The Role of G Proteins in Yeast Signal Transduction

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A family of guanine-nucleotide-binding proteins, G proteins, serves as modulators or transducers in various transmembrane signaling systems (Gilman 1987). Two G proteins, G_s and G_i, are involved in hormonal regulation of the adenylate cyclase activity; the former activates the cyclase in response to β-adrenergic stimuli, whereas the latter mediates inhibition of the enzyme (Gilman 1987). Transducin, G_t, in the retinal rod outer segment, regulates a cGMP-specific phosphodiesterase activity in the visual signal transduction system (Stryer and Bourne 1986). The other set of G proteins, G_o, abundant in brain tissues (Sternweis and Robishaw 1984), may be involved in neuronal responses.

Each G protein consists of three different subunits, α, β, and γ. The α subunit contains a guanine-nucleotide-binding site and determines the specificity of the protein for its receptor and effector. In response to interaction with a specific receptor, the α subunit binds GTP and dissociates from the $\beta\gamma$ subunit complex. The released α subunit, with GTP bound, is then able to interact with a specific effector and regulate its function. After the GTP bound to the α subunit is hydrolyzed to GDP, the α subunit reassociates with the $\beta\gamma$ complex, regenerating the inactive G protein (Stryer and Bourne 1986; Gilman 1987). The α subunit therefore seems to play an important role in G protein function.

Recently, cDNA sequences for α subunits of G_s, G_i, G_o, and G_t were determined. These studies revealed that the nucleotide and deduced amino acid sequences are highly homologous among these different G proteins. In view of the strong conservation of the amino acid sequences of each G protein species among different organisms, we searched for G-protein-homologous genes in yeast and isolated two different genes, *GPA1* and *GPA2*, from *Saccharomyces cerevisiae*, which are homologous to cDNAs for mammalian G protein α subunits (Fig. 1) (Nakafuku et al. 1987, 1988). Yeast cells offer several advantages over mammalian cells in the ease of applying genetic approaches. Studies of the function of G proteins in yeast are expected to shed more light on the role of these proteins in signal transduction in mammalian cells. In this paper, we describe the roles of yeast G proteins in the yeast signal transduction systems.

S. cerevisiae has two different signal transduction systems involved at the G_1 phase of the cell cycle (Fig. 2). One is mediated by nutrients, such as glucose, which regulate the early G_1 phase positively; the other is mediated by mating pheromones, which regulate the late G_1 negatively (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).

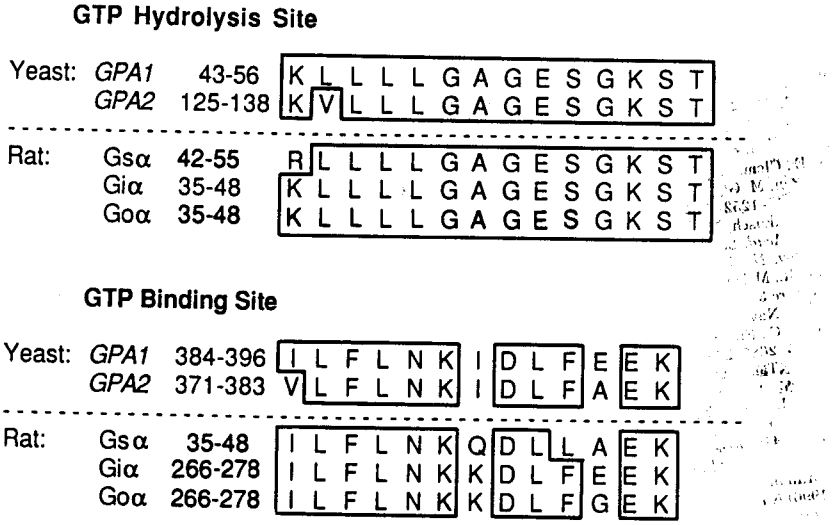

Figure 1. Two regions of amino acid sequence homology in yeast and rat G_α. Regions of exact homology are boxed.

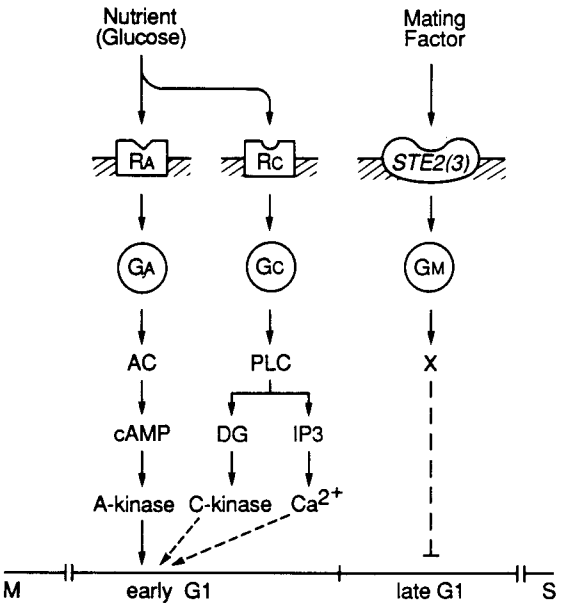

Figure 2. Yeast signal transducing system at the G_1 phase of the cell cycle. A terminal arrowhead indicates stimulation; a terminal bar indicates inhibition. (R) Receptor; (G) G protein; (AC) adenylate cyclase; (PLC) phospholipase C; (DG) diacylglycerol; (IP3) inositol triphosphate; (A-kinase) cAMP-dependent protein kinase; (C-kinase) protein kinase C.

Mating Factor Signal Transduction

Mating response in *S. cerevisiae* provides an important model for studying signal transduction events for eukaryotic cells. Yeast has three distinct cell types: two haploid cell types, **a**, and α, and an **a**/α diploid cell type. The **a** and α cell types can mate to yield **a**/α diploids through a multistep mating process. This mating process is initiated by peptide pheromones secreted by each haploid cell type. α cells secrete α-factor, which acts on **a** cells, whereas **a** cells produce **a**-factor, which acts on α cells. Both mating factors (1) arrest the cell cycle of target cells at late G_1 phase prior to the onset of DNA replication (S phase); (2) alter expression of a number of genes, some of which are known to be essential for mating; and (3) induce a morphological alteration ("shmoo" formation) and thereby initiate the mating program of a haploid cell (Sprague et al. 1983). It is believed that both mating factors generate an intracellular signal by interacting with their specific membrane receptors on the target cells. Genetic and biochemical studies have suggested that *STE2* and *STE3* encode the receptors for α-factor and **a**-factor, respectively (Jenness et al. 1983; Hagen et al. 1986). *STE2* and *STE3* proteins have been suggested to share a common signal transduction pathway by interacting with a common or interchangeable protein within the target cells (Bender and Sprague 1986; Nakayama et al. 1987). The *STE2* and *STE3* gene products are predicted to have seven transmembrane domains (Burkholder and Hartwell 1985; Nakayama et al. 1985), as is the case for rhodopsin (Nathans and Hogness 1984) and the β-adrenergic receptor (Dixon et al. 1986), which interact with mammalian G proteins in the signal transduction process (Stryer and Bourne 1986; Gilman 1987). Therefore, G protein(s) may interact with *STE2* and *STE3* proteins to transmit mating-factor signals in a manner analogous to that of mammalian G proteins.

The results of studies on the function of *GPA1*, summarized below, suggest that *GPA1* is involved in mating-factor-mediated signal transduction in yeast haploid cells (Miyajima et al. 1987, 1988). The *GPA1* transcript was found in haploid cells but was not detectable in **a**/α diploid cells. Disruption of *GPA1* (*gpa1::HIS3*) was lethal in haploid cells but not in **a**/α diploid cells, indicating that *GPA1* is a haploid-specific gene essential for cell growth (Table 1). Loss of *GPA1* function in haploid cells resulted in cell-cycle arrest at late G_1 phase and induced shmoo formation, both of which are commonly observed when haploid cells are exposed to mating factors. These results suggest that the *gpa1::HIS3* mutation causes constitutive expression from the mating-system. If *GPA1* protein acts downstream from the mating-factor receptor, cells lacking *GPA1* function would no longer need the receptor function to initiate the mating program. The *MAT***a** *gpa1::HIS3 ste2::LEU2* strain carrying the *GPA1* gene controlled by the galactose-inducible *GAL1* promoter grew in the presence of galactose and showed a sterile phenotype because of *STE2* disruption (*ste2::LEU2*). This strain ceased dividing when *GPA1* expression was repressed by glucose, mating efficiency of the cell increased 10^3-fold, and the steady-state level of expression of a pheromone-specific gene (*FUS1*) increased 5- to 10-fold (Fig. 3) (Nakayama et al. 1988). These results suggest that loss of *GPA1* function in haploid cells generates an intracellular signal that is equivalent to that generated through mating-factor receptor interaction. Dietzel and Kurjan (1987) have isolated a gene (*SCG1*) that suppresses the supersensitivity of *sst2* mutant cells to mating factors when present on a multicopy plasmid. Comparison of the sequences of *SCG1* and *GPA1* revealed only five differences, both in nucleotide and amino acid sequences, suggesting that they are the same gene from different strains of yeast. This result supports the view that *GPA1* is involved in the mating-factor-mediated signal transduction pathway.

The model in Figure 4 is one of the simplest candidates to explain the above observations. This model is based on the reaction mechanism of GTP-binding protein originally proposed for translational elongation factors and extrapolated to other systems (Kaziro 1978). The reaction mechanism consists of three basic concepts: (1) The protein has two qualitatively different conformations defined by the bound ligand, GDP or GTP; (2) the GTP-bound form is an active conformation that is able to activate biological effectors, whereas the GDP-bound form is inactive; and (3) hydrolysis of the bound GTP is therefore required to shut off the signal. According to this model (Fig. 4), haploid cell arrest in the presence of mating factors is mediated by the activity of GTP-bound *GPA1* protein in a man-

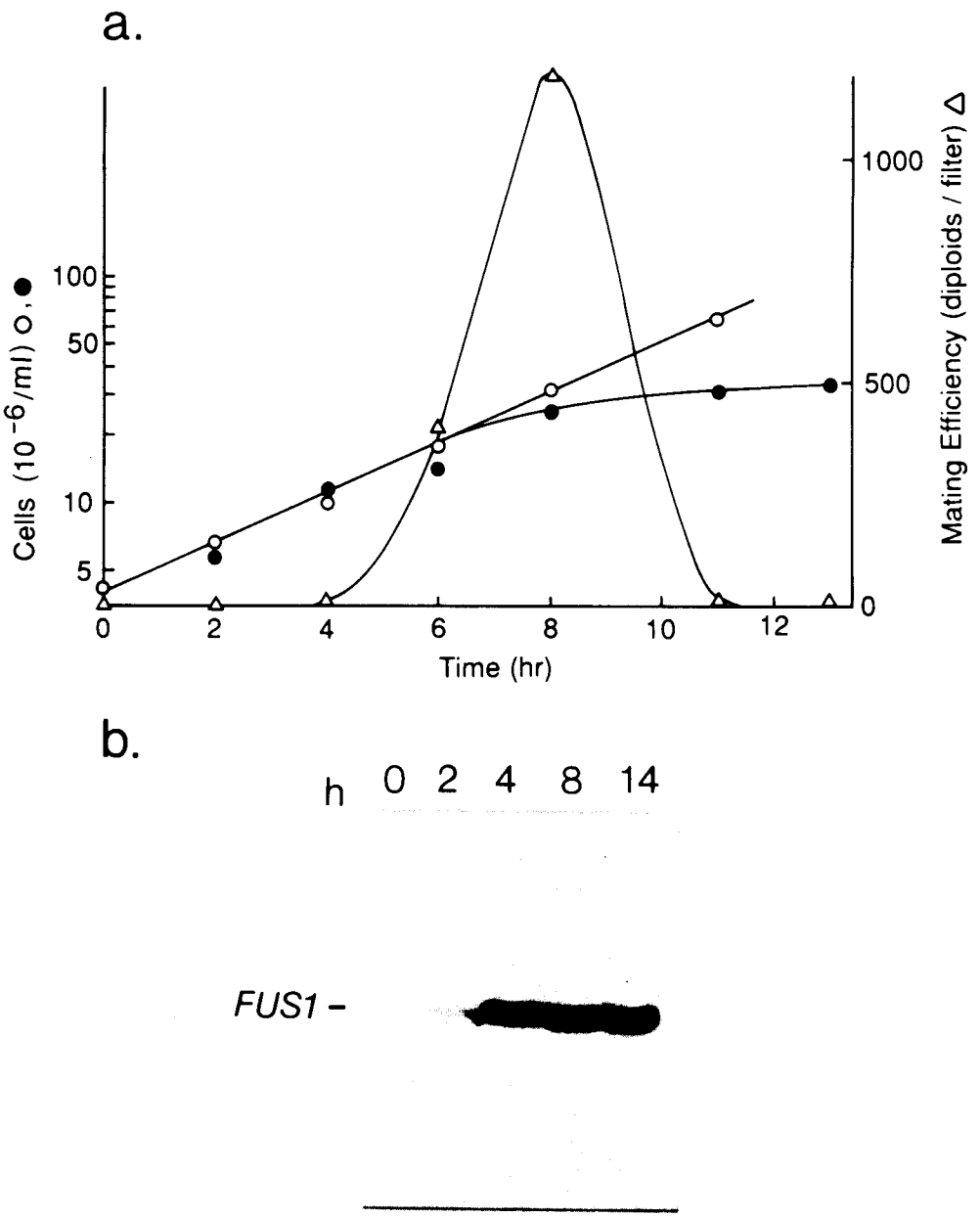

Figure 3. Effect of *GPA1* function on mating efficiency of *ste2::LEU2* and on expression of the *FUS1* gene. Cells of *MAT***a** *gpa1::HIS3 ste2::LEU2* carrying the *GPA1* gene controlled by the *GAL1* promoter were incubated in galactose-containing medium. At time 0, cells were harvested, washed, divided into two portions, and resuspended, one in galactose containing medium (○), the other in glucose containing medium (●). Both were incubated at 30°C. (*a*) Mating efficiency of cells grown in glucose-containing medium (△) and cell number (○, ●); (*b*) mRNA of *FUS1*.

ner similar to that of mammalian G proteins in hormonal signal transduction. In the absence of the mating factor, *GPA1* protein would bind GDP and restrain a putative effector molecule that is responsible for generation of a cell-cycle arrest signal (Fig. 4a). Binding of mating factor to its receptor would promote the exchange of the bound GDP to GTP. The GTP-bound form would then activate the effector to generate a signal for cell-cycle arrest of haploid cells (Fig. 4b). On the basis of this model, the lethality of the cell with disrupted *GPA1* can be explained by the uncoupling of the effector from the *GPA1* protein (Fig. 4c). The effector, unlocked from the mating-factor receptor complex, may elicit a constitutive signal for cell-cycle arrest, regardless of the presence of mating factors. Loss of *GPA1* function can suppress the sterile phenotype of *STE2* disruption (*ste2::LEU2*) (Fig. 3). This supports the idea that *GPA1* disruption results in continuous production of a cell-cycle arrest signal and in promotion of conjugation in the absence of a mating-factor signal. On the other hand, recent reports suggest that the activity of the effector is affected by the $\beta\gamma$

Table 1. Characterization of *GPA1*

Mating type	*GPA1* mRNA	Growth of *gpa1::HIS3*
*MAT***a**	+	lethal (late G_1 arrest)
MATα	+	lethal (late G_1 arrest)
*MAT***a**/*MATα*	−	viable

Modified from Miyajima et al. (1987).

subunits rather than the α subunit (Jelsema and Axelrod 1987; Logothetis et al. 1987). Dietzel and Kurjan (1987) suggested a positive transductive role for the $\beta\gamma$ subunits in the mating-factor signaling pathway. In their model, activation of the receptor allows association of *SCG1* (*GPA1*) protein-$\beta\gamma$ complex with the receptor, after which *SCG1* (*GPA1*) protein binds GTP and $\beta\gamma$ subunits are released from GTP-bound *SCG1* (*GPA1*) protein. Free $\beta\gamma$ subunits interact with the effector to stimulate its activity, leading to cell-cycle arrest. This model is consistent with the phenotypes associated with *GPA1*. In this case, a mutation-defective β or γ subunit will show a sterile phenotype. β or γ subunits have not been identified in yeast at this time; however, a remarkable conservation between the structure of mammalian and yeast G_α suggests that yeast may possess β and γ subunits of G proteins.

Suppressor mutations of the *gpa1::HIS3* mutation can be used to define further components involved in this system. Two recessive mutations, *sgp1* and *sgp2*, that suppress the lethality of *gpa1::HIS3* showed a cell-type-nonspecific sterile phenotype in combination with *gpa1::HIS3*, yet expressed the major α-factor gene (*MFα1*), as judged by the ability to express a *MFα1-lacZ* fusion gene (Table 2) (Miyajima et al. 1988). The *sgp1* mutaton is closely linked to *gpa1::HIS3* and probably occurs at the *GPA1* locus. The *sgp2* mutation is not linked to *GPA1* and is different from the previously identified cell-type-nonspecific sterile mutations (*ste4*, *ste5*, *ste7*, *ste11*, and *ste12*). The *SGP2* gene has been cloned (N. Nakayama and K. Matsumoto, in prep.). Disruption of *SGP2* confers temperature-sensitive growth and **a**-cell-specific sterile phenotypes. The *dpr1* (*ram*) mutant, a suppressor of $RAS2^{Val19}$, shows similar phenotypes (Powers et al. 1986; Fujiyama et al. 1987). It was found that (1) the cloned *SGP2* gene complements both the sterility and temperature-sensitive growth of *MAT***a** *dpr1*; (2) the cloned *DPR1* gene, in turn, complements the ability of *sgp2* to suppress the lethality of *gpa1::HIS3*; (3) the *dpr1* mutation suppresses the growth defect of *gpa1::HIS3*; (4) the *dpr1 gpa1::HIS3* strain is sterile not only as **a** cells, but also as α cells; (5) both *dpr1* and *sgp2* are mapped within a genetically indistinguishable locus; and (6) the restriction maps for both cloned genes are identical (N. Nakayama and K. Matsumoto, in prep.). These observations indicate that *SGP2* and *DPR1* are identical. The *DPR1* product has been shown to be responsible for the carboxy-terminal processing and fatty acid acylation of **a**-factor and *RAS* proteins (Powers et al. 1986; Fujiyama et al. 1987). Both **a**-factor- and *RAS*-coding sequences terminate

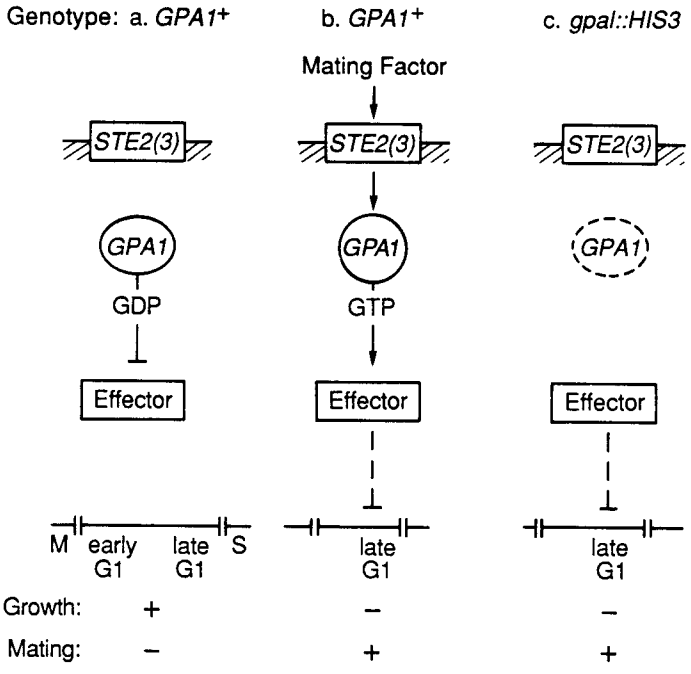

Figure 4. Model of the function of *GPA1* in the mating-factor signaling of yeast.

Table 2. Mating Efficiency and Expression of the *MFα1-lacZ* Fusion Product in *sgp* Mutants

Genotype	Mating efficiency	β-Galactosidase activity (units/OD_{660})
MATa GPA1[a]	1.00	220
MATα GPA1	1.00	—
MATa gpa1::HIS3 sgp1[a]	6.6×10^{-4}	205
MATα gpa1::HIS3 sgp1	4.4×10^{-4}	—
MATa gpa1::HIS3 sgp2[a]	$<4.6 \times 10^{-5}$	159
MATα gpa1::HIS3 sgp2	$<5.5 \times 10^{-5}$	—
MATα GPA1 sgp2	0.53	—

Modified from Miyajima et al. (1988).
[a]Strains carry the *MFα1-lacZ* fusion gene integrated at the *MFα1* locus on the chromosome.

with the potential acyltransferase recognition sequence, Cys-A-A-X, where A is an aliphatic amino acid. Therefore, the *SGP2* (*DPR1*) product may affect localization of an essential component in the mating-factor signaling pathway (Fig. 5). Interestingly, the γ subunit of transducin terminates with Cys-A(Val)-A(Ile)-X(Ser) (Hurley et al. 1984). If localization or function of a putative γ subunit of *GPA1* protein would be affected by the *sgp2* (*dpr1*) mutation, βγ subunits could have a primary transduction role, as suggested by Dietzel and Kurjan (1987).

We recently isolated an *ssg1* mutant that restored conjugational competence to *sgp1* mutants and simultaneously conferred a temperature-sensitive growth phenotype. *ssg1* mutants increased the amount of transcripts of the pheromone-inducible gene (*FUS1*) at a nonpermissive temperature, even in the absence of mating factors. *ssg1* suppressed the sterile phenotype of *ste4* and *ste5* but not that of *ste7*, *ste11*, or *ste12* (K. Matsumoto, unpubl.). These results suggest that the *SSG1* product is involved downstream from the *GPA1*, *STE4, and STE5* products and that the *ssg1* mutation may produce a constitutive signal for cell-cycle arrest (see Fig. 5).

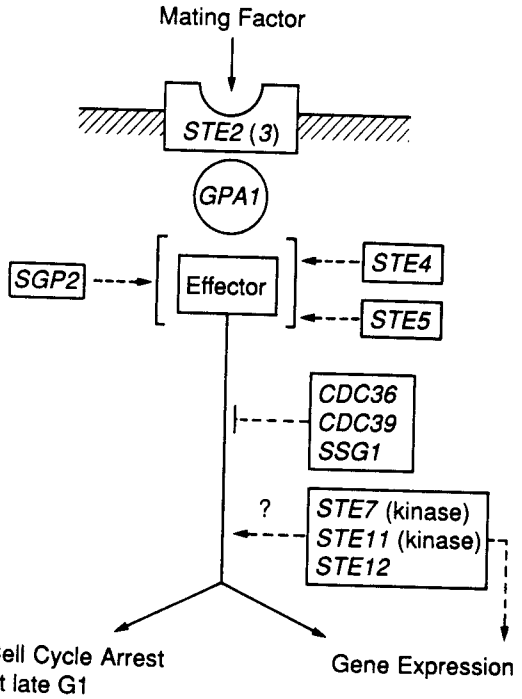

Figure 5. Schematic representation of the functional relations among *GPA1*, *SGP2*, *SSG1*, *STE*, and *CDC*.

The cell-type-nonspecific *STE* genes, *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*, are thought to be involved directly or indirectly in the mating-factor signal transduction. The growth defect of *gpa1::HIS3* was suppressed by all of these *ste* mutations (Nakayama et al. 1988). This suppression can be attributed to loss of activity and/or loss of expression of a component acting downstream from the *GPA1* product in the mating-factor signaling pathway. Because the *GPA1* product seems to be one of the components of the pathway, we examined the effect of *ste* mutations on the level of *GPA1* transcripts in order to shed light on the mode of action of these *STE* products. No significant alteration of *GPA1* transcript was observed in *ste4* and *ste5* mutants. This result suggests that suppression of *gpa1::HIS3* by *ste4* and *ste5* would be attributable to loss of activity of a component in the mating-factor signaling pathway. Because some alleles of *ste4* are suppressed by an elevated dosage of *STE5* (MacKay 1983), the *STE5* product may be functionally related to the *STE4* product or work downstream from the *STE4* product in the mating pathway (Fig. 5). Moderate alteration (a two- to threefold reduction compared to the wild-type strain) in the level of *GPA1* transcripts was observed in *ste7*, *ste11*, and *ste12* mutants, suggesting that the suppression by *ste7*, *ste11*, and *ste12* may be due to altered expression of genes whose products are probably functioning downstream from *GPA1*; these may include the effector. The class I "Start" genes, *CDC28*, *CDC36*, *CDC37*, and *CDC39* (Reed 1980), seem to be essential for cell-cycle progression at late G_1 phase, or they may negatively control an intracellular signal for mating factors that inhibit G_1-to-S transition. Among these *CDC* genes, *CDC36* and *CDC39* may be

involved in the mating-factor signaling pathway, because (1) *cdc36* suppresses the mating defect of the *ste2* disruption mutant and raises the level of a pheromone-specific transcript in a manner similar to the addition of mating factors (S. Reed, pers. comm.), and (2) sterility of *ste4* and *ste5* mutants is suppressed by *cdc36* or *cdc39* (Shuster 1982; Jenness et al. 1987). On the basis of these data, a scheme representing possible functional relationships among the *GPA1*, *SGP2*, *SSG1*, *STE*, and *CDC* genes is presented in Figure 5.

The predicted amino acid sequence of *GPA1* protein is homologous to those of mammalian G proteins (Nakafuku et al. 1987). The primary structures of the region for GTP hydrolysis and for GTP binding are nearly identical (Fig. 1). Dietzel and Kurjan (1987) showed that expression of the rat $G_{s\alpha}$ gene in yeast partially complements both the *sst2* and *scg1* (*gpa1*) defects. We compared the ability of rat $G_{s\alpha}$, $G_{i\alpha}$, and $G_{o\alpha}$ genes to suppress the lethality of *gpa1*::*HIS3* (I. Miyajima and K. Matsumoto, in prep.). In rat G_α-expression plasmids, transcription of each rat G_α is under the control of the galactose-inducible *GAL1* promoter. Rat $G_{s\alpha}$ and $G_{i\alpha}$ suppressed the growth defect of *gpa1*::*HIS3*, but rat $G_{o\alpha}$ did not (Table 3). The *gpa1*::*HIS3* mutants expressing rat $G_{s\alpha}$ or $G_{i\alpha}$ showed the mating defect, indicating that rat $G_{s\alpha}$ and $G_{i\alpha}$ can suppress the lethality resulting from *GPA1* disruption but cannot substitute for its function in mating-factor signal transduction.

cAMP Pathway

Cell growth is blocked in early G_1 stage by nutrient limitation. Genetic and biochemical studies have shown that cAMP plays an important role at this stage (Matsumoto et al. 1985). The regulatory role of cAMP in yeast has been studied by isolation of mutants defective in adenylate cyclase and cAMP-dependent protein kinase (Matsumoto et al. 1982). The *cyr1* mutants defective in adenylate cyclase activity were arrested at the early G_1 phase of the cell cycle in the absence of cAMP (Matsumoto et al. 1983a, 1984). cAMP exerts its effect through binding to the regulatory subunits (*BCY1* gene product) of cAMP-dependent protein kinase, thereby freeing active catalytic subunits (*TPK* gene product) (Fig. 6) (Matsumoto et al. 1982; Johnson et al. 1987; Toda et al. 1987a,b; Yamano et al. 1987). The *bcy1* mutation is defective in the regulatory subunit, thereby allowing the protein kinase to function in the absence of cAMP. Thus, *bcy1* can suppress the growth defect of *cyr1* (Matsumoto et al. 1982). In addition, the early G_1 arrest caused by nutritional limitation does not occur in *bcy1* (Matsumoto et al. 1983a,b).

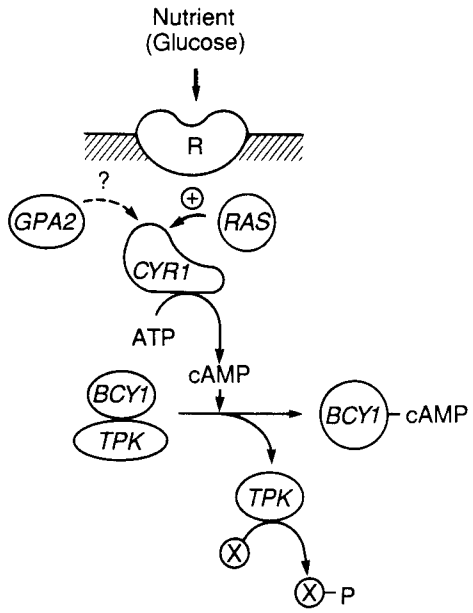

Figure 6. cAMP pathway in yeast. (R) Receptor; (X) substrate for cAMP-dependent protein kinase.

The signal transduction system regulating adenylate cyclase activity in mammalian cells is modulated by two G proteins. G_s and G_i (Gilman 1987). The binding of ligands to a receptor results in stimulation or inhibition of adenylate cyclase activity. The adenylate cyclase system of yeast consists of at least two protein components, the catalytic and regulatory subunits, and is regulated by guanine nucleotides in the presence of magnesium ions (Casperson et al. 1983). 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). Yeast strains containing a disruption in either *RAS1* or *RAS2* are viable, but disruptions in both are lethal (Kataoka et al. 1984; Tatchell et al. 1984). Thus, *RAS* functions are essential for yeast cell viability and proliferation. Moreover, yeast cells carry-

Table 3. Effect of Rat G_α Proteins on *gpa1*::*HIS3*

Plasmid[a]	Growth on YPGal[b]	Doubling time in YPGal[b] (hr)	Mating ability	Response to α-factor
GAL1-GPA1	+++	3.8	fertile	sensitive
GAL1-rat $G_{s\alpha}$	++	6.5	sterile	resistant
GAL1-rat $G_{i\alpha}$	+	9.8	sterile	resistant
GAL1-rat $G_{o\alpha}$	−			

Genotype of the strain is *MATa gpa1*::*HIS3*.

[a]Each plasmid carries either the *GPA1*, rat $G_{s\alpha}$, rat $G_{i\alpha}$, or rat $G_{o\alpha}$ gene controlled by the *GAL1* promoter.

[b]YPGal medium contains 1% yeast extract, 2% Bacto-peptone, and 5% galactose.

ing the *RAS2*^{Val19} gene, a mutation analogous to the oncogenic human Ha-*ras*^{Val17}, have a defective response to nutritional deprivation (Kataoka et al. 1984; Toda et al. 1985). Genetic and biochemical evidence indicates that yeast *RAS* proteins participate in the yeast adenylate cyclase system and are involved in controlling the intracellular level of cAMP (Broek et al. 1985; Toda et al. 1985). The *bcy1* mutation suppresses the lethality resulting from disruption of both endogenous *RAS* genes. Yeast cells lacking *RAS* function show no GTP stimulation of adenylate cyclase, whereas *RAS2*^{Val19} strains have high levels of adenylate cyclase activity. The addition of purified yeast or mammalian *ras* protein activates yeast adenylate cyclase in vitro. These results suggest that the yeast *RAS* proteins appear to stimulate adenylate cyclase in a manner analogous to the stimulation of mammalian adenylate cyclase by G_s protein (Fig. 6) (Gilman 1987).

On the basis of analogy to the G protein, *RAS* proteins may act as transducers to convey extracellular signals to an intracellular effector pathway. The presence of glucose and its derivatives results in a rapid increase in intracellular cAMP in yeast in a manner similar to the effect of various hormones and growth factors in mammalian cells (Eraso and Gancedo 1985). We tested the stimulation of glucose-mediated cAMP formation in *ras* mutants. In glucose-starved wild-type cells, glucose stimulated cAMP formation transiently (Fig. 7a). In *ras1::HIS3 ras2::LEU2 bcy1* cells, glucose increased cAMP formation slightly (K. Kaibuchi, unpubl.), suggesting that adenylate cyclase depends on more than just *CYR1* and *RAS* gene products. We have found that *GPA2* is involved in the regulation of cAMP levels (Nakafuku et al. 1988).

To examine the possibility that *GPA2* may be involved in the regulation of cAMP levels, we studied the effect of *GPA2* on cAMP formation. In the experiments shown in Figure 7, the kinetics of cAMP formation in response to glucose was measured. The *gpa2* disruption mutant (*gpa2::HIS3*) is viable and did not affect glucose-induced cAMP formation (Fig. 7b). On the other hand, introduction of a multicopy plasmid carrying *GPA2* (YEp*GPA2*) into the wild-type strain increased the level of glucose-induced synthesis of cAMP remarkably (Fig. 7c). This effect was not observed when a low-copy plasmid carrying *GPA2* (YCp*GPA2*) or YEp*GPA1* was introduced into wild-type cells.

The effect of introduction of YEp*GPA2* into various mutants that affect cAMP formation was examined. Although the introduction of YEp*GPA2* did not suppress the growth defect of a *ras1::HIS3 ras2::LEU2* double mutant or temperature-sensitive *cdc25* and *cyr1* mutants, we found that this plasmid can suppress a temperature-sensitive *ras2* mutant, *ras2*(ts). These results suggest that in addition to *RAS1* and *RAS2*, *GPA2* regulates the level of cAMP in *S. cerevisiae* (Fig. 6). Because the level of cAMP is determined by adenylate cyclase and phosphodiesterase activities, *GPA2* on multicopy plasmids may directly activate adenylate cyclase or inhibit phosphodiesterase. However, multicopy *GPA2* did not affect phosphodiesterase activity (T. Takagi and M. Nakafuku, unpubl.). If the function of *GPA2* is to regulate adenylate cyclase, the nonlethal phenotype of *gpa2::HIS3* suggests that an additional G protein may be involved in the activation of adenylate cyclase in *S. cerevisiae*. In view of the suppression of the growth defect in a *ras2*(ts) strain but not in a *ras1::HIS3 ras2::LEU2* strain by multicopy *GPA2*, *GPA2* protein may activate adenylate cyclase in a *RAS*-protein-dependent manner. Alternatively, the effect of *GPA2* could be indirect; thus, it may not be normally involved in the regulation of adenylate cyclase. In mammalian cells, hormonal stimuli dissociate $\beta\gamma$ sub-

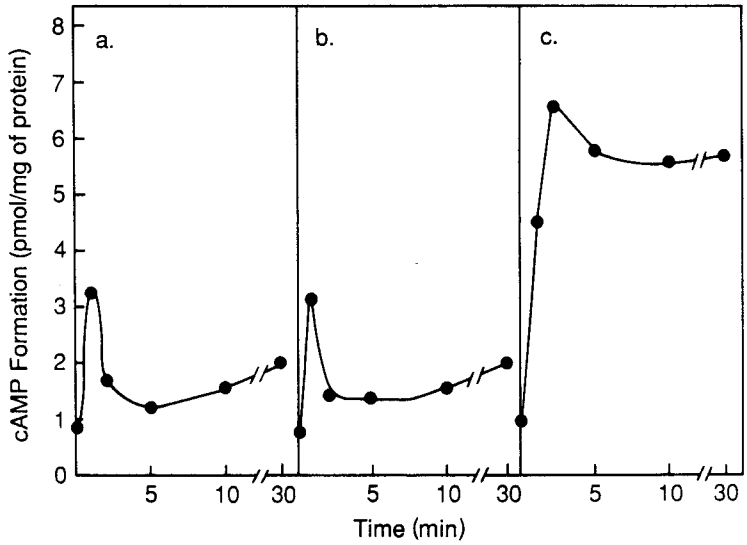

Figure 7. Kinetics of glucose-induced cAMP formation. Yeast cells were stimulated with 25 mM glucose and incubated at 30°C. (*a*) Wild-type strain; (*b*) *gpa2::HIS3* strain; (*c*) wild-type strain with YEp*GPA2* (Nakafuku et al. 1988).

units from α subunits of G proteins. Excess $\beta\gamma$ subunits are inhibitory to the activation of adenylate cyclase by the GTP-bound form of the $G_{s\alpha}$ (Katada et al. 1984). Likewise, *GPA2* protein, when expressed at a high level, may form a complex with free $\beta\gamma$ subunits, which are otherwise inhibitory to other G protein(s) involved in cAMP formation.

In the previous section, we presented the possibility that *GPA1* protein interacts with the mating-factor receptors (*STE2* and *STE3* products) in a manner analogous to mammalian G protein interaction with β-adrenergic-type receptor. Therefore, it may be reasonable to assume that *GPA2* protein also interacts with a receptor molecule in the cytoplasmic membrane of yeast cells to transmit a signal to an effector molecule.

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