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# *GPA1*, A Haploid-Specific Essential Gene, Encodes a Yeast Homolog of Mammalian G Protein Which May Be Involved in Mating Factor Signal Transduction

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## Summary

***GPA1* protein of Saccharomyces cerevisiae is homologous to the α subunit of mammalian G protein. *GPA1* transcript was found in haploid cells but was not detected in diploid cells. Disruption of *GPA1* resulted in a haploid-specific lethal phenotype, indicating that *GPA1* is a haploid-specific essential gene for cell growth. Upon regulation of expression of *GPA1* by the galactose-inducible *GAL1* promoter, the loss of *GPA1* function was found to lead to cell-cycle arrest at the late G1 phase. Mutants that suppress the lethality of the *gpa1::HIS3* mutation showed a sterile phenotype that was not cell-type-specific. These results suggest that *GPA1* protein may control the signal for mating-factor-mediated cell-cycle arrest.**

## Introduction

A family of guanine nucleotide-binding regulatory proteins (G proteins) is known to be involved in a variety of receptor-mediated signal transduction systems in mammalian cells (Gilman, 1984). Gs and Gi are involved in hormonal stimulation and inhibition of adenylate cyclase activity, respectively (Gilman, 1984), while transducin (Gt) in the retinal rod outer segment regulates a cGMP-specific phosphodiesterase activity in the visual signal transduction system (Stryer, 1986). The other set of G proteins, Go, found mainly in brain (Sternweis and Robishaw, 1984; Neer et al., 1984; Milligan and Klee, 1985; Katada et al., 1986), may be involved in neuronal responses.

Recently, the cDNA sequences for the α subunits of Gs, Gi, Go, and Gt have been determined (Lochrie et al., 1985; Medynsky et al., 1985; Tanabe et al., 1985; Yatsunami and Khorana, 1985; Itoh et al., 1986; Nukada et al., 1986a and 1986b; Robishaw et al., 1986). The nucleotide and amino acid sequences are highly homologous among these different G proteins. In view of the strong conservation of the amino acid sequences of Gsα and Giα in different species, we searched for G protein homologous genes in yeast and succeeded in isolating *GPA1* from Saccharomyces cerevisiae, which is homologous with cDNAs for mammalian G protein α subunits (Nakafuku et al., 1987). The *GPA1* gene encodes a protein of 472 amino acids that is highly homologous to rat brain Giα and Goα proteins. The sequence has less homology to rat brain Gsα. Comparison of the predicted amino acid sequence of *GPA1* protein with that of mammalian G proteins revealed a strong conservation in the region of the GTP binding and hydrolysis sites.

The occurrence of G proteins in yeast, where an extensive genetic analysis can be carried out, is expected to shed more light on the role of the proteins in signal transduction in mammalian cells. S. cerevisiae has two different signal transduction systems at the G1 phase of the cell cycle. One is mediated by nutrients such as glucose, which positively regulate the early G1 phase; the other is mediated by mating pheromones, which negatively regulate the late G1 phase (Reed, 1980). In the first case, glucose serves as an extracellular signal for the regulation of adenylate cyclase and inositide phospholipid turnover (Kaibuchi et al., 1986). It is already known that S. cerevisiae has one family of GTP-binding proteins, the *ras* family (DeFeo-Jones et al., 1983; Powers et al., 1984). *RAS1* and *RAS2* genes are involved in regulation of the early G1 phase. *RAS2* protein stimulates adenylate cyclase (Toda et al., 1985; Broek et al., 1985), while *RAS1* protein seems to negatively affect inositide phospholipid turnover (Kaibuchi et al., 1986).

Mating response of yeast is an important model system for studying signal transduction events for eukaryotic cells. Each haploid cell, **a** and α, produces specific mating pheromones, known as **a**- and α-factors. These mating pheromones interact with specific membrane receptors and cause cell-cycle arrest of cells of the opposite cell type at late G1 phase (Sprague et al., 1983). Mating between **a** and α haploid cells results in a third cell type, **a**/α diploids. Genetic and biochemical studies have suggested that *STE2* and *STE3* may encode the receptors for α-factor and **a**-factor, respectively (Jenness et al., 1983; Hagen et al., 1986). Bender and Sprague (1986) and we (Nakayama et al., 1987) suggested that *STE2* and *STE3* proteins share a common signal transduction pathway by interacting with a common or interchangeable protein within the target cells. The *STE2* and *STE3* proteins have seven potential transmembrane domains (Burkholder and Hartwell, 1985; Nakayama et al., 1985; Hagen et al., 1986) as do rhodopsin and the β-adrenergic receptor (Ovchinnikov, 1982; Dixon et al., 1986). Since these receptors interact with G proteins in the signal transduction process (Gilman, 1984; Stryer, 1986), we considered the possibility that *STE2* and *STE3* proteins interact with G protein(s) to transmit mating factor signals.

In this paper, we describe our initial characterization of the *GPA1* gene. We found that *GPA1* is a haploid-specific essential gene and may be involved in the mating factor signal transduction pathway. Recently, Dietzel and Kurjan independently isolated a gene (*SCG1*) which, when present on a multicopy plasmid, suppresses the supersensitivity of *sst2* cells to mating factor. Comparison of the nucleo-

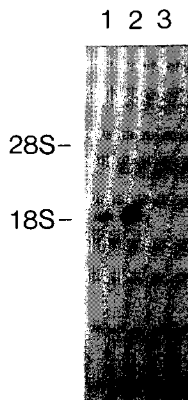



Figure 1. Northern Blotting Analysis of Poly(A)+ RNA from **a**, α, and **a**/α Cells

Twenty micrograms of poly(A)+ RNA isolated from **a**, α, and **a**/α cells were electrophoresed on a 1.2% agarose gel containing formaldehyde and hybridized with a $^{32}$P-labeled 1.9 kb EcoRI fragment of *GPA1* as described in Experimental Procedures. Lane 1, **a** cells (MNY1); lane 2, α cells (MNY2); lane 3, **a**/α cells (MNY3). The positions of rRNA markers are shown.

tide sequences of *GPA1* (Nakafuku et al., 1987) and *SCG1* showed that they may be alleles of the same gene (J. Kurjan, personal communication).

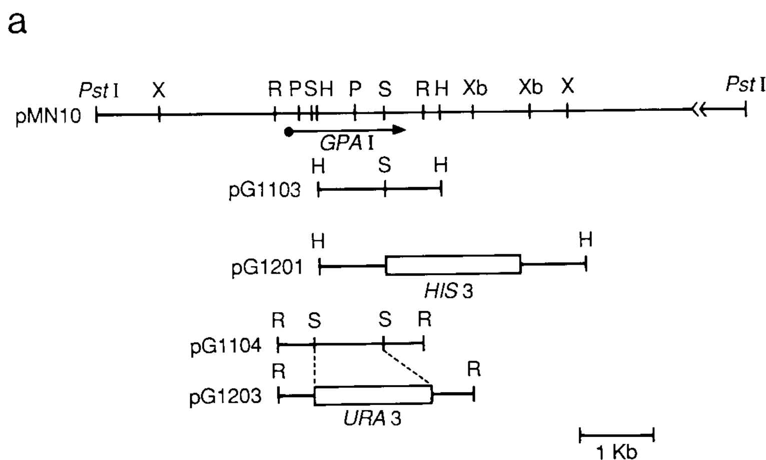
## Results

### Expression of *GPA1*

In order to study the expression of *GPA1*, poly(A)+ RNA was isolated from exponentially growing cells of **a**, α, and **a**/α strains and subjected to Northern blotting analysis using the 1.9 kb EcoRI fragment containing *GPA1* as a probe. *GPA1* transcript of approximately 1.7 kb was detected in RNA from both **a** and α haploid cells but not from **a**/α diploid cells (Figure 1). On the other hand, as a control, the transcript encoding polypeptide chain elongation factor 1α (*EF1αA*) was present at a similar level in all strains (data not shown). The amount of *GPA1* message in the **a** strain is about 2-fold lower than that of the α strain. This result was reproducible in wild-type **a** and α strains of a different background. We did not analyze this phenomenon further.

### Disruption of *GPA1*

To test whether *GPA1* is essential for cell growth, chromosomal *GPA1* was transplaced by a DNA fragment containing *GPA1* with a selectable marker replacing or inserted within part of the coding region (Figure 2a). A wild-type diploid strain KMG1 (*his3/his3*, *ura3/ura3*) was transformed to His+ by pG1201. Southern blotting analysis of one of stable His+ transformants, KMG2, showed



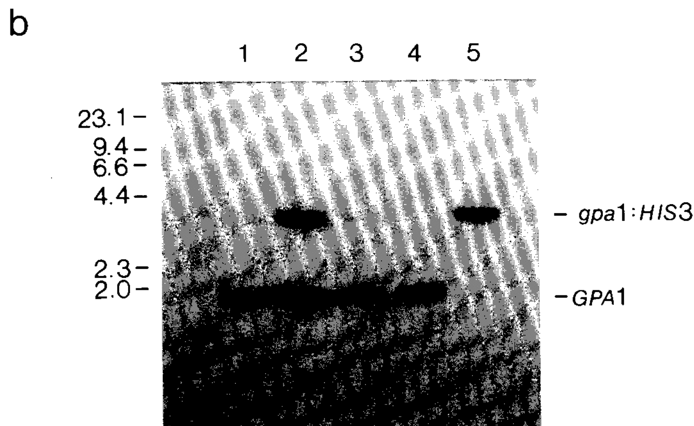




Figure 2. Structure of Disruption Plasmids and Southern Blotting Analysis of the Disrupted *GPA1* Gene

(a) pG1201 and pG1203 used for disruption of *GPA1* were constructed as described in Experimental Procedures. The coding region for *GPA1*, arrow; restriction enzyme recognition sites, EcoRI, R; HindIII, H; PvuII, P; SphI, S; XbaI, Xb; and XhoI, X; solid bar, fragment cloned into pUC8; open bar, yeast *HIS3* or *URA3*. pMN10 contains a 12.5 kb fragment including the entire *GPA1* gene on YEp24.

(b) Total cellular DNA was prepared from the wild-type diploid strain KMG1 (*GPA1/GPA1*), the heterozygous disruption diploid strain KMG2 (*GPA1/gpa1::HIS3*), its haploid progeny, and the homozygous disruption strain KMG3 (*gpa1::HIS3/gpa1::HIS3*). DNA digested with EcoRI was electrophoresed on an 0.8% agarose gel and hybridized with a $^{32}$P-labeled 1.9 kb EcoRI fragment as described in Experimental Procedures. Lane 1, KMG1; lane 2, KMG2; lanes 3 and 4, two progeny derived from KMG2 (KMG2-1A and KMG2-1B); lane 5, KMG3. The lower band (1.9 kb) and upper band (3.7 kb) correspond to the wild-type allele of *GPA1* and the disrupted allele *gpa1::HIS3*, respectively. Sizes are given in kilobases.

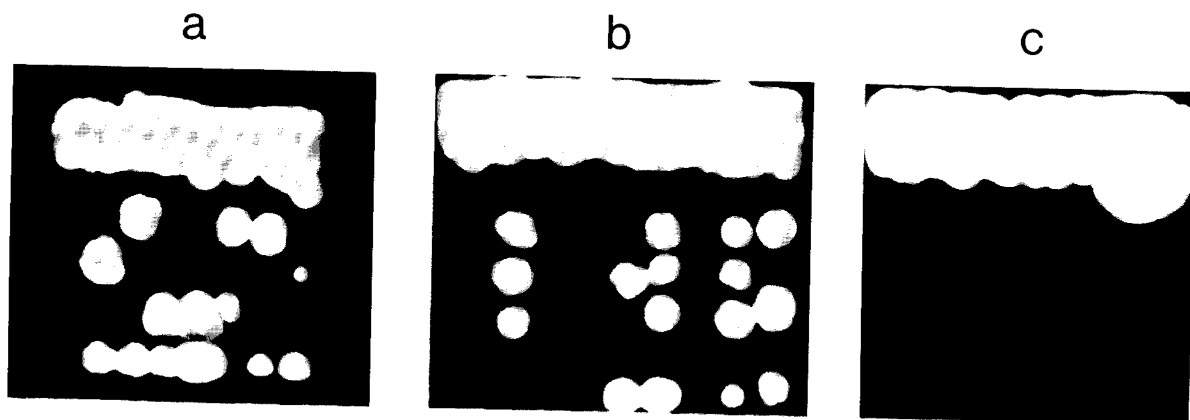



Figure 3. Dissection of Haploid Progeny Derived from *GPA1* Disrupted Diploid Cells
Diploid cells were sporulated and then tetrads were dissected by micromanipulation. The four spores from individual asci are aligned vertically. The spores were allowed to germinate on YPD medium at 30°C. The pictures were taken 7 days after dissection. a, KMG2; b, KMG3 carrying pMN10; c, KMG3.

two EcoRI fragments hybridized to a *GPA1* probe. One is the 1.9 kb fragment which is also found in a wild-type strain and the other is the 3.7 kb fragment generated by integration of the insertionally disrupted gene at the *GPA1* locus (Figure 2b, lane 2). Therefore, KMG2 is heterozygous for *GPA1* with the genotype *GPA1/gpa1::HIS3*. This strain was sporulated and dissected to separate the four haploid meiotic spores that were allowed to germinate. All dissected asci produced only two spores able to form colonies (Figure 3a). These colonies were all His$^-$ Southern analysis of DNA isolated from several viable colonies showed they contained the wild-type *GPA1* gene (Figure 2b, lanes 3 and 4). The lack of viable His$^+$ spores from KMG2 indicates that the presence of *GPA1* is either essential for cell growth or required for spore germination. Strain KMG2 was then transformed to Ura$^+$ with pMN10 (see Figure 2a), which consists of *GPA1*, the 2μ origin of replication and *URA3*. One of the resulting transformants was sporulated and haploid progeny strain KMG2-1D, which has the disrupted *GPA1* on a chromosome but carries intact *GPA1* on a plasmid, was obtained. Plasmids containing 2μ DNA replication origin (YEp and its derivatives) are easily lost in the absence of selective pressure. However, pMN10 was maintained in KMG2-1D even after the cells were grown for many generations in YPD medium (Table 1). This result strongly suggests that the cells are unable to grow in the absence of *GPA1*. The lethality of spores carrying the disrupted *GPA1* could have been due to the *HIS3* insertion near the COOH-terminus of *GPA1*, generating a truncated protein inhibitory for cell growth, and not due to the lack of a functional *GPA1*. To eliminate this possibility, a deletion mutant was constructed using pG1203 DNA (Figure 2a) by a method similar to that for insertion mutants. In this case, stable Ura$^+$ diploid transformants were obtained. Southern blotting analysis of the diploids confirmed that a deletion was introduced at one of the *GPA1* loci (data not shown). A heterozygous *GPA1/Δgpa1::URA3* diploid was sporulated. In all tetrads examined, each set of tetrads showed two viable and two nonviable spores. In every case, the viable spores were Ura$^-$, indicating they carry the normal *GPA1* gene. Since either insertion into *GPA1* or deletion of *GPA1* resulted in the same recessive lethal phenotype, we concluded that some function of *GPA1* is essential for cell growth.

**The Loss of *GPA1* Function Results in Late G1 Arrest**

Microscopic examination of the spores containing a disrupted *GPA1* showed that they germinated and went through several cell divisions. Micromanipulation of these cells revealed that most of the cells were unbudded and some of them showed an aberrant cell morphology, simi-

Table 1. Effect of *gpal::HIS3* Mutations on the Growth of Haploid and Diploid Cells

| Strain | Genotype[a] | Number of Ura$^+$ Colonies / Total Number of Colonies<br>Ura$^+$/YPD[b] |
|---|---|---|
| KMG2-1D+pMN10 | *gpa1::HIS3* + p*GPA1*(*URA3*) | 512/512 |
| KMG2-2D+pMN10 | *gpa1::HIS3* + p*GPA1*(*URA3*) | 337/337 |
| KMG3 + pMN10 | *gpa1::HIS3*/*gpa1::HIS3* + p*GPA1*(*URA3*) | 80/228 |

[a] p*GPA1*(*URA3*) indicates that the given strains contain the *URA3* plasmid expressing the *GPA1* product.
[b] The stabilities of the Ura$^+$ marker expressed as the ratio of the number of Ura$^+$ colonies to the number of colonies on the YPD plate are indicated.

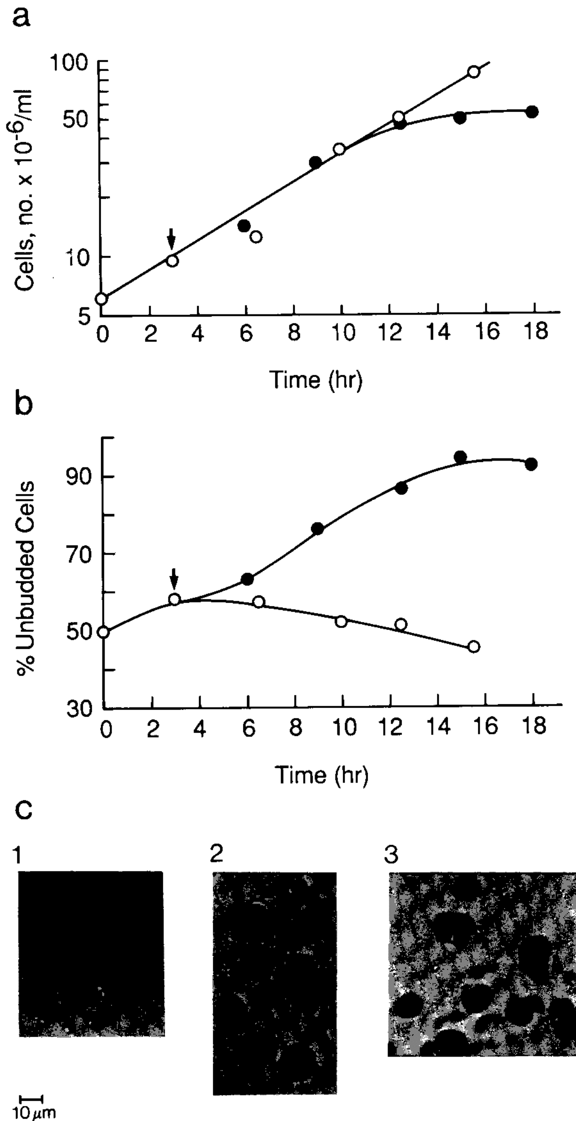



Figure 4. Loss of *GPA1* Function and Its Effect on Cellular Growth
KMG4-8C (*gpa1::HIS3*) carrying the *GPA1* gene controlled by the *GAL1* promoter (pG1501) was grown for 24 hr in SSG medium without uracil. A portion of the culture was inoculated into YPGal medium and shaken at 30°C. At the time indicated by the arrow, the culture was divided into two portions. Cells in one part (○) were grown under the same condition; those in the other part (●) were harvested, washed, and suspended in YPD medium, and the suspensions were shaken at 30°C. a, cell number; b, unbudded cells; c, light microscope photographs of cells growing exponentially in YPGal medium (1) (*GPA1* on) and arrested cells after 15 hr in YPD medium (2 and 3) (*GPA1* off).

lar to the "shmoo" of cells arrested by mating factors (data not shown). This phenotype is characteristic of cells arrested in late G1 phase.

To confirm the defect of the cell cycle in the *gpa1* mutant, we constructed a plasmid pG1501 in which the *GPA1* promoter and 5′ untranslated region were replaced by the *GAL1* promoter sequence plus the entire 5′ untranslated region of the *GAL1* transcript. In this way, the *GPA1* gene can easily be controlled by galactose and one can study the consequences of the loss of gene function in a large population of cells. Strain KMG4-8C (*gpa1::HIS3*) harboring pG1501 grew normally in YPGal medium with a generation time of about 4 hr, but cell growth ceased after several hours in YPD medium, which represses the *GAL1* promoter and hence the expression of the *GPA1* gene (Figure 4a). By microscopic inspection of cells 15 hr after the halt of *GPA1* gene expression, we observed that the size of these cells, as compared with logarithmically growing cells in YPGal medium, increased significantly (Figure 4c). In addition, about 90% of the total cell population was in the unbudded stage (Figure 4b), some of which exhibited shmoo formation (Figure 4c). These results suggest that the lack of *GPA1* product arrests the cell cycle in the late G1 phase.

### *GPA1* Is a Haploid-Specific Essential Gene

As shown in Figure 1, *GPA1* transcription is repressed in **a**/α cells. Thus, the effect of *gpa1* mutation on the cell growth of diploid cells was tested. We constructed a diploid KMG3 homozygous for *gpa1::HIS3* carrying pMN10 by crossing the strains KMG2-1D and KMG2-2D, both of which carry pMN10. First, the stability of the plasmid pMN10 in these strains was studied. Both haploid and diploid cells were grown without selection in YPD medium for many generations and plated onto YPD. The resulting colonies were replica plated on uracil prototrophic medium. As mentioned earlier, pMN10 plasmid was maintained in haploid cells. In contrast, pMN10 was readily lost from diploid cells (Table 1). This result shows that *gpa1::HIS3* homozygous diploids are viable without a functional *GPA1*. Southern blotting confirmed that intact *GPA1* is absent in KMG3 (Figure 2b, lane 5). No difference in cellular growth rates was observed between wild-type diploid cells (KMG1) and *gpa1::HIS3* homozygous diploid cells (KMG3) without pMN10. The generation time of each strain was 2 hr in YPD at 30°C. The *gpa1::HIS3* homozygous diploids in the presence or absence of pMN10 were sporulated for tetrad analysis. Haploid segregants recovered from a diploid containing pMN10 were all Ura$^+$ (Figure 3b). However, the diploid without the plasmid gave rise to no viable haploid segregants (Figure 3c). Based on these results, together with haploid-specific expression of *GPA1*, we concluded that *GPA1* is a haploid-specific essential gene.

### The Relationship between the *GPA1* Product and the Mating Pathway

In diploid cells, it is known that **a**- and α-specific genes are not expressed (Sprague et al., 1983; Wilson and Herskowitz, 1984). Furthermore, transcription of the set of haploid-specific genes, common to both haploid cell types, is repressed (Jensen et al., 1983). These haploid-specific genes are believed to be required for mating functions common to both cell types. To determine whether the *GPA1* protein is an element of the mating pathway, we tested whether suppressor mutations of the *gpa1::HIS3* mutant can affect mating ability. The *gpa1::HIS3* mutant cells carrying pMN10 plasmid were streaked on medium containing 5-fluoro-acetic acid (5-FOA). Wild-type strains

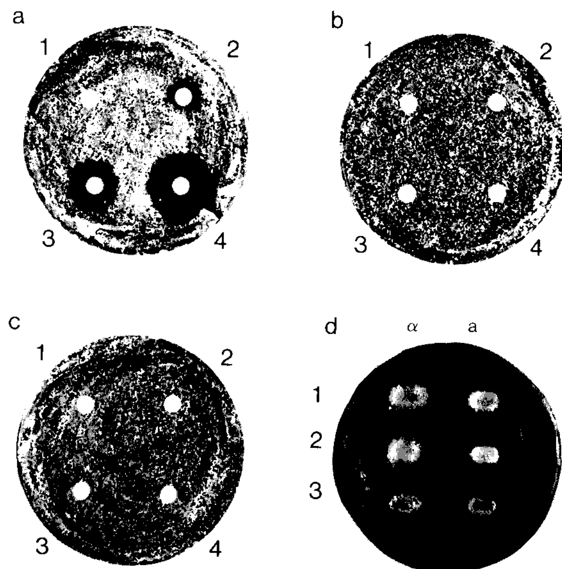



Figure 5. Response to α-Factor and Production of α-Factor of Mutants
YPD plates, buffered to pH 4 by the addition of succinic acid to 0.5%, were seeded with a lawn of each strain. Discs of Whatman chromatography paper (6 mm diameter) were placed on the nascent lawn and samples of synthetic α-factor (Sigma) were pipetted onto the discs. The amounts of α-factor were: (1) 0 (buffer alone); (2) 50 ng, (3) 500 ng, (4) 5 μg. (a) KMG2-1A (**a** wild-type). (b) KMG6-1B (**a** *gpa1::HIS3 sgp1*). (c) KMG9-1A (**a** *gpa1::HIS3 sgp2*).
(d) Each strain was grown as a patch on a YPD plate and replicated onto a YPD plate spread with strain RC631 (**a** *sst2-1*) which is supersensitive to α-factor. A "halo" around the patch indicates that growth of RC631 is inhibited by secreted α-factor. 1, KMG2-2C (α wild-type) and KMG2-1A (**a** wild-type); 2, KMG6-2A (α *gpa1::HIS3 sgp1*) and KMG6-1B (**a** *gpa1::HIS3 sgp1*); 3, KMG9-3D (α *gpa1::HIS3 sgp2*) and KMG9-1A (**a** *gpa1::HIS3 sgp2*).

(*URA3*$^+$) are unable to grow on 5-FOA medium, whereas *ura3*$^-$ strains grow normally (Boeke et al., 1984). Since the plasmid pMN10 has the *URA3*$^+$ gene as a selection marker, *gpa1::HIS3* mutants carrying pMN10 were unable to grow on 5-FOA medium. They also failed to grow without pMN10. Hence, it is possible to isolate suppressor mutations of *gpa1::HIS3* from 5-FOA resistant colonies. We selected 77 independent, spontaneously arising, 5-FOA resistant colonies. They could grow on YPD medium without pMN10 and they were phenotypically classified into three groups. The first group had the ability to mate and had a His$^-$ phenotype. They may have arisen as a result of homologous recombination between *gpa1::HIS3* and the plasmid-borne *GPA1*. Mutants of the second group was defective in mating and in production of mating factors. The third group was sterile but able to produce mating factors.

We characterized five 5-FOA resistant mutants (KMG2-1DR-2, KMG2-2DR-2, KMG2-2DR-12, KMG2-12CR-1, and KMG2-12CR-14) genetically. Mutant KMG2-1DR-2 fell into the second group and the remaining four mutants fell into the third. They were crossed to *gpa1::HIS3* strains (KMG2-1D and KMG2-2D) carrying pMN10 and tetrads were dissected after sporulation. All tetrads showed 2+:2− segregation for growth on 5-FOA medium and the sterile phenotype, which suggested that each trait was segregated as a single gene. All 5-FOA resistant segregants had very reduced mating proficiency compared with 5-FOA sensitive segregants, indicating that each of these five mutants has a single mutation that determines both the suppression of the growth defect of the *gpa1::HIS3* mutation and the sterile phenotype. The sterile phenotype of mutants of the second and third groups tested was not restricted to either haploid cell type. Since second group mutants lost the ability to produce mating factors, mutations of this group may have indirectly circumvented the requirement of *GPA1* for growth. It is known that *sir* mutations derepress the expression of silent mating type loci, leading to the expression of diploid phenotypes in haploid cells (Rine and Herskowitz, 1987). Since the *GPA1* protein is not essential for growth of diploid cells, it is expected that *sir* mutations would be found within this class of suppressors of *GPA1* disruption. On the other hand, because mutants of the third group produce but do not respond to mating factors (Figure 5), it is thought that they may be directly involved in mating factor signal transduction. The four mutants of the third group were assigned to two loci by pairwise crosses between different mutants (or their segregants). The segregation patterns indicated that mutants KMG2-2DR-2, KMG2-2DR-12, and KMG2-12CR-14 bear mutations on one of two loci (called *sgp1*; suppressor of *gpa1*), whereas mutant KMG2-12CR-1 has a mutation at the other locus (called *sgp2*). Detailed characterization of sterile suppressor mutations is underway. The fact that suppressor mutations of *gpa1::HIS3* showed a cell-type-nonspecific sterile phenotype suggests that the *GPA1* may be involved in mating-factor-mediated signal transduction.

Binding of α-factor and the mating ability of **a** cells is dependent on the expression of a functional *STE2* protein that is thought to be the α-factor receptor (Jenness et al., 1983). In order to test that the *GPA1* product functions downstream of the mating factor receptor, we characterized an **a** *gpa1 ste2* double mutant. A null mutation of *STE2* (Δ*ste2::URA3*) (Nakayama et al., 1987) was introduced into strain KMG4-8C (**a** *gpa1::HIS3*) carrying pG1302 to yield the strain KMG10 (**a** *gpa1::HIS3* Δ*ste2::URA3*). The plasmid pG1302 was not lost from KMG10 even when this strain was grown for about 20 generations in YPD medium. Under the same condition, 15% of wild-type cells (KMG4-7D) carrying pG1302 lost the plasmid. Thus disruption of *STE2* does not suppress the growth defect of the *gpa1::HIS3* mutation. Mating ability of KMG10 was also characterized qualitatively. In the presence of pG1302, KMG10 showed the sterile phenotype because of its *ste2* disruption mutation (Figure 6a). On the other hand, KMG10 showed a partially fertile phenotype in the absence of selective pressure for pG1302 (Figure 6b). No resulting diploid colony carried pG1302. These results strongly suggest that the *gpa1::HIS3* mutation suppresses the sterile phenotype of Δ*ste2::URA3*.

## Mapping of *GPA1*

Many genes affect either the cell-cycle or mating response. We decided to map the *GPA1* locus to see if it was distinct from previously mapped genes. *GPA1* was map-

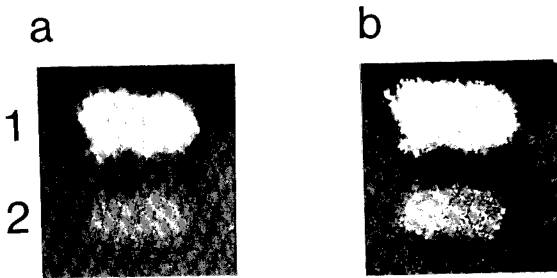



Figure 6. Mating Ability of *gpa1::HIS3* Δ*ste2::URA3* Strain

Each strain was grown as a patch on a YPD plate and replicated onto a lawn of strain KMG4-7D (α wild-type) on a YPD plate. After 24 hr at 30°C, the plate was replicated onto an SD plate containing uracil (a) and an SD plate containing uracil and tryptophan (b) to select diploid cells. Plates were photographed after 48 hr incubation at 30°C. 1, KMG4-8C (**a** *gpa1::HIS3*) carrying pG1302; 2, KMG10 (**a** *gpa1::HIS3* Δ*ste2::URA3*) carrying pG1302.

ped genetically by tetrad analysis of diploids KMG4 and KMG5 carrying pMN10 and physically by chromosome blotting. Strain KMG4 is heterozygous for *trp1*, which is closely linked to the centromere. In addition, the strain is homozygous for *his3* and carries the disrupted *gpa1::HIS3* allele at the *GPA1* locus on one homolog. Thus the segregation of the *gpa1::HIS3* allele in tetrads could be monitored by scoring *HIS3*. Analysis of 32 tetrads from the strain KMG4 carrying pMN10 indicates that the *GPA1* locus is about 1.6 cM from its centromere (Table 2).

*GPA1* probe hybridized to a Southern blot of intact chromosomal DNA separated by orthogonal-field-alternating gel electrophoresis (Carle and Olson, 1985) at a band corresponding to chromosome VIII (data not shown). This result was confirmed by the tetrad analysis of diploid KMG5 carrying pMN10. Strain KMG5 is heterozygous for *arg4*, which lies on chromosome VIII. The strain is homozygous for *his3* and heterozygous for the disrupted *gpa1::HIS3* allele. Analysis of 39 tetrads from strain KMG5 carrying pMN10 indicated that the *GPA1* locus is mapped to chromosome VIII, 10.3 cM from *arg4* (Table 2). This locus is not coincident with any previously described *cdc* or *ste* genes.

## Discussion

### Expression of *GPA1*

There is a group of genes whose transcripts are expressed in haploids but repressed in **a**/α diploids. Repression requires both the **a**1 protein, encoded at *MAT***a**, and the α2 protein, encoded at *MAT*α, which make up **a**1-α2 activity (Jensen et al., 1983). Our finding that *GPA1* mRNA is not produced in **a**/α cells is evidence that *GPA1* is a member of the set of haploid-specific genes. It is proposed that the transcription of *GPA1* would be under negative control by **a**1-α2 activity. *GPA1* would be expressed in haploid **a** cells because of the absence of the α2 product, and in α cells because of the absence of the **a**1 product. Miller et al. (1984) have described a 20 bp consensus sequence common to the 5′ ends of haploid-specific genes that are negatively regulated by **a**1-α2. We searched for the haploid-specific regulatory sequence in *GPA1*. While there is a sequence of some homology (TGGTATTTTAGCATCACATCA) 50 bp upstream of the translational start (Nakafuku et al., 1987), we may have to search further upstream to find a more compelling match.

### The Possible Role for *GPA1* on Signal Transduction

We propose a model of the function of *GPA1* in light of the unique sensitivity of haploids to mating-factor-mediated G1 arrest. Five key observations support a role for *GPA1* in the mating factor signal transduction pathway: one, *GPA1* mRNA is expressed only in haploid cells but not in **a**/α diploids; two, disruptions of *GPA1* are lethal in haploid cells but not in **a**/α diploid cells, which indicates that *GPA1* is a haploid-specific essential gene for cell growth; three, the arrest phenotype of haploids, when *GPA1* expression was turned off, is characteristic of cells arrested in late G1 phase and some of these arrested cells exhibit shmoo morphology; four, suppressors of *gpa1::HIS3* have been isolated that produce mating factors but do not respond to mating factors; five, loss of *GPA1* function can relieve the sterility caused by a *ste2* mutation.

The model depicted in Figure 7 is one of the simplest but generally acceptable candidates to explain these observations. This model is based on the reaction mechanism of GTP-binding protein originally proposed for translational elongation factors and then extrapolated to other systems (Kaziro, 1978). This reaction mechanism consists of three basic concepts: first, the protein has two qualitatively different conformations defined by the bound ligand, GDP or GTP; second, the GTP-bound form is an active conformation that is able to activate biological effectors, while the GDP-bound form is inactive; and third, hydrolysis of the bound GTP is, therefore, required to shut off the signal.

According to this model (Figure 7), haploid cell arrest in the presence of mating factors is mediated by the activity

Table 2. Mapping of *GPA1*

| Strain | Markers | PD | NPD | T | Linkage[a] |
|---|---|---|---|---|---|
| KMG4 + pMN10 | *gpa1–trp1* | 15 | 16 | 1 | 1.6[b] |
| KMG5 + pMN10 | *gpa1–arg4* | 31 | 0 | 8 | 10.3[c] |

[a] Linkage is given in centimorgans (cM).
[b] The *gpa1–CEN8* distance was calculated as one-half of the second-division segregation frequency.
[c] The *gpa1–arg4* distance was calculated by the formula: 100 (T + 6NPD)/2 (PD + NPD + T), where PD is the number of parental ditype, NPD the number of nonparental ditype, and T the number of tetratype.

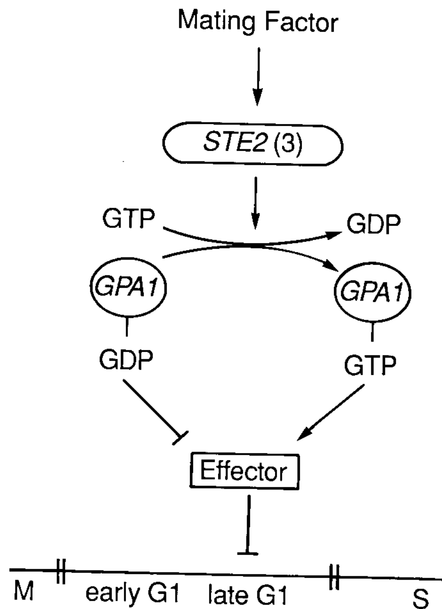



Figure 7. Model of the Function of *GPA1* Protein in the Mating Pathway

of GTP-bound *GPA1* protein in a manner similar to mammalian G proteins in hormonal signal transduction. In the absence of mating factor, the *GPA1* protein binds GDP and restrains a putative effector molecule that is responsible for generation of a cell-cycle arrest signal. Binding of mating factor to its receptor promotes the exchange of the bound GDP with GTP. The GTP-bound form of the *GPA1* protein activates the effector, and the effector is then able to transmit a signal to arrest the cell cycle at late G1 phase. Based on this model, the lethality of the cell with disrupted *GPA1* can be explained by the uncoupling of the effector molecule from *GPA1* protein. The effector, which may be unlocked from the mating factor receptor complex, may elicit a constitutive signal for cell-cycle arrest regardless of the presence of mating factors. We found that loss of the *GPA1* plasmid in a *gpa1::HIS3 Δste2::URA3* strain results in cells which cannot grow but can mate. This supports the idea that *GPA1* disruption results in continuous production of a cell-cycle arrest signal and promotion of conjugation in the absence of a mating factor signal. Suppressor mutations of *gpa1::HIS3* (*sgp1* and *sgp2*), which showed a cell-type-nonspecific sterile phenotype but produced mating factors, can be used to define further components involved in this system. Some of the suppressor mutations may occur in the effector molecule. In this case, the mutant effector may be altered not to generate the arrest signal. Therefore, this type of mutation may be able to suppress the lethality of the *gpa1::HIS3* mutation and may show a cell-type-nonspecific sterile phenotype in the background of *GPA1*$^{+}$. Other cell-type-nonspecific sterile mutations may have occurred within the *GPA1* gene. These may not be able to activate the effector even in the presence of mating factor. To confirm the above model, we are interested in isolating *gpa1* mutations that show a sterile phenotype or temperature-sensitive growth phenotype by using in vitro mutagenesis of *GPA1*.

From our experiments reported in this paper, we have not yet demonstrated a direct interaction between mating-factor receptors and *GPA1* protein. In the case of hormonal inhibition of cAMP synthesis mediated by Gi, evidence points to indirect inhibition by βγ complexes released from Gi that bind and inactivate Gsα (Gilman, 1984). By analogy with this mammalian system, an alternative possibility, although less likely, is that *GPA1* protein is indirectly involved in the mating-factor transducing pathway. In this case, when *GPA1* gene is disrupted, free β and γ subunits not bound to *GPA1* protein would affect the function of another G protein involved in the mating pathway.

Both biochemical and genetic studies are certainly necessary to clarify the real reaction mechanisms of the *GPA1* product during signal transduction. For example, based on existing biochemical observations on the mechanism of signal transduction for mammalian hormones, it will be interesting to test for an in vitro mating-factor-dependent GTPase activity. Further analysis of the suppressor mutants obtained from *gpa1::HIS3* mutation will be useful to elucidate the function of *GPA1* protein in the signal transducing pathway.

## Experimental Procedures

### Yeast Strains and Media

The yeast strains used in this study are listed in Table 3. Medium YPD contained 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% dextrose; YPGal contained 1% Bacto-yeast extract, 2% Bacto-peptone, and 4% galactose; SD contained 0.7% yeast nitrogen base without amino acids (Difco) and 2% dextrose; SSG contained 0.7% yeast nitrogen base without amino acids, 0.2% sucrose and 4% galactose. SD and SSG were supplemented with auxotrophic requirements. 5-fluoro-

Table 3. Yeast Strains Used in This Study

| Strains | Genotype | |
|---|---|---|
| KMG1 | **a**/α | *ura3/ura3 his3/his3 trp1/+ lys2/+ +/leu2 ade8/+* |
| KMG2 | **a**/α | *ura3/ura3 his3/his3 trp1/+ lys2/+ +/leu2 ade8/+ gpa1::HIS3/+* |
| KMG2-1A | **a** | *ura3 his3 lys2 ade8* |
| KMG2-1B | **a** | *ura3 his3* |
| KMG2-1D | α | *ura3 his3 trp1 leu2 gpa1::HIS3* |
| KMG2-2C | α | *ura3 his3 trp1 lys2 ade8* |
| KMG2-2D | **a** | *ura3 his3 lys2 gpa1::HIS3* |
| KMG2-12C | α | *ura3 his3 trp1 leu2 gpa1::HIS3* |
| KMG3 | **a**/α | *ura3/ura3 his3/his3 +/trp1 lys2/+ +/leu2 gpa1::HIS3/gpa1::HIS3* |
| KMG4 | **a**/α | *ura3/ura3 his3/his3 +/trp1 +/leu2 +/gpa1::HIS3* |
| KMG4-7D | α | *ura3 his3 trp1* |
| KMG4-8C | **a** | *ura3 his3 trp1 leu2 gpa1::HIS3* |
| KMG5 | **a**/α | *ura3/ura3 his3/his3 trp1/+ +/leu2 arg4/+ +/gpa1::HIS3* |
| KMG6-1B | **a** | *ura3 his3 trp1 lys2 gpa1::HIS3 sgp1* |
| KMG6-2A | α | *ura3 his3 trp1 gpa1::HIS3 sgp1* |
| KMG9-1A | **a** | *ura3 his3 trp1 ade2 gpa1::HIS3 sgp2* |
| KMG9-3D | α | *ura3 his3 leu2 ade2 gpa1::HIS3 sgp2* |
| KMG10 | **a** | *ura3 his3 trp1 leu2 gpa1::HIS3 Δste2::URA3* |
| MNY1 | α | *arg* |
| MNY2 | **a** | *ura3 trp1 leu2 ade* |
| MNY3 | **a**/α | *ura3/+ trp1/+ leu2/+ ade/+ +/arg* |
| RC631 | **a** | *rme his6 met1 ade2 ura1 can1 cyh2 GAL sst2-1* |

orotic acid (5-FOA) medium was prepared as described by Boeke et al. (1984).

### Northern and Southern Blotting Analysis

Total cellular RNA was isolated as described previously (Nakafuku et al., 1987). Poly(A)$^+$ RNA selected by oligo (dT)-cellulose column chromatography was electrophoresed on a 1.2% agarose gel containing formaldehyde and blotted onto a nitrocellulose membrane filter (Maniatis et al., 1982). The $^{32}$P-labeled 1.9 kb EcoRI fragment of *GPA1* (1.0 $\times$ $10^8$ cpm/µg DNA) was used for hybridization under highly stringent conditions (Nakafuku et al., 1987).

Total yeast DNA prepared from saturated cultures (Davis et al., 1980) was digested with EcoRI and electrophoresed on 0.8% agarose gel. DNA transferred to a nitrocellulose membrane filter was hybridized with $^{32}$P-labeled 1.9 kb EcoRI fragment of *GPA1* in the same condition as described above.

### Construction of the Disruption Strains of *GPA1*

The plasmids, pG1201 and pG1203, were constructed to perform one-step gene replacement (Rothstein et al., 1983). The 1.7 kb HindIII fragment and the 1.9 kb EcoRI fragment containing *GPA1* were first cloned into pUC8 to obtain pG1103 and pG1104, respectively. The resulting plasmids were digested with SphI and blunt-ended with Klenow fragment. The 1.8 kb BamHI fragment of *HIS3* and the 1.3 kb HindIII fragment of *URA3* were also blunt-ended and ligated with the SphI-cleaved pG1103 and G1104 to yield pG1201 and pG1203, respectively. The HindIII-cleaved pG1201 and the EcoRI-cleaved pG1203 were introduced into yeast cells by the lithium acetate transformation method (Itoh et al., 1983). Transformants were selected by histidine or uracil prototrophy.

### Construction of pG1302 and pG1501

The 4.0 kb XbaI-XhoI fragment carrying *GPA1* was cloned into a YCp-based plasmid. The resulting plasmid pG1302 also contains *TRP1-ARS1*, *CEN3*, and pBR322.

The 2.4 kb EcoRI fragment carrying the *GAL1* promoter (Johnston et al., 1984) was cloned into M13mp8 (Vieira and Messing, 1982) and EcoRI and XbaI sites were introduced just in front of the initiator codon of *GAL1* using oligonucleotide directed mutagenesis (Zoller and Smith, 1982). The 0.8 kb TaqI-XbaI fragment carrying the promoter and the 5′ untranslated region of *GAL1* were isolated from the mutated *GAL1* gene and cloned into pUC18. Then, the KpnI-HindIII fragment was inserted into the KpnI-HindIII sites of pTRP56 (Miyajima et al., 1984). The 1.3 kb HindIII fragment of *URA3* was inserted into the HindIII site of the resulting plasmid to obtain pGT3. The 0.9 kb BamHI-HindIII fragment carrying *CEN3* was cloned into the AatII site of pGT3 to yield pGT5.

The 1.9 kb EcoRI fragment containing the entire coding region of *GPA1* was cloned into M13mp11 and an XbaI site was introduced just in front of the initiation codon of *GPA1* using oligonucleotide-directed mutagenesis. The 1.7 kb XbaI fragment carrying *GPA1* from the ATG to the 3′ untranslated region was then inserted at the XbaI site of pGT5 to obtain pG1501.

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