Truncated Forms of a Novel Yeast Protein Suppress the Lethality of a G Protein α Subunit Deficiency by Interacting with the β Subunit*

(Received for publication, June 1, 1995, and in revised form, August 18, 1995)

Brian H. Spain†, Derrick Koo, Meenakshi Ramakrishnan, Bartholomew Dzudzor, and John Colicelli§

From the Department of Biological Chemistry and Laboratory of Structural Biology and Molecular Medicine, UCLA School of Medicine, Los Angeles, California 90024

In Saccharomyces cerevisiae, the mating pheromone-initiated signal is transduced by a heterotrimeric G protein and normally results in transient cell cycle arrest and differentiation. A null allele of the Gα (GPA1/SCG1) subunit results in cell death due to unchecked signaling from the Gβγ (STE4, STE18, respectively) heterodimer. We have identified three high copy suppressors of gpa1 lethality. Two of these genes encode known transcription factors, Mata2p and Mcm1p. The third is a truncated form of a novel gene, SYG1. Overexpressed wild type SYG1 is a weak suppressor of gpa1. In contrast, the isolated mutant allele SYG1-1 is a strong suppressor that completely blocks the cell cycle arrest and differentiation phenotypes of gpa1 cells of both mating types. One deletion mutant (SYG1Δ340) can suppress the cell cycle arrest associated with gpa1, but the cells retain a differentiated morphology. SYG1-1 can suppress the effects of overexpressed wild type Gβ but is not able to suppress the lethality of an activated Gβ mutant (STE4HPl). Consistent with these genetic observations, the suppressing form of Syn1p can interact with the STE4 gene product, as determined by a yeast two-hybrid assay. SYG1-1 is also capable of promoting pheromone recovery in wild type cells, as judged by halo assay. SYG1 predicts eight membrane-spanning domains. Deletion mutants of SYG1 indicate that complete gpa1 suppression requires removal of all of these hydrophobic regions. Interestingly, this truncated protein localizes to the plasma membrane-enriched subcellular fraction as does full-length Syn1p. Three hypothetical yeast proteins, identified by their similarity to the SYG1 primary sequence within the gpa1 suppression domain, also appear to have related structures. The properties of Syn1p are consistent with those of a transmembrane signaling component that can respond to, or transduce signals through, Gβ or Gβγ.

† This work was supported by National Institutes of Health Grant RO1 CA 56301 and Department of Energy Contract DE FC03 87ER60615. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Supported in part by United States Public Health Service National Research Service Award GM-07104.
§ To whom correspondence should be addressed. Tel.: 310-206-7800; Fax: 310-825-9433; E-mail: colicelli@bes.medsch.ucla.edu.
with the plasma membrane and that it acts to block G protein signaling at the level of Gβγ.

**MATERIALS AND METHODS**

Strains, Transformation, Selection, and Propagation Procedures—Strains are described in Table 1. Strains SP1 and RS22–6C were mated to make diploid strain SR. Strain SRgCU was created by sequentially transforming strain SR with a KpnI-blunted p416-SYG1-1 fragment from pgH into diploid strain SR. Strain SRgHU was created by inserting blunt-ended LEU2 fragments between the internal SspI sites of pUV1-SYG1-1 and replacing by the LEU2 marker, the HindIII/EcoRI fragment of SYG1-1 was removed from pKS-SYG1-1 and replaced by the LEU2 marker, the HindIII/EcoRI fragment of SYG1-1 was removed from pKS-SYG1-1 and replaced by the LEU2 marker. Each PCR product was sequenced and primer annealing positions were confirmed by DNA sequencing. Each product was sequenced and primer annealing positions were confirmed by DNA sequencing. Each product was sequenced and primer annealing positions were confirmed by DNA sequencing.

### Table 1

| Strain | Genotype | Ref. |
|--------|----------|------|
| SP1    | MATa ade8 his3 leu2 trp1 ura3 can1 |     |
| RS22-6C| MATa ade8 his3 leu2 trp1 ura3 can1 |     |
| FY250  | MATa ade8 his3 leu2 trp1 ura3 |     |
| FY251  | MATa ade8 his3 leu2 trp1 ura3 |     |
| SRgCU  | MATa/MATa ade8/EDE8 his3/3 his3 leu2/leu2 trp1/ TRP1 ura3/ura3 can1/can1 gpa1/HIS3/gpa1:URA3 | This work |
| GU1    | MATa ade8 his3 leu2 trp1 ura3 can1 gpa1:HIS3 plgC | This work |
| GU2    | MATa ade8 his3 leu2 trp1 ura3 can1 gpa1:HIS3 plgC | This work |
| LG1TG  | MATa ade8 his3 leu2 trp1 ura3 can1 gpa1:HIS3 ptcG | This work |
| LG2TG  | MATa ade8 his3 leu2 trp1 ura3 can1 gpa1:HIS3 ptcG | This work |
| GU15S  | MATa ade8 his3 leu2 trp1 ura3 can1 gpa1:HIS3 pUV1-SYG1-1 | This work |
| DE6    | MATa ade2 ade4 his4 leu2 lys2 trp3 ura3 cry1 can1 SUP4-3 | This work |
| pDJ117 |   | 8   |
| DBC    | MATa ade2 ade4 his4 leu2 lys2 trp1 ura3 cry1 can1 SUP4-3 | This work |
| STE411CpUV2-2C |   |      |
| GPY74-15Ca | MATa his4 or 6 leu2 trp1 ura3 sst1 | 63   |
| YDM400 | MATa ade2 ade4 his3 leu2 trp1 ura3 sst2 |     |
| DC14   | MATa his1 |     |
| DC17   | MATa his1 |     |
| DC224  | MATa ade8 his4 leu2 trp1 ura3 |     |
| SP1-SN | MATa ade6 his3 leu2 trp1 ura3 can1 syg1:: LEU2 | This work |
| FY250-SN| MATa his3 leu2 trp1 ura3 syg1::LEU2 | This work |
| L40    | MATa his3 leu2 trplYS2::leAop-HIS3 URA3::leAop-lacZ |     |

* Cold Spring Harbor yeast collection.
  † F. Winston, C. Dollard, and S. Ricapero-Hovasse, personal communication.
  ‡ D. Ma and J. Thorner, personal communication.
  § S. Hollenberg, R. Sterniglantz, and H. Weintraub, manuscript in preparation.

3 The abbreviations used are: SC, synthetic complete; MOPS, N-morpholinoethanesulfonic acid; kb, kilobase(s); PCR, polymerase chain reaction; bp, base pair(s); HA, hemagglutinin.
were assayed with the same result. plUV1-SYG1A340 was constructed by removing the 5'-Sad restriction fragment from plUV1-SYG1-1 using the internal Sad site and a polylinker Sad site. plUV1-SYG1199 was constructed by removing the 5'-NheI/NNot fragment from plUV1-SYG1-1 by digesting with Nol and NheI, blunting the termini with Klenow, and then ligating. For plUV1-SYG1A340 and plUV1-SYG1199, the sequence was provided with alkaline phosphatase-conjugated goat anti-rabbit antibody (Bio-Rad, 1:250 dilution). For the membrane stripping experiments, aliquots of the 10,000 x g pellets were diluted into 0.5 ml of stripping buffer (0.5 M NaCl, 2 mM urea, 10 mM Tris-HCl, pH 0.5, 0.5% mercaptoethanol, 0.5% Triton X-100, or 1% SDS) and incubated for at least 1 h on ice. The samples were then centrifuged at 10,000 x g for 10 min at 4°C. The pellets were resuspended in lysis buffer, and the supernatants were either concentrated at 4°C using Microcon-10 microconcentrators (Amicon) or acetone-precipitated.

Mating, Halo, Two-hybrid, STE4′′′′, and Overexpressed Wild Type

STE4 Suppression Assays—Quantitative mating assays were conducted essentially as described (20) except that approximately 105 cells of the tester strains DC14 or DC17 were used, and the experimental trials were cultured in plasmid maintenance synthetic medium before being switched into YPD medium. Mating efficiencies were calculated as the ratio of diploid colonies formed to the titer of experimental haploid cells used.

Halo assays were performed essentially as described (21) except that the NH2-terminal strains were cultured as for the quantitative mating assay and approximately 5 x 106 cells were inoculated into either SC or YPD soft agar which was poured onto the corresponding media. Results shown used the YPD conditions which facilitated a slightly enhanced response to a-factor over the synthetic medium conditions. Synthetic a-factor (Sigma) was added to the filter discs in a volume of 10 μl of water.

FUS1 induction analyses were done using the bar1strain strain GPY74–15Ca which was treated with a-factor at a concentration of 1 μg/ml. After 2 h of incubation, >80% of the cells were unbudded. Time points for RNA analyses were taken prior to addition of pheromone (T = 0) and 15, 60, 120, and 180 min after addition of pheromone. FUS1 induction assays were measured by reverse transcribed-PCR occurred as described (22). FUS1 induction was also measured from a 2 μg based FUS1-lacZ reporter construct, pSB234 (23). This was transformed into GPY74–15Ca cells, followed by plUV1, plUV1-SYG1-1, YEPα2, and YEP-MCM1. Cells were grown overnight in selective media, switched to YPD, treated with a-factor for 1 h, lysed, and assayed for β-galactosidase activity (24). The two-hybrid β-galactosidase liquid assays were done similarly except that lysis was accomplished by two cycles of freeze-thaw using liquid nitrogen. To test for His complementation, individual transformants were patched on media selecting for each of the two introduced plasmids and then replica plated to media that also selected for HIS3 expression. The patches on His selection media were replica plated a second time onto the same media to eliminate background growth. Under assay, β-galactosidase assays were done essentially as described.

Halo STE4” suppression was assayed by transforming DBC cells, culturing them for 1–2 days in SC-leucine media, and then plated 1–10 μl of culture onto SC-leucine-arginine+ canavamine media to select for loss of the pU2C maintenance plasmid. Suppression of overexpressed wild type STE4 was done essentially as described (10) except that individual transformants were first patched onto media containing sucrose and subsequently replica plated onto media containing galactose (3%). To enhance the growth difference, these were replica plated onto the same media a second time.

RNA Analysis, Hybridizations, Phage Library Screening, Sequencing, and PCR—Total RNA was prepared using the hot phenol method (26). For Northern analysis, total RNA was fractionated on a 0.8% agarose, 2% formaldehyde gel prior to transfer to reinforced nitrocellulose. Reverse transcribed-PCR studies employed conditions as described (27) except that 2 μg of total RNA was used for cDNA synthesis and an annealing temperature of 54°C was used for all PCR reactions. The number of PCR amplification cycles and agarose percentage required for sufficient amplification varied depending on the template. The upstream and downstream primers, respectively, and the fragment sizes were as follows: ACT1 (5’T-GAGTGAACGTGGTTAC-3’), 750 bp; TTP1 (5’T-TCCTGGAGAAGGATAGAT-3’), 500 bp; STE2 (5’T-GATGCTGAGCTGATATC-3’), 800 bp; CACATGCACTCCT-GATC-3’), 750 bp; STE3 (5’T-GCTAAGGAGCTTGTAG-3’), 1000 bp; STE4 (5’T-ACTAGGAGCTGAGCTGATATC-3’), 800 bp; YEPα2 (5’T-CACCACTGGTTAATACCCC-3’), 375 bp.

Pheromone Signal Suppression

2 P. L. Bartel and S. Fields, unpublished results.
The S. cerevisiae cDNA library used was created by Jeff Kuret (Cold Spring Harbor Laboratory) (28). Standard screening and hybridization conditions were used. Sequencing was performed using dideoxy termination reactions. Data base searches were performed using the BLAST algorithm (29).

RESULTS

Isolation of gpa1 Suppressors—To identify genes involved in regulating the pheromone response in S. cerevisiae, a genetic selection for yeast sequences capable of rescuing gpa1 cells was undertaken using a plasmid exchange protocol. Strain GU1 has a gpa1 null allele but is viable because it has an extrachromosomal copy of GPA1 carried on the plasmid pLG3, which also encodes a positive selectable marker (LEU2) and a negative selectable marker (CAN1). GU1 cells were transformed with a library of yeast genomic fragments carried on a 2µ-based high copy plasmid. Approximately 5 x 10^7 transformants were obtained from seven separate transformations. These cells were grown in synthetic media which selected for the library plasmid but allowed for the loss of pLG3. Transformants which lost the plasmid were identified using the negative selectable marker. Plasmid DNA was isolated from individual colonies and used to transform fresh GU1 cells which were then tested for suppression of gpa1 lethality.

A total of seven independent plasmids that could suppress gpa1 were isolated. Following restriction mapping, cross-hybridization and sequence analysis, it was determined that five clones contained the MATα2 gene and one clone carried MCM1. The remaining clone was found to contain a portion of a novel gene, SYG1 (suppressor of yeast gpa1). The cloned form of the gene, SYG1-1, was also capable of suppressing gpa1 in an α strain (GU2) that is otherwise isogenic with GU1. This demonstrated that suppression is likely to involve a generalization of the cell cycle arrest and not a mating type-specific interference.

Sequence analysis revealed that SYG1-1 had a 417-amino acid open reading frame that is a carboxyl-terminal truncation of SYG1 (Fig. 1). A portion of this sequence was used as a probe to isolate the remainder of the SYG1 coding region from a S. cerevisiae cDNA library. Four distinct SYG1 cDNAs were isolated and sequenced. All were colinear with the original SYG1-1 clone and contained additional 3′ sequences that extended the open reading frame to 902 amino acids (Fig. 1B). The cDNA sequences were used to reconstruct a full-length SYG1 coding region attached to the SYG1 promoter. Full-length SYG1, expressed from the same high copy expression plasmid as SYG1-1, was an extremely weak suppressor of gpa1. Relative to SYG1-1, suppression by SYG1 resulted in fewer colonies (Fig. 2), and these took twice as long to appear.

A striking feature of the SYG1 primary sequence was revealed by hydropathy and hydrophobic moment analyses. As shown (Figs. 1 and 3), several distinct hydrophobic domains are located within the full-length protein, and eight of these are predicted to span the membrane. The eighth domain was noted to be amphiphilic, raising the possibility that it may form a pore. The truncation giving rise to SYG1-1 resulted in disruption of the first hydrophobic domain and deletion of the others. This suggested that removal of these domains conferred upon SYG1-1 its potent gpa1 suppression activity.
Northern analysis (Fig. 4) showed that SYG1 is expressed as a 2.8-kb transcript in both a and a haploids as well as in a/a diploids. Expression in GU1 and GU2 cells which overexpress GPA1 (lanes 1 and 2), appeared to be slightly repressed compared to levels in a wild type haploid cell (lane 4) or a diploid cell (lane 3), suggesting possible regulation by some component(s) of this pathway. Two smaller transcripts also appeared on the blot. These are likely to be SYG1 derived since they were eliminated in a syg1 deletion strain (lane 5). No change in SYG1 expression level was seen following treatment with α-factor (data not shown).

Strains that carry a syg1::LEU2 mutation showed no noticeable change in mating efficiency. These cells did not display any generalized defect in cell growth under normal conditions nor when exposed to stresses including growth at 37 °C, salt shock treatment at 55 °C, and hyperosmotic media. They were also able to switch to alternate carbon sources, undergo meiosis, and give rise to viable spores. We considered the possible existence of genes that are functionally redundant with SYG1 and can thereby mask the effects of the syg1 null mutation. Although we have not detected any related sequences using low stringency hybridizations, four sequences with significant, though limited, similarity to SYG1 were identified through a data base search (Fig. 1C). The two regions of similarity with SYG1 lie within the sequence of SYG1-1. In addition, three of these sequences (N2052, J0336, and YCR524) encode predicted proteins that, like SYG1, have amino-terminal spans of about 400 hydrophilic residues followed by multiple strongly hydrophobic domains. Overexpression of the amino termini of these proteins does not confer any detectable gpa1 suppression, however (data not shown).

We performed a carboxyl-terminal deletion analysis of SYG1 to further examine structure-function relationships (Fig. 5). SYG1Δ554, SYG1Δ519, and SYG1Δ464 showed no gpa1 suppression activity in either GU1 or GU2 cells. These constructs, then, did not display even the very weak suppression seen with full-length SYG1, perhaps due to altered protein conformation. The SYG1Δ400 mutant, in which all hydrophobic domains were eliminated, rescued gpa1 in both GU1 and GU2 as effectively as SYG1-1 (Fig. 1Δ17). The SYG1-1 and SYG1Δ400 rescued cells showed normal morphologies (Fig. 6, data not shown for SYG1Δ400). In contrast, deletion of 60 amino acids from SYG1Δ400 produced a surprising result. SYG1Δ340 was capable of suppressing the growth arrest associated with gpa1, but not the differentiation. These cells were able to undergo mitosis while maintaining a shmoo morphology (Fig. 6). Deletion of another 141 amino acids (SYG1Δ199) prevented any rescue.

Subcellular Localization of Full-length and Truncated Syg1p—Initially, we hypothesized that the dramatic increase in gpa1 suppressor activity of SYG1Δ400 compared to full-length SYG1 was attributable to altered subcellular localization of the truncated protein. To test this model, crude extracts containing epitope-tagged Syg1pΔ400p and Syg1p were subjected to differential centrifugation. It should be noted that the epitope fusion product of SYG1Δ400 retained gpa1 suppression activity (data not shown). Immunological analysis of marker proteins confirmed organelar separation. Surprisingly, both Syg1pΔ400p and Syg1p localized to the particulate fraction, which was greatly enriched for plasma membrane (Fig. 7A). Therefore, altered subcellular localization of Syg1pΔ400p does not account for its potent gpa1 suppression.

This subcellular localization result suggested that Syg1pΔ400p may associate with another membrane-localized protein or contain its own membrane targeting signal, even though no such signal is predicted by the sequence. Treatment of the plasma membrane-enriched fraction with reagents (high salt, high pH, urea and β-mercaptoethanol) that typically disrupt peripheral protein-protein interactions did not release Syg1pΔ400p (Fig. 7B). Triton X-100 and SDS were used to detect the presence of protein-membrane interactions. 1% SDS completely solubilized Syg1pΔ400p, but 2% Triton X-100 was unable to do so for both Syg1pΔ400p (Fig. 7B) and Syg1p (data not shown).

SYG1-1 is a High Copy Suppressor in Mutant and Wild Type Cells—The ability to rescue gpa1 requires high level expression of SYG1-1. This was demonstrated by expressing SYG1-1 on a centromere-based plasmid that is maintained as a low copy...
episome. This construct did not suppress gpa1, demonstrating that high levels of Syg1p are needed.

To address the possibility that SYG1-1 was interfering with signal transduction by directly or indirectly altering expression of signaling components, we performed quantitative RT-PCR. High copy expression of SYG1-1 in the wild type strains SP1 and FY250, as well as in the gpa1 mutant strains SP1 and FY250, had no effect on mating type-specific expression of pheromone receptors (in a MATa background STE2 is expressed at normal levels and STE3 is not expressed, as in wild type cells) nor on the normal abundance of STE4 message (data not shown). In addition, overexpression of SYG1-1 did not appear to block the induction of FUS1 after pheromone treatment. This result was confirmed using direct detection of a FUS1 promoter-driven LacZ construct (23). As seen in Table II, expression of SYG1-1 only slightly dampens FUS1 induction. As expected, expression of MATα2 had a strong inhibitory effect. Thus, for all of these signaling components, we found no evidence that message regulation is significantly altered by SYG1-1.

The ability of SYG1 and SYG1-1 to modulate response to pheromone was tested using a halo assay. Application of pheromone was used to create a zone of growth inhibition that reflects the normal cell cycle arrest response following activation of the mating pathway. Although wild type cells with SYG1 gave normal halos, cells overexpressing SYG1 gave rise to halos that became partially filled with colonies (Fig. 8A). Colonies within the halo were visible soon after those that formed the lawn. A similar result was seen when GPA1 was overexpressed in wild type cells (Fig. 8B), probably due to the sequestering of Ste4p into an inactive complex with Gpa1p. In both cases, the diameter of the halo, representing the initial sensitivity to pheromone, was unchanged from wild type cells. These data are consistent with the FUS1-lacZ data in which SYG1-1 had little effect on initial FUS1 induction. Simultaneous overexpression of GPA1 and SYG1-1 did not further enhance the growth of cells within the halo (Fig. 8B). This indicated that Gpa1p and Syg1p may compete for the same target (Ste4p). It also suggested that there may be a limit to the level
of signal repression and/or adaptation enhancement that can be achieved in this way.

The turbid halo results indicated that SYG1-1 may function to relieve cell cycle arrest by stimulating adaptation. Sst1p and Sst2p mediate separate adaptation pathways which, when disrupted (sst1 or sst2), result in a supersensitive response to pheromone. The Bar1p/Sst1p protease normally contributes to pheromone recovery mediated by SYG1-1 in sst1 or sst2 cells whose diameters were unchanged (Fig. 8, C and D). These data indicated that SYG1-1 promotes pheromone recovery is independent of SST1p and SST2p.

For GPA1, the co-overexpression of full-length SYG1 did not alter the halo fill-in effect (Fig. 8B). Similarly, overexpression of non-suppressing truncations of SYG1 did not modulate pheromone recovery mediated by SYG1-1 (data not shown). In addition, syg1::LEU2 mutants showed no alteration in halo formation from wild type cells (Fig. 8E). In cells expressing SYG1-1 that are also gpai1, no halo is ever visible (Fig. 8G).

Expression of SYG1-1 had very little effect on mating efficiency. Both wild type cells (SP1) and GU1 cells transformed with pUV1-SYG1-1 mated at levels that were essentially unchanged from untransformed controls (within 2-fold, data not shown). gpai1 cells that carried the SYG1-1 suppressor did show reduced mating efficiency (about 20-fold, data not shown). Given these cells showed no pheromone response in a halo assay, it was surprising that they mated at this level of efficiency (no mating with cells of the same mating type was observed). This is, however, similar to the level of mating seen in gpai1 receptorless strains (33). Thus, our data confirm that gpai1 cells are indeed mating competent.

SYG1-1 Suppresses Excess Wild Type STE4 but Not a Dominant Active STE4—To genetically localize the effects of SYG1-1 action, we examined the ability of this allele to suppress the effects of high levels of wild type STE4. The overexpression of STE4 is known to give a phenotype similar to that of the gpai1 mutation, and it can be suppressed by the overexpression of GPA1 (10). We used an inducible construct in which STE4 overexpression and the resulting cell cycle arrest were triggered by galactose. As seen in Fig. 9, SYG1-1 was able to suppress the cell cycle arrest caused by STE4 overexpression, although the full-length SYG1 clone was not.

To test whether Syg1-1p might be suppressing through a direct interaction with Ste4p, or as is the case for Gpa1p, we examined the ability of this allele to suppress STE4D400p, a dominant mutant in the Gβ subunit (8). The STE4D400p mutant is lethal because the mutant protein is less able to bind Gpa1p and thereby escapes negative regulation, resulting in a constitutive signal for cell cycle arrest (9). This mutation is suppressed by the overexpression of GPA1p (34) or by the expression of the MATα locus when an a strain is used (8). Strain DBC was used to test for suppression of STE4D400p. Although both MATα2 and MCM1 constructs allowed for plasmid exchange, neither SYG1-1 nor SYG1 overexpression did (Fig. 10), indicating that STE4D400p is epistatic to SYG1-1 overexpression. The inability of SYG1-1 to block the effects of STE4D400p is likely due to the activation mutation itself (Gly1 to Asp at residue 124). This alteration appears to affect directly the interaction of Ste4p with Gpa1p (9) and might also prevent protein-protein interactions critical for SYG1-1-mediated suppression. The STE4D400p result, in conjunction with the overexpressed STE4 data, suggested that Syg1-1p suppresses gpa1 by interacting with Ste4p.

Suppressing Forms of Syg1p Can Physically Interact with Ste4p—The ability of Syg1-1p to bind Ste4p was examined using the two-hybrid reporter system (35). The LexA-coding sequence was fused to the carboxyl terminus of Syg1p400p, and the resulting hybrid construct was shown to retain gpa1 suppression activity (amino-terminal fusion constructs last this function). Fig. 11 shows that hybrid fusions of Syg1p400p or Gpa1p can interact with Ste4p to induce expression of a HIS3 reporter gene. The same constructs also induced lacZ expression as judged by a filter lift β-galactosidase assay (data not shown).

The interaction between Syg1p400p and Ste4p was quantitatively analyzed (Table III). This combination gave rise to signals that were consistently 10-fold above all combinations of vectors and non-interacting controls. However, signals for Gpa1p with Ste4p, which were similar to what has been reported (18), were significantly higher than those for Syg1p400p with Ste4p. This presumably results in part from Syg1p400p-LexA, like Syg1p400p, having a strong propensity for membrane attachment and thereby being resistant to nuclear localization. It may also reflect an inherently lower binding affinity. Also, although Syg1p400p does not appear to interact with Ste18p (Fig. 11, Table III), this or some other protein may strengthen Ste4p/Syg1p400p binding.

**DISCUSSION**

We have shown that the gpai1 mutation, which leads to constitutive Gβγ signaling, can be suppressed by overexpression of MCM1, MATα2, and SYG1-1. MCM1 and MATα2 are both known to encode transcription factors that control the
Figs. 10, 11. Suppression of STE44[+]

Fig. 10. Suppression of STE44[+]. DBC cells carrying the STE44[+]
mutation were transformed with the indicated plasmids (vector = pADNS, MCM1 = YEp-MCM1, SYG1 = pADNS-SYG1, a2 = YEp-a2). They were grown in liquid media for 2
and plated on selection media (SC-leucine-arginine+canavanine). Growth requires loss of the pU2C plasmid.

Fig. 11. Interaction between Syg14400p and Ste4p using a H133 reporter. Four independent L4 transformants were patched
onto plasmid-selecting media (SC-Leu-Trap) and then replica plated to
the same media or to media that also selected for His6p. The latter was double replica plated to eliminate background
growth. Following are the plasmids that correspond to the indicated
fusion gene products. For the GAL4 activation domain: pGAD2F.N (Gal4[768–881]) only, pKB40.1 (Gal4[768–
881]-Ste4p), and pGAD424-STE18 (Gal4[768–881]-Ste18p). For the LexA DNA binding domain: pBTM116 (LexA only), pCLA-SYG1400
(Syg11p-LexA), and pBMT116-GPA1 (LexA-Gpa1p).

TABLE III
Interaction between Syg14400p and Ste4p

| Fusion partners* | LexA-DNA binding | IaC activityb |
|------------------|------------------|---------------|
| GAL4 activation domain | Syg14400p | Ste4p | Gpa1p | Syg14400p | Ste18p |
|------------------|------------------|---------------|
| NF | + NF | 0.28 ± 0.09 | 0.31 ± 0.00 | 0.22 ± 0.05 | 0.30 ± 0.05 |
| NF | Syg14400p | 0.26 ± 0.04 | 0.31 ± 0.00 | 0.22 ± 0.05 | 0.30 ± 0.05 |
| Ste4p | + NF | 0.28 ± 0.04 | 0.31 ± 0.00 | 0.22 ± 0.05 | 0.30 ± 0.05 |
| Ste18p | + Gpa1p | 1.41 ± 0.06 | 2.85 ± 0.53 | 0.20 ± 0.03 | 0.10 ± 0.01 |

*Nonfusion (NF) vectors were pGAD2F.N and pBMT116. Syg14400p-LexA, LexA-Gpa1p, and Gal4[768–881]-Ste18p fusions were expressed from pCLA-SYG1400, pBMT116-GPA1 and pGAD424-STE18, respectively (see “Materials and Methods”). Gal4[768–881]-
Ste4 was expressed from pKB40.1 (18).

Values are in modified Miller units (24) and represent the mean ± standard deviation.

expression in α cells and for MCM1 expression in a cells, however, the basis for gpa1 suppression is not entirely clear. Both MCM1 and MATα2 have the capacity to work as het-
erodimeric partners and can act through interactions with
other transcription regulating proteins (37). Therefore, increased amounts of these proteins may result in a stoichiom-
tric imbalance resulting in blocked expression of signaling com-
ponents or induced expression of factors that suppress the
mating pathway.

Other cases of high copy or dominant suppressors of gpa1
have been reported, and all seem to function downstream of
Gβγ. The MSG5 gene encodes a protein phosphatase that,
when overexpressed, is apparently able to counteract some
protein phosphorylation events that propagate the mating sig-
nal (38). Expression of a mutant b-factor receptor (STE4A2F[+])
in a cells also suppresses the gpa1 mutation (39). The gpa1
suppression action of STE4A2F[+] is carried out downstream
of Gβγ and results in altered FUS1 expression (36). Other,
uncharacterized gpa1 suppressors have also been reported
(38, 40).

We have described the isolation and characterization of
SYG1-1 (a truncated form of the SYG1 gene) that is a novel
high copy suppressor of gpa1. SYG1 itself does not have ex-
pression characteristics common to many genes directly in-
volved in the mating pathway; its transcription is not induced
by pheromone nor is it restricted to haploids. Although SYG1
may not normally be involved in the pheromone response path-
way, we present data consistent with its participation in G
protein-mediated signaling.

The ability of SYG1-1 and SYG14400 to suppress the gpa1
mutation and overexpressed STE4 is most easily explained by
their gene products’ binding and sequestering Ste4p (Gq). This
interaction is at least partially dependent on substrate 124 of
Ste4p since the dominant STE4A4[+]
mutant allele is not sup-
pressible. Interestingly, this residue is also involved either
directly or indirectly in Gpa1p interactions (9). Although full-
length Syg1p probably has Ste4p binding capability (high copy
SYG1 is a weak suppressor of gpa1), the removal of putative
transmembrane domains from Syg1p is required for its strong
suppression of the pheromone pathway.

Despite lacking predicted transmembrane domains and a
signal sequence, Syg14400p localizes to the same plasma mem-
brane-enriched subcellular fraction as does Syg1p. That
Syg14400p can not be released by a panel of reagents that
disrupt protein-protein interactions suggested that it is tightly
associated with the plasma membrane. Furthermore, both
Syg14400p and Syg1p were insoluble in the nonionic detergent
Triton X-100, which disrupts hydrophobic but neither polar,
protein-protein, nor protein-lipid interactions (41), thereby
suggesting that the NH2-terminal portion of Syg1p is sufficient
to confer this characteristic upon Syg1p. The overall solubility
profiles for Syg14400p and Syg1p may be indicative of protein-
protein interactions involving the cytoskeleton or membrane
skeleton (42). Any interacting partner would, of course, need to
be sufficiently abundant to accommodate the high levels of
overexpressed Syg14400p and Syg1p. Alternatively, the Triton
X-100 insolubility of Syg14400p and Syg1p may be an inherent
characteristic, perhaps occurring by self-aggregation or by com-
plexing with other insoluble material. Further biochemical
studies should reveal the basis of Syg14400p association with
the plasma membrane and may help determine both the nor-
mal function of Syg1p and how Gβγ relates to that function.

In mammalian cells, as in yeast, Gβγ heterodimers bind
tightly to Gα proteins and mediate receptor coupling. Mamm-
alian Gβγ heterodimers are also involved in numerous cellular
processes mediated by direct interactions with a variety of

...
downstream effectors including some forms of adenylyl cyclase (43), ion channels (44, 45), phosphducin (46), PI3 kinase (47), and β-adrenergic receptor kinase (48, 49). As in mammalian cells, there may be multiple effectors responding to each Gβγ heterodimer in yeast. Data from studies of Gap2p, a Gap1-related Gγ protein known to activate adenylyl cyclase in yeast (50), support the existence of other Gβγ complexes. Gap2p is expressed not only in haploid cells but also in diploids which are devoid of Ste4p and Ste18p. In addition, overexpression of Gap2p does not suppress gap1 (51). These findings suggest that another Gβγ may indeed exist in yeast, a premise which should be settled by the S. cerevisiae genome sequencing project. We are currently using synthetic lethal and yeast two-hybrid screens to elucidate the normal function of Syg1p, to identify functionally redundant gene products, and to test whether interactions with Ste4p or any other Gβ or Gβγ proteins, play a role in Syg1p function.

We have demonstrated that in otherwise wild type cells, high level expression of SYG1-1 stimulates recovery from pheromone, as judged by halo assay. Many factors required for adaption and resumption of vegetative growth have been identified. These include pheromone receptors (21, 52), Sst2p (21, 43), Sst1 (21, 18), and this localization is consistent with a role in G protein interactions. In addition, the identification of genes structurally related to SYG1 suggests the existence of a family of genes with shared functional characteristics. Finally, truncation mutants of SYG1 should continue to prove useful in the analysis of signals leading to cell cycle arrest and differentiation and may also help reveal other G protein-mediated pathways.

Acknowledgments—We thank George Sprague, Steven Reed, Duane Jenness, Jeremy Thorner, David Meyer, Doreen Ma, Henrik Dhilman, and Malcolm Whiteway for providing various reagents and for useful discussions. We also thank Susanne Flügkiger Staub for technical assistance and Greg Payne and Harvey Herschman for their critical reading of the manuscript.

REFERENCES
1. Sprague, G. F. (1990) Adv. Genet. 27, 33–62
2. Herskowitz, I. (1989) Nature 342, 749–757
3. Kurjan, J. (1992) Annu. Rev. Biochem. 61, 1097–1129
4. Herskowitz, I. (1995) Cell 80, 187–197
5. Hirsh, J. P., and Cross, F. R. (1992) Biotechnology 10, 373–377
6. Dietzel, C., and Kurjan, J. (1987) Cell 50, 1001–1010
7. Miyajima, I., Nakafuku, M., Nakayama, N., Brenner, C., Miyajima, A., Katohshi, K., Ari, K., Kaziro, Y., and Matsumoto, K. (1987) Cell 50, 1011–1019
8. Blinder, D., Bouvier, S., and Jenness, D. E. (1989) Cell 56, 479–486
9. Whitleay, M., Clark, K. L., Leberer, E., Dignard, D., and Thomas, D. Y. (1994) Mol. Cell. Biol. 14, 3223–3230
10. Cole, G. M., Stone, D. E., and Reed, S. I. (1990) Mol. Cell. Biol. 10, 510–517
11. Peter, M., Gartner, A., Horecka, J., Ammerer, G., and Herskowitz, I. (1993) Cell 73, 747–760
12. Dolan, J. W., and Fields, S. (1990) Genes & Dev. 4, 492–502
13. Pillai, R., Kytle, K., Reyes, A., and Colicelli, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11970–11974
14. Brauch, J. R., Strathern, J. N., and Hicks, J. B. (1979) Gene (Amst.) 8, 121–133
15. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
16. Colicelli, J., Birdmeier, C., Michail, T., O’Neill, K., Riggs, M., and Wiger, M. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 3590–3593
17. Bartel, P. L., Chien, C.-T., Stenglaniz, R., and Fields, S. (1993) in Cellular Interactions in Development: A Practical Approach (Hartley, D. A., ed) pp. 153–170, Oxford University Press, Oxford
18. Clark, K. L., Dignard, D., Thomas, D. Y., and Whitleay, M. (1993) Mol. Cell. Biol. 13, 1–8
19. Blumers, K. J., Reneke, J. E., and Thorner, J. (1988) J. Biol. Chem. 263, 10836–10842
20. Hagen, D. C., McCaffrey, G., and Sprague, G. F. (1991) Mol. Cell. Biol. 11, 2592–2561
21. Reneke, J. E., Blumer, K. J., Courchesne, W. E., and Thorner, J. (1988) Cell 55, 221–234
22. McCaffrey, G., Clay, F. J., Kelsay, S., and Sprague, G. F. (1987) Mol. Cell. Biol. 7, 2680–2690
23. Trueheart, J., Boeke, J. D., and Fink, G. R. (1993) Mol. Cell. Biol. 7, 2136–2238
24. Guarente, L. (1981) Methods Enzymol. 171, 181–191
25. Cherevay, P. M., and Nathans, D. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5789–5793
26. Kohler, K., and Domdey, H. (1991) Methods Enzymol. 194, 398–405
27. Srinivasa, R. T., Gilbert, R. S., Xie, W., Lener, S., and Herschman, H. R. (1994) J. Cell Biol. 125, 192–200
28. Field, J., Vojtek, A., Ballester, R., Bolger, G., Colicelli, J., Ferguson, K., Gerst, J., Kataoka, T., Michaeli, T., Posers, S., Riggs, M., Rodgers, L., Wieland, I., Y-encoded, B., Wieland, L., and Wiger, M. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 1319–1327
29. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
30. MacKay, V. L., Welsh, S. K., Isley, M. Y., Manney, T. R., Holly, J., Saari, G. C., and Parker, M. L. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 55–59
31. Dietzel, C., and Kurjan, J. (1987) Mol. Cell. Biol. 7, 4169–4177
32. Wein, J. L., Guiterrez-Stel, C., and Blumer, K. J. (1993) J. Biol. Chem. 268, 4062–4077
33. Jang, K.-Y., Ferguson, J., and Reed, S. I. (1988) Mol. Cell. Biol. 8, 2484–2493
34. Zhang, M., and Tipper, D. J. (1993) Mol. Microbiol. 9, 813–821
35. Finkel, S., and Stenglaniz, R. (1994) Trends Genet. 10, 286–292
36. Hirsch, J. P., and Cross, F. R. (1993) Genetics 135, 943–953
37. Komachi, K., Redd, M. J., and Johnson, A. D. (1994) Genes & Dev. 8, 2857–2867
38. Do, K., Gartner, A., Ammerer, G., Errede, B., Shinkawa, H., Sugimoto, K., and Matsumoto, K. (1994) EMBO J. 13, 61–70
39. Cross, F. R. (1990) Genetics 126, 301–308
40. Kopp, J. B. (1993) Mol. Cell. Biol. 13, 8767–8788
41. Luna, E. J., and Hilt, A. L. (1992) Science 258, 955–964
42. Hare, J. F., and Hohol, A. (1994) J. Biol. Chem. 269, 5981–5988
43. Tang, W.-J., and Gilman, A. G. (1991) Science 254, 1500–1503
44. Logothetis, D., Kurachi, Y., Gally, J., Neer, E. J., and Clapham, D. E. (1987) Nature 325, 321–326
45. Widnall, K. D., Iniguez-Lluhi, J. A., Davenport, P. A., Taussig, R., Krakowsky, G. B., Linder, M. E., Gilman, A. G., and Clapham, D. E. (1994) Nature 370, 255–257
46. Lee, R. H., Ting, T. D., Lieberman, B. S., Tobias, D. E., Hitt, A. L., and Hitt, A. L. (1992) J. Biol. Chem. 267, 2321–2327
47. Stephens, L., Smrcka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C., and

Hawkins, P. T. (1994) Cell 77, 83–93
48. Haga, K., and Haga, T. (1992) J. Biol. Chem. 267, 2222–2227
49. Pitcher, J. A., Inglese, J., Higgins, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwatra, M. M., Caron, M. G., and Lefkowitz, R. J. (1992) Science 257, 1264–1267
50. Nakafuku, M., Obara, T., Kaibuchi, K., Miyajima, I., Miyajima, A., Itoh, H., Nakamura, S., Arai, K-I., Matsumoto, K., and Kaziro, Y. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1374–1378
51. Papasavvas, S., Arkinstall, S., Reid, J., and Payton, M. (1992) Biochem. Biophys. Res. Commun. 184, 1378–1385
52. Konopka, J. B., Jenness, D. D., and Hartwell, L. H. (1988) Cell 54, 609–620
53. Cole, G. M., and Reed, S. I. (1991) Cell 64, 703–716
54. Grishen, A. V., Weiner, J. L., and Blumer, K. J. (1994) Genetics 138, 1081–1092
55. Michaeli, T., Field, J., Ballester, R., O’Neill, K., and Wigler, M. (1989) EMBO J. 8, 3039–3044
56. Colicelli, J., Nicolette, C., Birchmeier, C., Rodgers, L., Riggs, M., and Wigler, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2913–2917
57. Han, L., and Colicelli, J. (1995) Mol. Cell. Biol. 15, 1218–1223
58. Elion, E. A., Grisafi, P. L., and Fink, G. R. (1990) Cell 60, 649–664
59. Chang, F., and Herskowitz, I. (1990) Cell 63, 999–1011
60. Schwartz, R. M., and Dayhoff, M. O. (1979) in Atlas of Protein Sequence and Structure (Dayhoff, M. O., ed) pp. 353–358, National Biomedical Research Foundation, Washington, D. C.
61. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
62. Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. (1984) J. Mol. Biol. 179, 125–142
63. Tan, P. K., Davis, N. G., Sprague, G. F., and Payne, G. S. (1993) J. Cell Biol. 123, 1707–1716