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Effects of Expression of Mammalian Gα and Hybrid Mammalian-Yeast Gα Proteins on the Yeast Pheromone Response Signal Transduction Pathway

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Scg1, the product of the *Saccharomyces cerevisiae SCG1* (also called *GPA1*) gene, is homologous to the α subunits of G proteins involved in signal transduction in mammalian cells. Scg1 negatively controls the pheromone response pathway in haploid cells. Either pheromonal activation or an *scg1* null mutation relieves the negative control and leads to an arrest of cell growth in the G1 phase of the cell cycle. Expression of rat Gαs was previously shown to complement the growth defect of *scg1* null mutants while not allowing mating. We have extended this analysis to examine the effects of the short form of Gαs (which lacks 15 amino acids present in the long form), Gαi2, Gαo, and Scg1-mammalian Gα hybrids. In addition, we have found that constructs able to complement *scg1* are also able to inhibit the response to pheromone and mating when expressed in a wild-type *SCG1* strain. Overexpression of Scg1 has a similar inhibitory effect. These results are consistent with a model proposed for the action of Scg1 as the α component of a heterotrimeric G protein in which the βγ component (Ste4/Ste18) activates the pheromone response after dissociation from Scg1. They suggest that the Gα constructs able to complement *scg1* can interact with βγ to prevent activation of the pathway but are unable to interact with pheromone receptors to activate the pathway.

The G protein family transduces hormonal and sensory signals received by a major class of transmembrane receptors in eucaryotic cells. These receptors respond to an extracellular signal by directly activating a G protein on the cytoplasmic face of the membrane (for a review, see references 12, 26, and 29). Mammalian G proteins are heterotrimers of α (39 to 52 kilodaltons), β (35 to 36 kilodaltons), and γ (8 to 10 kilodaltons) subunits. The α subunits have highly conserved regions that are similar to the guanine nucleotide-binding pockets of the structurally defined GTP-binding proteins, EF-Tu and c-H-ras (9, 31). In the unstimulated state, the nucleotide-binding domain of α is occupied by GDP, and the G protein exists as a stable, membrane-bound heterotrimer. Interaction of an agonist with the appropriate receptor induces a receptor-G protein interaction that results in replacement of GDP by GTP and dissociation of α(GTP) from βγ. In the phototransduction and β-adrenergic systems, α(GTP) subunits directly activate effectors; however, recent evidence suggests that βγ can stimulate phospholipase A_2 activity in cardiac cells (4, 17).

In the yeast *Saccharomyces cerevisiae*, haploid cells of **a** and α mating types are prepared for mating by mutual interactions mediated by peptide pheromones (for a review, see reference 8). α cells secrete α-factor, a tridecapeptide that binds to the α-factor receptor present on **a** cells, and **a** cells secrete **a**-factor, a modified dodecapeptide that binds to the **a**-factor receptor present on α cells. Binding of either pheromone to its receptor results in an arrest of the cell cycle in G1 and other responses that are necessary for mating. Sequencing of the structural genes for the **a**- and α-factor receptors (*STE3* and *STE2*, respectively; 5, 13, 25) suggests that both have the seven transmembrane-spanning domain topology characteristics of mammalian G protein-coupled receptors.

Genes involved in pheromone response and mating that encode α, β, and γ homologs have been identified. The α homolog (Scg1) is encoded by *SCG1* (10) (also called *GPA1*; 22, 23), and the β and γ subunits (Ste4, Ste18) are encoded by *STE4* and *STE18*, respectively (32). Null mutations in these genes result in phenotypes in haploids but not diploids. The *ste4* and *ste18* null mutants are sterile (i.e., are defective in pheromone response and mating), indicating that wild-type Ste4 and Ste18 play positive roles in activation of the pheromone response pathway. The *scg1* null mutants show cell cycle arrest and morphological changes characteristic of pheromone response; this phenotype represents constitutive activation of the pheromone response pathway, indicating that wild-type Scg1 plays a negative or inhibitory role (10, 14, 22, 24).

Genetic results indicate that β and γ act downstream of α in the pathway (1, 24, 32). A model consistent with these results suggests that, in the resting state, the G protein exists as a heterotrimer, α(GDP)βγ (10, 14, 32). After activation of the pathway and guanine nucleotide exchange, α(GTP) and βγ dissociate, and βγ stimulates pheromone response by activation of a downstream effector. The negative role of α results from inhibition of βγ activity.

It was previously shown that expression of rat Gαs (the α subunit of the G protein that activates adenylate cyclase) under the control of the yeast *CUP1* promoter complemented the growth and morphological defects of an *scg1* null mutant (10). The *scg1*[Gαs] cells were sterile. In this paper, we have extended the analysis of the complementation of *scg1* by mammalian Gα genes to Gαi2 (one of three related Gα proteins involved in inhibition of adenylate cyclase) and

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TABLE 1. Yeast strains

Strain	Genotypic[a] or phenotypic characteristics	Source or reference
W303	*MATa/MATα [ade2-1 his3-11,5 leu2-3,112 trp1-1 ura3-1 can1-100]*	R. Rothstein
D109	*SCG1/scg1::URA3* derivative of W303	10
D111	*SCG1/scg1::LacZ-[LEU2]* derivative of W303	10
381G	*MATa cry1 ade2 his4*^{am} *lys2 trp1*^{am} *tyr1 SUP4-3*^{ts}	L. Hartwell
DJ147-1-1	*MATα ura3-52*, otherwise isogenic to 381G	D. Jenness
DJ147-1-2	*MATa ura3-52 leu2-3,112* Tyr$^+$, otherwise isogenic to 381G	D. Jenness
DJ211-3-4	*bar1-1* derivative of 147-1-2	D. Jenness

[a] Markers shown in brackets are homozygous.

Gαo (a G protein of unknown function abundant in brain tissue). We have also constructed and tested hybrid Scg1 and mammalian Gα genes. We show that expression of many of these constructs efficiently complements the *scg1* growth defect but does not allow mating. In addition, their expression exerts a dominant inhibitory effect on the response to α-factor and on mating in both **a** and α cells. Overexpression of *SCG1* has the same inhibitory effect.

MATERIALS AND METHODS

Yeast strains, transformations, and expression vectors. The yeast strains used are described in Table 1. Transformants of these strains with the plasmid constructs described below were selected by uracil or tryptophan prototrophy and maintained on SC-Ura medium (synthetic complete medium lacking uracil; 3) or SC-Trp medium. All media contained 2% glucose except media designed for induction of pGAL constructs, which contained 2% sucrose and 1% galactose. YEP-Gal medium, used for tetrad analysis, is yeast extract-peptone (3) containing 2% galactose. All plasmid constructs were propagated in *Escherichia coli* AKE28 (20) under ampicillin selection.

Expression vectors, pPGK (YEpDT-PGK; Fig. 1). The segment comprising Amp^R, Ori^E, and *URA3* was derived from YIp5. The 2-μm replication origin (a 1,424-base-pair (bp) *Hae*III-to-*Hpa*I fragment from YEp24) was inserted at the *Eco*RI site of YIp5, as in p1A1 (3). The *PGK* expression cassette was inserted between the *Cla*I and *Nru*I sites (converted by linker insertion to *Xho*I and *Sal*I, respectively) and consisted of two fragments derived from pMA91 (20). pMA91 contained a 2.9-kilobase *Hin*dIII fragment of yeast DNA comprising the *PGK* gene and flanking sequences and a deletion which joined nucleotide −2 to the *Bgl*II site 30 codons from the C terminus of the gene. The 672-bp fragment comprising UAS_{PGK} was derived by inserting an *Xho*I linker at the *Dra*I site at −670 and an *Eco*RI linker at the *Bgl*II site of pMA91 at −2. The 300-bp translation and transcription termination fragment was derived by *Bal* 31 recision at the *Bgl*II site followed by insertion of a *Bam*HI linker one codon upstream of the terminator codon (TAA) in the PGK reading frame. Terminators occurred in all three reading frames within 22 bp of the *Bam*HI site, immediately followed by a *Cla*I site (GGATCCGGGA AATAAATTGAATTGAATTGAAATGCAT). A *Sal*I linker was inserted at the *Hin*dIII site 300 bp from this *Bam*HI site, downstream of the *PGK* transcription terminator. Filling in of the two *PGK* fragments cut at the their *Eco*RI and *Bam*HI sites, followed by religation with a *Hin*dIII linker, regenerated both sites (GAATTCCAAGCTTGGGATCC), producing YEpDT-PGK with unique *Eco*RI, *Hin*dIII, and *Bam*HI cloning sites for expression (Fig. 1).

pGAL (YCpDT-GAL; Fig. 1). The 672-bp *Xho*I-*Eco*RI fragment comprising UAS_{PGK} in pPGK was replaced with the 685-bp *Bam*HI-*Eco*RI UAS_{GAL} fragment from pBM272 (provided by Mark Johnston) after filling in both *Xho*I and *Bam*HI sites. The *Xho*I site was regenerated. The product was YEpDT-GAL. The 985-bp *Xho*I-*Sal*I fragment of YEpDT-GAL comprising the expression cassette was reinserted into pBM272 between the *Eco*RI and *Sal*I sites after filling in both the *Eco*RI and *Xho*I sites, producing YCpDT-GAL (Fig. 1). This vector contained the *ARS1*, *CEN4*, and *URA3* yeast fragments (indicated in Fig. 1) derived from pBM272.

pCUP (pYSK-136; Fig. 1). The pCUP vector (11) comprised the Amp^R and Ori fragments of pBR322, the 2-kilobase *Eco*RI origin fragment of 2 μm, a 900-bp *TRP1* fragment, and an expression cassette consisting of a 430-bp UAS_{CUP1} fragment and a 310-bp *CYC1* terminator fragment separated by unique *Nco*I and *Asp*718I expression sites.

cDNAs and expression of full-length constructs. Gα cDNAs. Rat Gα cDNAs were provided by Randall R. Reed (15) as *Eco*RI fragments in λ phage or by Gary Johnson as sub-

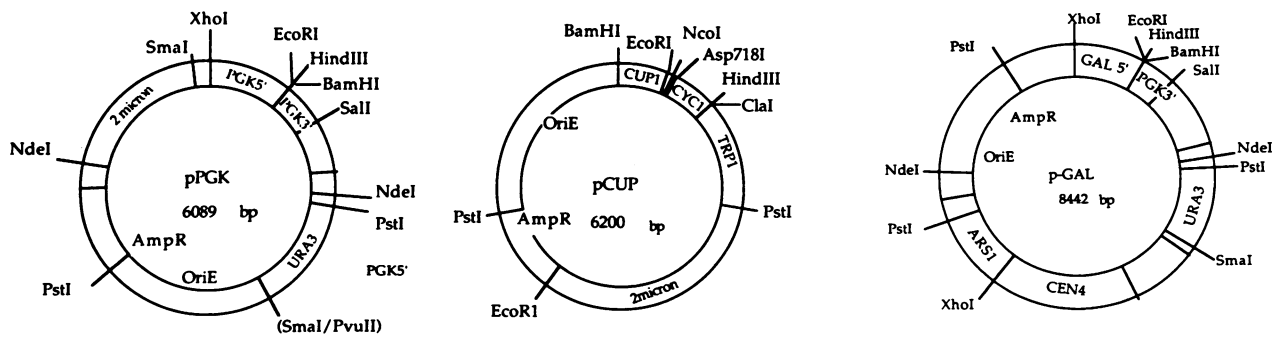

FIG. 1. Expression vectors. pCUP (pYSK; 11) contains an expression cassette consisting of the *CUP1* promoter and *CYC1* transcription terminator. pGAL contains an expression cassette consisting of the *GAL1,10* promoter and the *PGK* transcription terminator. pPGK contains an expression cassette consisting of the *PGK* promoter and transcription terminator sequence. Gα gene fragments were inserted into the unique *Eco*RI, *Hin*dIII, or *Bam*HI sites in pPGK and pGAL or at the *Nco*I site in pCUP. All three vectors contained the pBR322 Amp^R gene and replication origin and the indicated yeast sequences (see Materials and Methods). 2 micron, 2 μm.

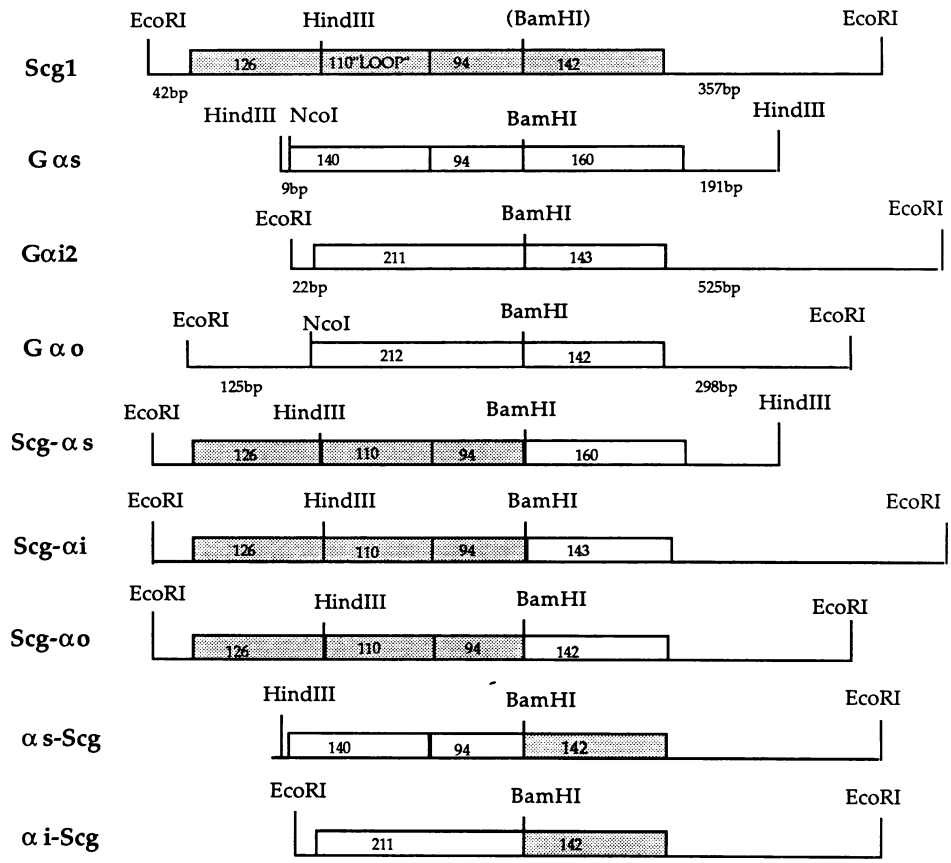

FIG. 2. Gα proteins and fusions: open reading frames with the lengths (in amino acid residues) of various regions indicated. Symbols: ▥, *SCG1*; □, mammalian Gα cDNAs. The *Hin*dIII site in *SCG1* is approximately at the start of the unique 110-residue loop in Scg1 which has no counterpart in the mammalian Gα proteins (10, 23). A *Bam*HI site was introduced into *SCG1* at a position homologous to a *Bam*HI site in Gαs, Gαi2, and Gαo (see Discussion), and the hybrid genes shown were constructed. The sizes (in base pairs) of the 5′ and 3′ sequences included when these constructs were inserted in the pGAL and pPGK expression vectors (Fig. 1) are also indicated. The pCUP constructs utilized an *Nco*I site at the Gα ATG initiation codon; therefore, no rat 5′ sequences were present upstream of the initiation codon in these constructs (data not shown).

clones in pGEM2. They included Gαi2, Gαo, and both Gαs long (Gαs) and short [Gαs(sh)].

pCUP expression constructs. The Gαs cDNA clone was cleaved at the *Afl*II site 27 bp past the translation termination codon. After being filled in with DNA polymerase (Klenow fragment) and cut with *Nco*I at the initiation codon (boldface) (CC**ATG**G), the 1,218-bp fragment of Gαs was cloned into pCUP between the *Nco*I and filled-in *Asp*718I sites. The Gαs(sh) construct was made by exchanging the internal *Bam*HI fragment of Gαs in pCUP-αs for the equivalent fragment of Gαs(sh). Similar procedures were used to insert minimal-length fragments of Gαi2 and Gαo cDNAs lacking rat 5′ sequences into the same expression sites.

pGAL and pPGK expression constructs (Fig. 2). To insert rat Gαs into pPGK, the pGEM clone was cleaved at the unique *Nco*I site at the translation start point. Insertion of a 10-bp *Hin*dIII linker after filling in produced a *Hin*dIII site at −9, leaving only 1 bp of rat 5′ sequence before the initiation codon (CCAAGCTTGG**CATG**). The 1,374-bp *Hin*dIII fragment starting at this point and ending 191 bp downstream of the translation terminator for Gαs was inserted into the *Hin*dIII sites of pPGK and pGAL for expression.

Rat Gαi2 cDNA was provided as a 1,750-bp *Eco*RI fragment in pGEM2. pPGK-αi was produced by partial *Bgl*I digestion of the pGEM2 clone followed by treatment with the Klenow fragment of DNA polymerase and insertion of a 10-bp *Eco*RI linker, producing an *Eco*RI site at −22 and retaining 14 bp of rat 5′ sequence (CCGAATTCGGCGG CGGACGGCAGGA**TG**). The 1,619-bp *Eco*RI fragment from this site to the *Eco*RI site 525 bp downstream of the translation terminator for Gαi2 was inserted into the *Eco*RI sites of pPGK and pGAL for expression (Fig. 2).

The yeast *SCG1* gene was cloned into M13 mp10 as a 1,931-bp *Eco*RI fragment. Mutagenesis with the oligonucleotide 5′-TTAGCATCACATCAAGAATTCAGAG-3′ (underlined nucleotides indicate positions resulting in nucleotide changes) was employed to insert an *Eco*RI site at −42 (Fig. 2). The new 1,762-bp *Eco*RI fragment was cloned into M13 mp19 and mutated with 5′-GAAGTGGATCCATTGTT TCG-3′ to create a silent *Bam*HI site at +989, producing Scg1(B). The 1,762-bp *Eco*RI fragment, with and without this mutation, was cloned into pGAL for expression, producing pGAL-Scg and pGAL-Scg(B) (Fig. 2). pPGK-Scg was constructed similarly.

Gα fusion constructs (Fig. 2). Scg-αi. The 1,031-bp N-terminal fragment of *SCG1* from the *Eco*RI site at −42 to the mutant *Bam*HI site at 989 and the 1,018-bp C-terminal fragment of Gαi2 from the *Bam*HI site at 634 to the downstream *Hin*dIII site of the pGEM2 multicloning site were inserted simultaneously into pGAL and also into pPGK, both cut with *Eco*RI and *Hin*dIII. The sizes of the protein

fragments in the hybrids resulting from this and other fusions are indicated in Fig. 2.

Scg-αs. The same *SCG1* fragment and the 666-bp C-terminal fragment of Gαs from the *Bam*HI site at 707 to the downstream *Hin*dIII site were inserted into the expression vectors as described above.

Scg-αo. Rat Gαo cDNA, provided as a 1,485-bp *Eco*RI fragment in pGEM2, was cloned directly into the *Eco*RI site of pPGK, producing pPGK-αo. This construct had 116 bp of 5′- and 298 bp of 3′-flanking cDNA sequence (Fig. 2). The fragment of Gαo from the *Bam*HI site at 635 to the *Bam*HI site just downstream of the *Eco*RI site in pPGK-αo (Fig. 1) was isolated and used to replace the *Bam*HI fragment from 989 to the same vector site in pPGK-Scg(B), producing pPGK-Scg-αo (Fig. 2).

αi-Scg. The 656-bp N-terminal fragment of Gαi2 from the *Eco*RI site at −22 to the *Bam*HI site at 634 and the 783-bp C-terminal fragment of *SCG1* from the mutant *Bam*HI site at 989 to the downstream *Hin*dIII site were inserted into expression vectors simultaneously, as described above.

αs-Scg. The 710-bp N-terminal fragment of Gαs from the *Hin*dIII site at -9 to the second *Bam*HI site was obtained by partial *Bam*HI hydrolysis and cloned into the pPGK and pGAL vectors cleaved with *Hin*dIII and *Bam*HI. The 752-bp C-terminal *SCG1 Bam*HI fragment from 989 to the downstream *Bam*HI site was subsequently inserted in the correct orientation to create the αs-Scg fusions.

Assays for effects on the pheromone response pathway. Complementation of the growth defect due to *scg1* null mutations was tested by transformation of strain D109 or D111 (Table 1) and sporulation and dissection of the diploids. Diploids containing pGAL constructs were dissected on YEP-Gal medium, diploids containing pCUP constructs were dissected on SC-Trp+Cu (100 μM) medium, and diploids containing the constitutive pPGK constructs were dissected on SC-Ura medium. Tetrads were monitored for growth of more than two spores per tetrad, and viable segregants were picked and tested for the marker present at *scg1* and for mating.

Response to α-factor was tested by monitoring growth inhibition. Transformants of strain DJ211-3-4 (Table 1) were plated as lawns in soft agar overlays on SC-Ura medium (pH 4.7) with appropriate carbon sources. Disks containing different quantities of α-factor (10 μl of 0×10^{-6}, 2×10^{-6}, 5×10^{-6}, and 10×10^{-6} M) were placed on the lawns. After 24 and 48 h at 30°C, zones of growth inhibition surrounding the disks were inspected for diameter and density of growth compared with the rest of the lawn. Clear, sharp-edged zones whose diameters were proportional to the log of α-factor concentration were produced on controls containing the vectors without inserts. Constructs that inhibited pheromone response allowed some growth within the α-factor zones; estimates of growth density were made visually. Repetition with multiple independent transformants gave reproducible results.

Inhibition of mating was tested by quantitative mating assays. Transformants of strains DJ147-1-1 and DJ147-1-2 (Table 1) were grown to mid-exponential phase on SC-Ura medium (pH 4.7) containing appropriate carbon sources, and mating efficiency was assayed by a membrane filter procedure (1).

RESULTS

Expression of heterologous and hybrid Gα proteins in yeast. In mammalian cells, major forms of Gαs are expressed that differ by the presence or absence of 15 amino acids due to differential splicing (12). The form previously shown to complement the growth and morphological defects of *scg1* null mutants was Gαs under the control of the copper-inducible *CUP1* promoter (10). We have extended this analysis to Gαs(sh), Gαi2, and Gαo (Fig. 2).

To examine the functional aspects of Gα protein domains, hybrids were made between *SCG1* and rat Gα cDNAs (diagrammed in Fig. 2) as described in Materials and Methods. Construction of these hybrids involved introducing a silent mutation in the Ile-coding codon 331 of *SCG1* that introduces a *Bam*HI site to produce *SCG1(B)*. *Bam*HI sites were present in Gαs, Gαo, and Gαi2 at the analogous position and were used to construct in-frame fusions between *SCG1* and the three rat Gα cDNA genes.

The heterologous and hybrid Gα proteins were expressed in yeast by using three different vectors, pCUP, pGAL, and pPGK (Fig. 1). To allow efficient expression, the heterologous or hybrid gene in each of the plasmids was flanked by a yeast upstream regulatory region and a yeast terminator. pGAL is a YCp (stable, low-copy-number) vector with the galactose-inducible *GAL1,10* promoter and *PGK* terminator. pPGK is a YEp (high-copy-number) vector with the constitutive *PGK* promoter and the *PGK* terminator. pCUP is a YEp vector with the copper-inducible *CUP1* promoter and the *CYC1* terminator.

The differences in plasmid copy number and promoter strength should have resulted in different expression levels for each of the vectors. Relative steady-state levels of transcripts, as determined by Northern (RNA) blot analysis with appropriate probes for the heterologous Gα proteins, were estimated to be 1:50:250 for pGAL, pPGK, and pCUP constructs, respectively (data not shown). The induced pGAL-Scg transcript in strain DJ211-3-4 was present at about twice the level of the endogenous 1,700-bp transcript derived from the wild-type gene (data not shown).

A polyclonal antiserum raised against a fusion of Gαs to the N-terminal 81 residues of the influenza virus NSI protein (34) was used to detect total cellular Gαs proteins by Western blot (immunoblot) (data not shown). Protein was undetectable in cells of strain DJ211-3-4 expressing pGAL-αs. Proteins of the expected sizes were detected in cells expressing pCUP-αs, pPGK-αs, pPGK-αs-Scg, and pPGK-Scg-αs. All were detected at similar levels in spite of the higher transcript levels expressed by pCUP-αs. For some constructs (e.g., pCUP-αs and pPGK-αs), therefore, protein and RNA levels did not correlate well. Protein levels in cells expressing pCUP-αs(sh) were 5- to 10-fold higher than for pCUP-αs.

Complementation of *scg1* null mutations. In haploid yeast, *scg1* null mutations result in a growth arrest due to constitutive activation of the pheromone response pathway (10, 14, 24). The *scg1* spores that arise from dissection of an *SCG1/scg1* diploid form microcolonies (see Fig. 3; tetrads with vector alone). It was previously shown that expression of pCUP-αs in *scg1* spores resulted in spore colonies that were smaller than those of the wild type (10; see Fig. 3) but that growth was similar to that of the wild type after the colonies were picked to fresh plates. The reason for the initial growth lag is unclear; however, the size of *scg1* spore colonies expressing the various Gα constructs provided a measure of complementation of the *scg1* growth defect. The ability of mammalian Gα genes and hybrids between the *SCG1* and mammalian genes to complement *scg1* was tested by transformation of *SCG1/scg1* diploids with the various constructs and examination of the growth and mating of

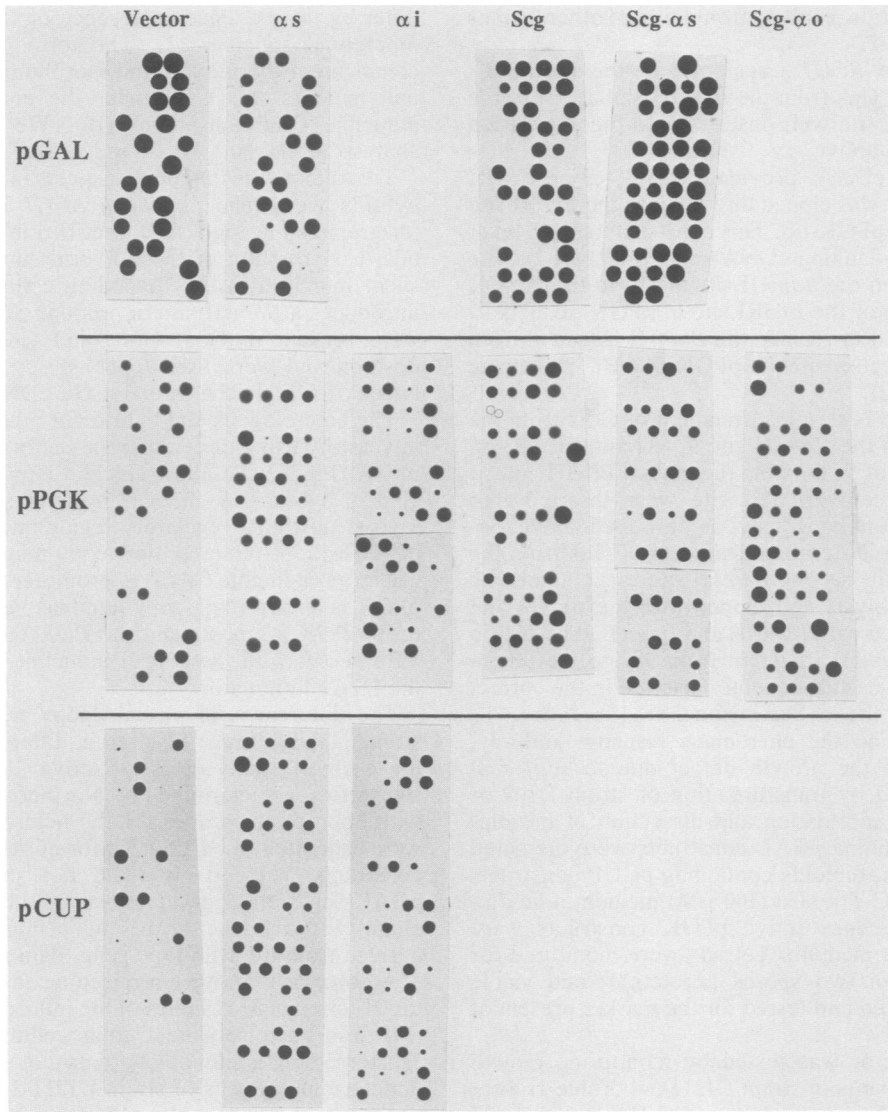

FIG. 3. Complementation of *scg1* growth defect by Gα constructs. Strain D111 (*SCG1/scg1*) containing the indicated constructs was sporulated and dissected onto medium that provided the appropriate selection and induction of the various plasmid constructs (see Materials and Methods). With vector alone, tetrads gave rise to two wild-type colonies and two barely visible colonies. The larger colony size for more than two spores per tetrad indicates complementation of the *scg1* growth defect. The relative complementation by these constructs is summarized in Table 2.

spores obtained by sporulation of the diploids and tetrad analysis. Representative tetrads are shown in Fig. 3.

All of the Gαs constructs complemented the *scg1* growth defect, but none allowed mating (Fig. 3 and Table 2). Complementation was poorest with pGAL-αs, intermediate with pCUP-αs, and best with pPGK-αs. Expression of Gαs(sh) under the control of the *CUP1* promoter [pCUP-αs(sh)] produced results indistinguishable from those produced with the long form (pCUP-αs; Fig. 3).

Gαi2 expressed from pCUP or pPGK but not from pGAL weakly complemented the *scg1* growth defect (Fig. 3 and Table 2). Again, pPGK-Gαi2 gave the strongest effect. The weakly complemented cells were sterile. No complementation of *scg1* was seen in cells expressing Gαo from either pCUP or pPGK.

A silent *Bam*HI site was introduced into *SCG1* to allow construction of the hybrid genes. Growth and mating were normal in both *scg1* and *SCG1* cells expressing either pGAL-Scg or pGAL-Scg(B) (Fig. 3), consistent with the presumptively silent mutation. The Scg-αs, Scg-αi, and Scg-αo hybrids efficiently complemented the *scg1* growth defect on either pGAL or pPGK vectors (Fig. 3 and Table 2), and the resulting cells were sterile. Again, the pPGK constructs were more effective than the pGAL constructs. The remaining hybrids, αs-Scg and αi-Scg, expressed from either pGAL or pPGK, were unable to complement *scg1*.

Inhibition of pheromone response and mating by expression by Gα proteins. The sterility of *scg1* cells expressing heterologous or hybrid Gα proteins suggested that these proteins were able to interact with a downstream component of the pheromone response pathway to keep the pathway inactivated but were unable to interact with the pheromone receptors to elicit activation of the pathway in response to pheromone (see Discussion). If the heterologous and hybrid

TABLE 2. Effects of Gα protein expression

Plasmid	Growth rate[a]	Inhibition of α-factor response[b]	% Inhibition of mating[c]
PGK, GAL, CUP	−	−	0
GAL-αs	(+)	++	35
CUP-αs	++	+++	ND
CUP-αs(sh)	++	+++	ND
PGK-αs	+++	++++	94
GAL-αi	−	(+)	0
CUP-αi	(+)	+	ND
PGK-αi	+	++	47
CUP-αo	−	−	0
GAL-Scg-αs	++	++	22
GAL-Scg-αi	+++	+++	62
PGK-Scg-αs	+++	+++	64
PGK-Scg-αi	+++	++++	100
PGK-Scg-αo	+++	++++	80
PGK-αs-Scg	−	−	0
PGK-αi-Scg	−	−	0
GAL-Scg	+++	−	0
GAL-Scg(B)	+++	−	0
PGK-Scg	+++	++++	86

[a] Colony size was estimated for *scg1* segregants carrying the indicated plasmids (Fig. 3). Symbols (colony size): +++, indistinguishable from Scg1$^+$; ++, somewhat smaller; +, small; (+), very small; −, identical to *scg1* null spores.

[b] Assessed from the relative densities of growth within zones of inhibition surrounding α-factor disks on test plates (Fig. 4). Symbols: ++++, lack of a detectable inhibition zone; +++, density 60 to 80% of lawn; ++, density 30 to 60% of lawn; +, density 10 to 30% of lawn; (+), several isolated colonies inside zone of inhibition; −, normal response.

[c] Assayed as described in Materials and Methods. Mating efficiency was normalized to matings of pairs of cells containing the PGK vector. ND, Not determined.

Gα proteins are able to interact with the downstream component in the presence of Scg1, they should inhibit activation of the response pathway in the presence of pheromone, resulting in dominant inhibition of mating. We therefore tested α-factor response and mating in wild-type *SCG1* strains expressing the various constructs.

Pheromone response was assayed by a plate assay for the inhibition of growth by α-factor. Disks containing α-factor produced clear, sharp-edged zones of growth inhibition on lawns of the *SCG1 bar1* strain (DJ211-3-4) carrying the vectors without insert (Fig. 4). Cells containing constructs that complemented the *scg1* growth defect also showed zones of growth inhibition of similar sizes, but this inhibition was partial; i.e., a variable extent of growth was seen within the α-factor zone. It has been suggested that growth within the zone of inhibition reflects more rapid recovery from growth inhibition, and a role has been proposed for Scg1(GTP) in this adaptation phenotype (6, 7, 21). Analysis of growth kinetics in *scg1* strains expressing the heterologous and hybrid Gα constructs (C. Mang and D. J. Tipper, unpublished data) suggested that the primary effect was on initiation of the response rather than on adaptation. We therefore interpret our assay as measuring inhibition of pheromone response. The relative levels of growth within the α-factor zones were used to estimate the degree of inhibition (Table 2).

Expression of the same constructs also resulted in an inhibition of mating as determined by quantitative mating assays (Table 2). Inhibition was more effective when a particular construct was expressed in cells of both mating types rather than in only one of the mating pair; e.g., 94% inhibition of mating was seen when both **a** and α cells contained pPGK-αs, whereas inhibition was about 85% when cells of either mating type contained pPGK-αs and the strain of the opposite mating type contained the pPGK vector. The results summarized in Table 2 represent mating frequencies with strains of both mating types expressing identical constructs.

An excellent correlation between the ability to complement the *scg1* growth defect and the ability to inhibit pheromone response and mating in the *SCG1* strain was seen (Table 2). Inhibition by Gαs was considerably greater than that by Gαi2 expressed from the same construct, and Gαo did not cause detectable inhibition. Inhibition was greater for a particular Gα protein expressed from pPGK or pCUP than when expressed from pGAL. The Scg-αi and Scg-αo constructs had the strongest effects in all three assays of any construct tested.

Overexpression of SCG1 inhibited mating and response to α-factor. Growth rates were normal in both *scg1* and *SCG1* cells expressing pPGK-Scg. However, while mating was normal in both *scg1* and *SCG1* cells expressing pGAL-Scg, and expression of pGAL-Scg did not affect the α-factor

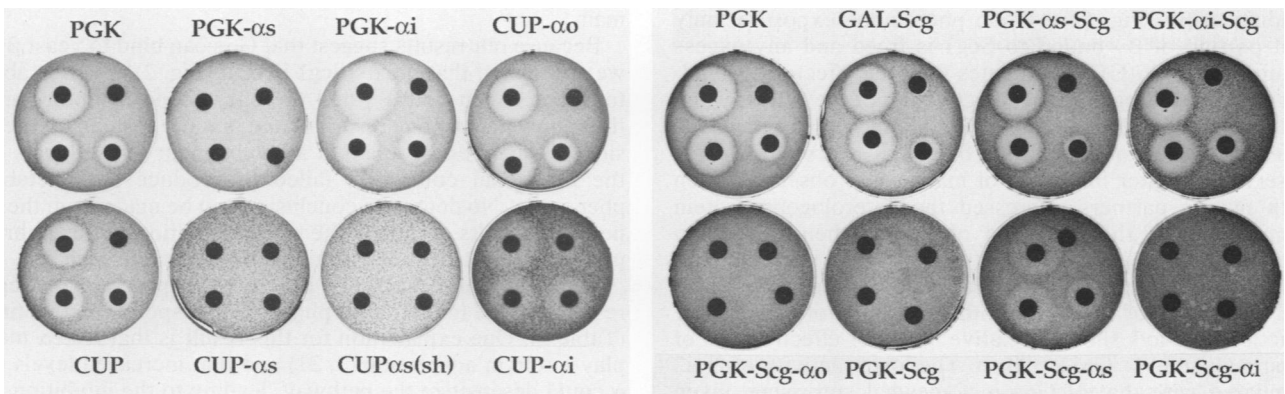

FIG. 4. Plate assays of α-factor sensitivity. Cells of strain DJ211-3-4 (*MAT***a** *SCG1 bar1*), transformed with the indicated plasmids, were spread as a soft agar overlay onto SC-Ura or SC-Trp (100 μM Cu^{2+}) buffered to pH 4.7 and exposed to disks containing increasing quantities of α-factor (see Materials and Methods). Zones are shown after 48 h at 30°C.

response in the wild-type **a** strain (DJ211-3-4) (Fig. 4), expression of pPGK-Scg efficiently inhibited α-factor response in this strain. The test plates failed to show even faint zones of inhibition surrounding α-factor disks (Fig. 4). Mating efficiency was also reduced by 86% when pPGK-Scg was expressed in both **a** and α cells (Table 2).

DISCUSSION

Scg1, the product of the yeast *SCG1* gene, is a yeast Gα homolog involved in pheromone response and mating (10). It was previously shown that the expression of the long form of rat Gαs in yeast was able to complement the growth and morphological defects resulting from null mutations in *scg1* (10). In this paper, we have extended this analysis to examine the effects of Gαs(sh) (which lacks 15 amino acids present in the long form; 12), Gαi2, Gαo, and Scg1 and mammalian Gα hybrids. In addition, we have found that constructs able to complement the growth defect in an *scg1* mutant are also able to inhibit the response to α-factor and mating when expressed in a wild-type *SCG1* strain. Overexpression of Scg1 has a similar inhibitory effect.

These results are consistent with a simple model (Fig. 5A) for the role of the yeast Gα(Scg1), Gβ (Ste4), and Gγ (Ste18) subunits in the activation of the pheromone response pathway. Genetic results (1, 24, 32) indicate that βγ functions downstream of α to activate the pathway, presumably by activating a downstream effector which is currently unidentified. In the absence of pheromone, α is presumed to bind GDP tightly and interact with βγ to inhibit the pathway. In the presence of pheromone, the pheromone receptor interaction relieves this negative control by promoting GDP and GTP exchange on α, resulting in dissociation of α from βγ; free βγ then activates the pathway. In an *scg1* null mutant, free βγ is present and constitutively activates the pathway (Fig. 5B), leading to G1 arrest and morphological alterations.

The ability of a mammalian Gα protein to complement the *scg1* growth defect suggests that the heterologous protein is able to interact with yeast βγ as an analog of Scg1(GDP), thus preventing activation of the pathway (Fig. 5C). The resulting cells are sterile and unable to respond to pheromone, suggesting that the heterologous protein cannot interact functionally with the pheromone receptors. The ability of heterologous proteins to complement the *scg1* growth defect correlates well with their ability to inhibit pheromone response and mating when expressed in wild-type *SCG1* cells. This effect is also consistent with the model, because the heterologous or hybrid Gα protein competes with Scg1 for binding to βγ (Fig. 5D). Upon pheromone exposure, only that portion of βγ bound to Scg1 is freed and any excess heterologous Gα(GDP) competes with the effector for binding to βγ, resulting in reduced activation of the pathway and reduced mating. The degree of inhibition should reflect the level of expression of the heterologous Gα protein, as was observed. Greater inhibition of mating was observed when both mating partners expressed the heterologous protein compared with the inhibition observed when expression occurred in only one of the two partners, because both mating partners must respond for mating to occur.

Comparison of Gαs with other rat G proteins by our functional assays showed relative levels of effectiveness of Gαs = Gαs(sh) > Gαi2 > Gαo. The lower activity of Gαi2 compared with that of Gαs is somewhat surprising, given that sequence conservation between Scg1 and Gαi2 (10, 23) is significantly higher than for Gαs. Intriguingly, in vitro studies of Gα proteins (2, 12, 16, 27, 28) indicate relative affinities of mammalian βγ of Gαs > Gαi >> Gαo, which correlates with relative activities in our assays. Direct assays of G protein subunit interactions will be necessary to determine the relevance of this correlation.

The construction of Scg1 and mammalian Gα hybrids was undertaken to analyze the role of various domains of Gα subunits (18) in the yeast signal transduction pathway. Fusions between *SCG1* and rat Gα cDNAs were made at a site within a highly conserved sequence of 32 amino acids predicted by analogy to *ras* (9) and EF-Tu (18) to comprise a critical component of the nucleotide-binding pocket of Gα proteins. The fusions, therefore, should be likely to retain normal structure in this region, and this region is unlikely to be involved in differential Gα functions. A Gαi-Gαs hybrid with the analogous fusion position has been shown to function in mammalian systems (19). The Scg1-αs, Scg1-αi2 and Scg1-αo hybrids (Fig. 2) inhibited pheromone response and mating in an *SCG1* strain and allowed growth but not mating of the *scg1* null mutant. These results indicate sufficient conservation of structure between the yeast and mammalian Gα proteins to allow the function of appropriate domains in the hybrids and suggest that, like intact Gαs and Gαi, these hybrid proteins can interact with βγ but not with the pheromone receptors (Fig. 5). The sterility of *scg1* strains and inhibition of mating in *SCG1* strains expressing the hybrids are consistent with an essential role for the C terminus of Scg1 in receptor interactions, as demonstrated in mammalian systems (18, 19, 30). High-level expression of the N-terminal 330 amino acids of Scg1 by itself was without effect in our assays (data not shown), and a 22-amino-acid C-terminal truncation of Scg1 results in an *scg1* null phenotype (S. Hirsch, C. Dietzel, and J. Kurjan, unpublished data). Thus, the C-terminal region of Scg1 is essential to prevent activation of the pheromone response pathway by Scg1 in the absence of pheromone but can be replaced by heterologous Gα sequences.

The efficient activity of the Scg1-αi2 and Scg1-αo hybrids, in contrast to that of the intact Gαi2 and Gαo proteins, suggests that the domain critical for interaction with βγ lies within the N-terminal 330 amino acids of Scg1. These results, therefore, are consistent with data from mammalian systems that have indirectly implicated the amino termini of Gα proteins in βγ interactions (18). The relative activities of the fusion proteins (Scg1-αi = Scg1-αo > Scg1-αs), unlike those of the intact proteins, do reflect sequence similarity to Scg1. The effectiveness of these fusions may depend, therefore, on the extent to which these heterologous C-terminal sequences interact appropriately with the N-terminal domain.

Because our results suggest that Gαs can bind to yeast βγ, we speculated that the αs-Scg1 hybrid (Fig. 2) might be able to interact with both βγ and the pheromone receptors and thus allow pheromone response and mating. Instead, expression of this hybrid protein at a level similar to the levels of the functional constructs failed to produce a detectable phenotype. No definitive conclusion can be made from these negative results, although the lack of function of this hybrid protein suggests that its structure may be abnormal.

We also found that very high level expression of Scg1 resulted in the inhibition of pheromone response and mating (Table 2). One explanation for this result is that free α may play a role in adaptation (7, 21) and that increased levels of α could desensitize the pathway, leading to the inhibition of pheromone response and mating. Alternatively, this result may reflect the importance of the balance between levels of the G protein subunits for normal activation of the pathway.

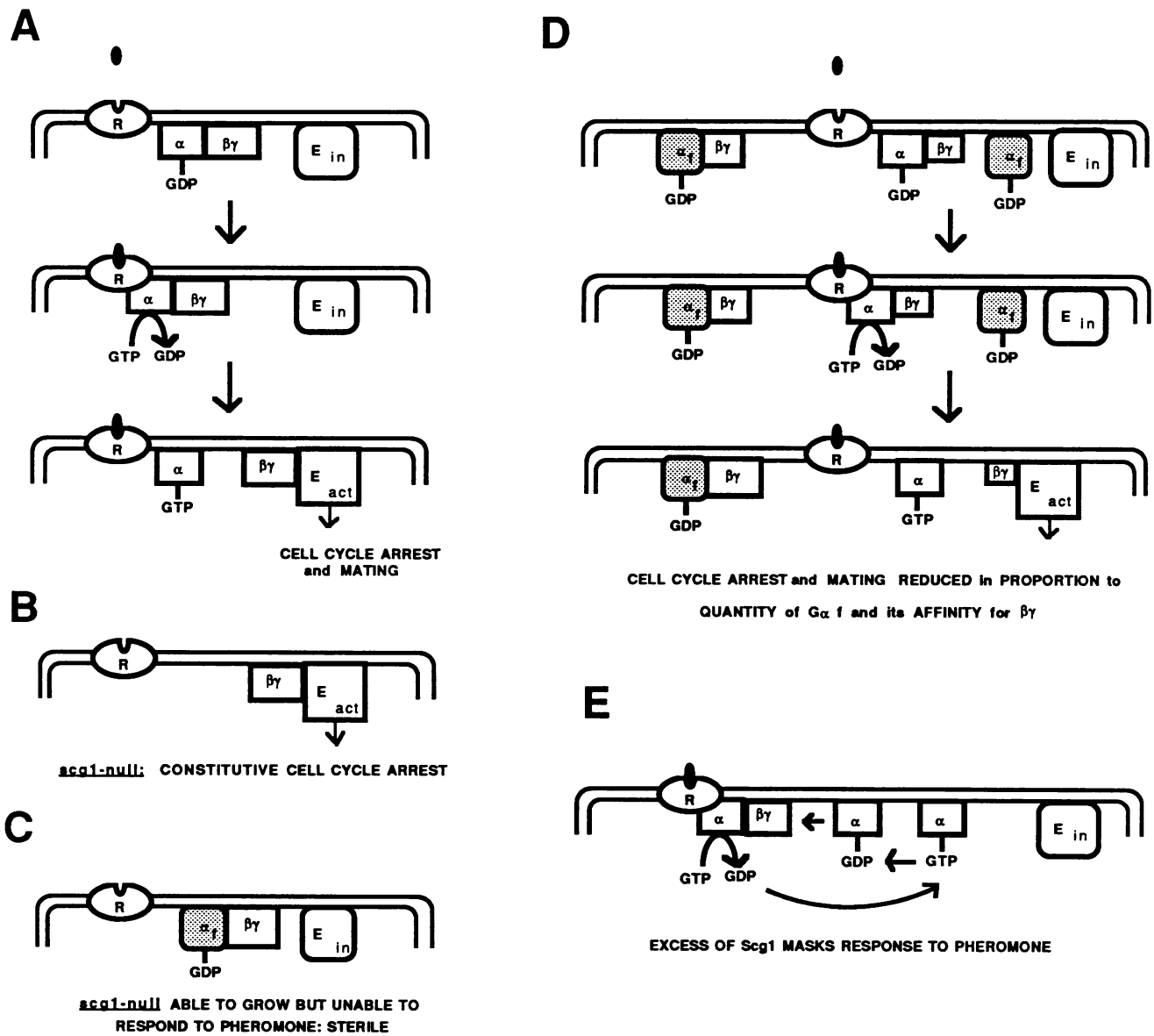

FIG. 5. Model for pheromone response pathway and effect of expression of heterologous or hybrid Gα proteins. (A) In the absence of pheromone (●), the pheromone response pathway (E) is inactive (in) because Gα(GDP) (Scg1) binds Gβγ (Ste4/Ste18). Interaction of pheromone with its receptor induces a GDP-GTP exchange on Gα, resulting in the release of Gβγ, which positively activates (act) the effector pathway, leading to cell cycle arrest and other aspects of the pheromone response. (B) In an *scg1* mutant, Gβγ is free, leading to constitutive activation of the pathway and cell cycle arrest. (C) A heterologous or hybrid Gα protein ($G\alpha_f$) complements the *scg1* growth defect in proportion to its ability to bind Gβγ. Because it cannot interact with the receptor, the pathway cannot be activated, so the cells are sterile. (D) When both Scg1 and a $G\alpha_f$ are present, they compete for binding to Gβγ. $G\alpha_f$ remains bound to Gβγ after exposure to pheromone, and any excess $G\alpha_f$ will compete with effector in binding Gβγ released by Scg1(GTP), reducing activation of the pathway and mating. The degree of inhibition should be proportional to the level of $G\alpha_f$ and its affinity for Gβγ. (E) In a strain greatly overproducing Scg1, conversion of Scg1(GDP)βγ to dissociated Scg1(GTP) and βγ by activated receptor is unaffected, but excess Scg1(GDP) is present and binds to freed βγ. The presumptive intrinsic GTPase activity of Scg1 may result in the maintenance of Scg1(GDP) levels sufficient to keep the pathway inactivated.

It has recently been shown (6, 33) that increasing the level of Ste4 (β) constitutively activates the pathway, while a simultaneous increase in the level of Scg1 (α) prevents this constitutive activation. Our data suggest that greatly increasing α alone may result in the persistence of sufficient α(GDP) to complex the available βγ, even in the presence of a fully activated receptor (Fig. 5E). Increased levels of either receptor or βγ, therefore, might alleviate the inhibitory effect of increased α on the pheromone response.

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