (5, 9). When *sst2* cells (created by deletion of the chromosomal *SST2* locus in the wild-type 15Dau strain) were treated with low doses of pheromone (Fig. 4C), large halos of growth inhibition were observed. The same strain induced for *GPA1* showed a significant suppression of the *sst2* phenotype (Fig. 4D). These cells were about 100 times more resistant to α-factor, as measured by halo diameter. *GPA1* overexpression appeared to very nearly completely suppress the *sst2* phenotype, since the halos in Fig. 4D are only slightly larger than those in Fig. 4A, the wild-type strain. However, the mutant *sst2* strain expressing inducible *GPA1* did not show the renewed growth of cells within the halos seen in Fig. 4B. The colonies seen inside the halos of both the *sst2* and *sst2* YCpGAL GPA1 strains are spontaneous pheromone-resistant mutants, as we determined by retesting three colonies growing inside the largest halo of each plate (not shown). Such mutants occur at high frequency in *sst2* cells (5). Similar assays on colonies taken from within the halos of wild-type cells overexpressing *GPA1* yielded halos equivalent in diameter to those of the initial assay (not shown), indicating that these cells are not pheromone-resistant mutants. In addition, *GPA1* overexpression suppresses the pheromone-sensitive phenotype of mutants carrying *ste2*T-326 (23), a truncation of the α-factor receptor (halo assays not shown).

Previous genetic data have indicated that *GPA1* and *SST2* act independently in the generation of a desensitization response to pheromone (28). Also, cells carrying both *ste2*T-326 and *sst2* mutations are significantly more sensitive than cells with either single mutation, suggesting that pheromone receptor and *sst2* act independently in desensitizing to the pheromone signal. Our results show that hyperexpression of *GPA1* is capable of compensating for a defect in an adaptive response dependent on either *SST2* or *STE2* receptor.

## DISCUSSION

A number of independent lines of evidence indicate that the putative βγ-subunit complex encoded by the *STE4* and *STE18* genes in yeast cells functions as the positive transducer of the signal generated by pheromone-receptor interaction (8, 16, 32, 33, 52). Our results corroborate these conclusions and extend them significantly. We found that conditional high-level expression of either *STE4* or *STE4* and *STE18* together yielded constitutive activation of the pheromone response. Overexpression of *STE18* alone did not activate the response. Therefore, *STE4* is probably limiting for the response in wild-type cells, with the intracellular concentration of the *STE4* gene product likely to be lower than that of the *STE18* product. This is no longer the case when *STE4* is overexpressed, since in this case overexpression of *STE18* further potentiates the response. It is clear that both *STE4* and *STE18* are essential for the response, since high-level *STE4* expression has no effect in the absence of *STE18*. *STE18* could be required for proper localization of *STE4* to the plasma membrane, since it contains a conserved cysteine-aliphatic-aliphatic-X motif at its carboxy terminus (52). This consensus motif has been shown to be required for membrane localization of mammalian *ras* proteins, probably via a palmitylation or farnesylation of the cysteine residue (45; reviewed in reference 1). Alternatively, *STE18* could interact directly with an effector in conjunction with the *STE4* product.

The role of *GPA1* in the pheromone response appears more complex. Hyperexpression of *GPA1* suppressed the constitutive response phenotype of *STE4* overexpression. At least two mechanisms for this suppression can be imagined. *GPA1* could attenuate the *STE4*-induced signal by sequestering the $G_{\beta\gamma}$ subunit complex into an inactive $G_{\alpha\beta\gamma}$ trimer, or activated *GPA1* alone could be capable of generating an adaptive response to pheromone. In in vitro mammalian systems, $G_{\alpha}$ and $G_{\beta\gamma}$ subunits have been demonstrated to modulate antagonistic responses to signaling in two distinct ways: (i) excess $G_{\beta\gamma}$ is capable of inhibiting $G_{s\alpha}$-stimulated adenylate cyclase indirectly (4, 21, 38, 39), probably by promoting reassociation of $G_{s\alpha}$ and $G_{\beta\gamma}$, and (ii) $G_{\beta\gamma}$ alone inhibits adenylate cyclase either directly (although at concentrations too high to be physiologically relevant) or through interaction with calmodulin (20, 22). The antagonism to pheromone stimulation observed in cells overexpressing *GPA1* may be due to analogous effects. That is, *GPA1* could lead to an enhanced adaptive response both as a result of its ability to interact directly with $G_{\beta\gamma}$, and thus inactivate it, and as a consequence of a stimulation of an adaptive pathway (or inhibition of the signaling pathway). *GPA1* hyperexpression rendered cells about five times more resistant to pheromone than wild-type cells in growth inhibition assays. In addition, inhibition zones of *GPA1*-induced cells showed a significant level of subsequent growth not seen in equivalent-size wild-type inhibition zones. These results suggest that high-level *GPA1* expression leads to a qualitatively different response as well as a quantitative reduction in sensitivity to pheromone. Also, overproduction of *GPA1* suppressed the supersensitive phenotypes of both an *sst2* mutant and a *ste2*T-326 receptor truncation. Since both *SST2* and *STE2* have been demonstrated to function in the recovery of cells exposed to pheromone, the suppression we observed suggests that *GPA1* may be involved in a similar process. Indeed, *GPA1* was originally isolated by one group on the basis of its ability to rescue the supersensitive phenotype of an *sst2* mutant when on a multicopy plasmid (8).

The hypothesis that *GPA1* may interact with a component other than $G_{\beta\gamma}$ in an adaptive response is supported by an analysis of a mutation of *GPA1* homologous to a constitutively activated mutation in *RAS2*, $RAS2^{Val\text{-}19}$ (28). This mutation, $GPA1^{Val\text{-}50}$, exhibits two distinct phenotypic responses to pheromone: supersensitivity upon initial exposure, but enhanced adaptation and renewed growth over time. The supersensitive phenotype of $GPA1^{Val\text{-}50}$ is recessive, whereas the adaptation phenotype is dominant to wild type. Assuming that $GPA1^{Val\text{-}50}$ behaves analogously to activated *ras*, that is, as a constitutively activated GTP-binding protein with greatly reduced GTP hydrolysis, the

dominant adaptation phenotype is difficult to reconcile with a simple sequestration model. If the sole function of *GPA1* is to sequester $G_{\beta\gamma}$ in an inactive $G_{\alpha\beta\gamma}$ heterotrimer, *GPA1*$^{Val-50}$, which presumably cannot bind $G_{\beta\gamma}$, should be recessive to wild-type *GPA1*, which can bind $G_{\beta\gamma}$. These results are more consistent with *GPA1* stimulation of an adaptive pathway independent of its role of sequestering $G_{\beta\gamma}$ in its inactive GDP-bound form.

The *GPA1*-mediated partial suppression of the constitutively activated *STE4*-overexpressing cells is interesting, since it demonstrates that the pathway is capable of existing in states other than fully active or inactive. As such, cells are capable of exhibiting a graded response to pheromone, depending on the strength of the signal. It has previously been observed that different concentrations of α-factor are required to activate various phenotypic aspects of the pathway. Agglutination induction (presumably as a result of increased agglutinin transcription [11]) requires low concentrations of α-factor ($\sim 10^{-12}$ M), whereas cell cycle arrest requires several orders of magnitude more α-factor ($\sim 10^{-10}$ M), and projection formation requires even more ($\sim 10^{-8}$ M) (17, 30). Our results reflect this asymmetric response, since the eightfold induction of *FUS1* levels in cells containing YCpGAL GPA1 and YCpGAL STE4 is still inadequate for cell cycle arrest and projection formation. However, only a small increase in *FUS1* expression (three times this amount, the 25-fold increase seen in YCpGAL STE4 cells) correlates with $G_1$ arrest and projection formation.

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