The carboxyl terminus of Scg1, the G α subunit involved in yeast mating, is implicated in interactions with the pheromone receptors

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The carboxyl termini of α subunits of mammalian G proteins have been implicated in receptor interactions. We have used a genetic analysis to test such a role for the carboxyl terminus of Scg1, the α subunit involved in the yeast pheromone response pathway. A 22-amino-acid truncation (*scg1^{Amb451}*) resulted in defects in growth and cellular morphology. This phenotype is similar to the null phenotype and represents constitutive activation of the pheromone response pathway; it could result from various effects, including protein instability or constitutive guanine nucleotide exchange, as reported for some altered mammalian G α s constructs. A 5-amino-acid truncation (*SCG1^{Och468}*) resulted in pheromone response and mating defects in both **a** and α cells, which is consistent with defects in interactions with the pheromone receptors. Lysine-to-proline mutations near the carboxyl terminus (*SCG1^{Pro467}* and *SCG1^{Pro468}*) resulted in pheromone response and mating defects, the severity of which differed in **a** and α cells. This differential effect in the two mating types suggests that the specificity for the interactions with the two pheromone receptors may involve different residues of the Scg1 carboxyl terminus. Mutations leading to constitutive activation of the pathway were recessive, whereas mutations that result in decreased pheromone response and mating were partially dominant. These relationships are consistent with the model for the mechanism of action of the G protein subunits in the pheromone response pathway and indicate the importance of the stoichiometry of components of this system.

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Signal transduction mediated by regulatory G proteins in mammalian systems involves the interaction of a ligand-receptor complex with an intracellular G protein composed of α , β , and γ subunits (for review, see Gilman 1987). Before activation, the α subunit has GDP bound and is complexed with $\beta\gamma$. After receptor activation, GDP is replaced by GTP, and the α subunit dissociates from the $\beta\gamma$ complex. The intrinsic GTPase activity of the α subunit hydrolyzes the bound GTP, regenerating the GDP-bound form of the α subunit, which reassociates with $\beta\gamma$ to complete the cycle.

A similar system in yeast is involved in the response of cells to peptide pheromones and is required for mating (for review, see Cross et al. 1988). Cells of **a** and α mating type secrete **a**-factor and α -factor, respectively, and respond to the pheromone secreted by the opposite mating type. Response to pheromone causes arrest of cells in the

G₁ phase of the cell cycle, morphological changes (called shmooing), and induction of a number of genes, many of which play roles in pheromone response or other aspects of mating. The **a**-factor receptor (Ste3) and α -factor receptor (Ste2) are expressed by α and **a** cells, respectively. These receptors share no sequence homology; however, they are both proposed to form a structure with seven membrane-spanning domains similar to mammalian and other G protein-coupled receptors (Herskowitz and Marsh 1987). Ste2 and Ste3 activate a common intracellular signaling pathway (Bender and Sprague 1986; Nakayama et al. 1987).

Genes encoding the three subunits of the regulatory G protein involved in this pathway have been identified. The α subunit is encoded by the *SCG1* (or *GPA1*) gene (Dietzel and Kurjan 1987a; Nakafuku et al. 1987), and the β and γ subunits are encoded by the *STE4* and *STE18* genes, respectively (Whiteway et al. 1989). Null mutations of *scg1* result in constitutive activation of the pathway, indicating that Scg1 plays a negative role (Dietzel and Kurjan 1987a; Miyajima et al. 1987; Jahng et al. 1988; Nakayama et al. 1988). Null mutations of *ste4* and

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ste18 result in an inability to activate the pathway, indicating that they play positive roles (Whiteway et al. 1989). Analysis of double mutants has shown that the β and γ subunits act downstream of the α subunit (Nakayama et al. 1988; Whiteway et al. 1989; Blinder et al. 1989). These observations are consistent with a model similar to the mammalian model, except that after dissociation of $\beta\gamma$ from α -GTP, $\beta\gamma$ activates an as yet unidentified effector that acts to stimulate all aspects of pheromone response.

Several lines of evidence have implicated the carboxyl termini of $G\alpha$ subunits in receptor interactions. A hybrid $G\alpha$ protein composed of the amino-terminal 212 amino acids of $G\alpha_i$ and the carboxy-terminal 160 amino acids of $G\alpha_s$ displays the same effector and receptor specificity as $G\alpha_s$, indicating that both of these functions reside in the carboxy-terminal 40% of the protein (Masters et al. 1988). Modification of $G\alpha_t$ (transducin) by pertussis toxin, which catalyzes ADP ribosylation of a cysteine residue 4 amino acids from the carboxyl terminus, creates a protein with a lower affinity for rhodopsin (Van Dop et al. 1984). Polyclonal antibodies raised against the terminal decapeptide of $G\alpha_t$ (Cerione et al. 1988) or monoclonal antibodies that bind to this region (Hamm et al. 1987) also block the G protein–rhodopsin interaction. In addition, binding of $G\alpha_t$ to rhodopsin can be competed by peptides corresponding to regions near the carboxyl terminus (Hamm et al. 1988). Rat $G\alpha_s$ *unc* mutants are defective in the interaction with the β -adrenergic receptor (Haga et al. 1977); this defect results from a single arginine to proline substitution 6 amino acids from the carboxyl terminus of $G\alpha_s$ (Rall and Harris 1987; Sullivan et al. 1987).

To investigate the role of the carboxyl terminus of the yeast $G\alpha$ protein, *Scg1*, a genetic analysis of this region was undertaken. Site-directed mutations were made, and genomic replacements of the wild-type gene with the mutant genes were constructed in yeast. The phenotypes observed in these strains are consistent with a role for the *Scg1* carboxyl terminus in receptor interactions and therefore provide further evidence for functional homology between yeast and vertebrate systems.

Results

The *Scg1* protein, the $G\alpha$ subunit of the yeast pheromone response pathway, is proposed to be involved in two protein–protein interactions. In the absence of pheromone, it binds to *Ste4/Ste18* ($\beta\gamma$), thus keeping the pathway inactive. Null *scg1* mutations prevent this function, resulting in constitutive activation of the pathway, that is, growth arrest and morphological changes (Dietzel and Kurjan 1987a; Miyajima et al. 1987; Jahng et al. 1988; Nakayama et al. 1988). The second interaction involves binding of the heterotrimeric G protein ($\alpha\beta\gamma$) to the activated pheromone receptors *Ste2* and *Ste3*, resulting in activation of the pathway. If the carboxyl terminus of *Scg1* is involved in the receptor interactions, carboxy-terminal mutations might affect receptor interactions without affecting the $\beta\gamma$ interaction.

A 22-amino-acid truncation of *SCG1* results in constitutive activation of the pathway

A lysine codon was changed to an amber codon to produce a 22-amino-acid truncation (*scg1^{Amb451}*) and was initially tested on a replicating plasmid (YE352). The assay used an *a scg1 :: LEU2* disruption strain carrying the *TRP1* and *MAT α* genes on a plasmid (pTRP1–MAT α). Expression of both *MAT α* and *MAT α* information represses components of the pheromone response pathway, resulting in suppression of the haploid *scg1* growth defect and providing a selection for retention of pTRP1–MAT α . The wild-type *SCG1* gene on a high-copy plasmid (YE352) allowed loss of pTRP1–MAT α (Table 1). YE352 with the *scg1^{Amb451}* gene, however, did not allow loss of pTRP1–MAT α (Table 1), indicating that the *scg1^{Amb451}* gene could not complement the growth defect resulting from a null mutation.

Gene replacements were made in a haploid strain containing pTRP1–MAT α . The *scg1^{Amb451}* [pTRP1–MAT α] strain did not show loss of pTRP1–MAT α upon growth in nonselective medium. An *a/a SCG1/scg1^{Amb451}* diploid was constructed and dissected to determine the mutant phenotype. Mutant colonies were very small and resembled colonies formed by *scg1* disruption mutants (Dietzel and Kurjan 1987a). When examined microscopically, the cells of these small colonies were morphologically aberrant, similar to the *scg1* disruption mutants (Dietzel and Kurjan 1987a). The *scg1^{Amb451}* phenotype therefore was similar to the null phenotype.

For dominance tests, the *HO* gene expressed under galactose control (Jensen and Herskowitz 1984) was used to switch mating types of the *a/a SCG1/scg1^{Amb451}* diploid to produce *a/a* and *a/a SCG1/scg1^{Amb451}* diploids. For controls, *SCG1/SCG1* and *SCG1/scg1 :: LEU2* diploids homozygous for mating type were also constructed. Growth, cellular morphology, and mating of the *SCG1/scg1^{Amb451}* and *SCG1/scg1 :: LEU2* heterozygotes and the *SCG1/SCG1* homozygote were similar (Fig. 1).

Response to α -factor was tested using several assays. One assay involved spotting α -factor on thin lawns of cells, which results in inhibition of growth of wild-type

Table 1. Plasmid loss

<i>SCG1</i> gene on plasmid	Number of Trp [−] colonies ^a
Wild type	10,12
Pro467	14,20,18,23
Pro468	27,28,23,15
Och468	27,22,27,25
Amb451	0,0,0,0

^aThe initial *MAT α* strains contain the *scg1 :: LEU2* null mutation, which prevents growth of haploid strains, the pTRP1–MAT α plasmid, which allows growth due to the presence of both *MAT α* and *MAT α* information, and a *URA3* plasmid containing either a mutant or wild-type *SCG1* gene. The strains were grown overnight under nonselective conditions and tested for loss of the pTRP1–MAT α plasmid. For each strain, several independent trials in which the numbers of Trp[−] colonies out of a total of 40 colonies tested are shown.

Figure 1. Mating efficiencies. Mating ability of haploid strains and diploids homozygous for mating type were tested. Mating of the haploid mutants that were capable of growth ($SCG1^{Pro467}$, $SCG1^{Pro468}$, and $SCG1^{Och468}$) were compared to the wild-type $SCG1$ strains. **a/a** $SCG1/SCG1$ and **α/α** $SCG1/SCG1$ diploids were compared to isogenic strains heterozygous for the $scg1^{Amb451}$, $SCG1^{Pro467}$, $SCG1^{Pro468}$, $SCG1^{Och468}$, and null ($scg1 :: LEU2$; Dietzel and Kurjan 1987a) mutations.

cells. A second assay involved confrontation tests in which individual cells were separated by micromanipulation on spots of α -factor on agar plates and cell division and cellular morphology were monitored under the microscope. Response to pheromone in both assays was similar for the **a/a** $SCG1/SCG1$, **a/a** $SCG1/scg1^{Amb451}$, and **a/a** $SCG1/scg1 :: LEU2$ diploids, although the heterozygous strains showed slightly stronger response; the morphological changes in the confrontation assays were somewhat more extreme with the heterozygotes than with the $SCG1/SCG1$ homozygote. The third approach involved assaying induction of a pheromone-inducible reporter gene containing *FUS1* regulatory sequences (McCaffrey et al. 1987; Trueheart et al. 1987) upstream of a β -galactosidase fusion. The $SCG1/scg1^{Amb451}$ and $SCG1/scg1 :: LEU2$ heterozygotes showed higher basal expression than the $SCG1/SCG1$ homozygote and induced expression levels similar to the wild-type homozygote (Fig. 2). Except for fairly minor differences, the heterozygotes are similar to the $SCG1/SCG1$ diploids; therefore, both the $scg1^{Amb451}$ and $scg1 :: LEU2$ mutations are recessive.

Mutations of the SCG1 carboxyl terminus result in defects in pheromone response and mating

Mutations near the *SCG1* carboxyl terminus that resulted in lysine to proline substitutions ($SCG1^{Pro467}$

and $SCG1^{Pro468}$) and in a 5-amino-acid truncation ($SCG1^{Och468}$) were constructed. The effect of these mutations was initially assayed on a replicating plasmid as described above. The **a** $scg1 :: LEU2$ [pTRP1-MAT α] strains containing any of these three mutated *SCG1* genes on YE352 were able to lose pTRP1-MAT α (Table 1), indicating that these mutations were able to complement the $scg1 :: LEU2$ growth defect when present on a high-copy plasmid. The three resulting strains showed normal growth and cellular morphology. The **a** $scg1 :: LEU2$ [YE352- $SCG1^{Och468}$] and **a** $scg1 :: LEU2$ [YE352- $SCG1^{Pro468}$] strains were sterile (mating defective), and the **a** $scg1 :: LEU2$ [YE352- $SCG1^{Pro467}$] strain showed reduced mating.

Gene replacements were made with these mutant genes. All three mutants showed normal growth and cellular morphology. The $SCG1^{Och468}$ mutation resulted in mating defects in both **a** and α strains (Fig. 1). Quantitative mating assays indicated that mating was reduced by several orders of magnitude (Table 2). No response to pheromone was detectable by the pheromone spotting or confrontation assays. In the assay of the pheromone-in-

Figure 2. Expression of a pheromone-inducible β -galactosidase construct. Strains were transformed with plasmid pSL973, which contains the *FUS1* pheromone-inducible UAS upstream of a *cyc1 :: lacZ* fusion. β -Galactosidase activity was assayed in the presence and absence of 10^{-7} M α -factor. The level of induction is shown beneath the strain names. (A) **a** haploid mutants capable of growth were compared to wild-type (WT) strain W303-1A. (B) **a/a** diploids heterozygous for *SCG1* and the mutation indicated were compared to the wild-type ($SCG1/SCG1$). (Solid box) $-\alpha$ -Factor; (hatched box) $+\alpha$ -factor.

Table 2. *Mating ability*

Strain	Mating as α^a	Mating as α^a
Wild type	1.0	1.0
Pro467	1.3×10^{-3}	5.4×10^{-5}
Pro468	1.7×10^{-4}	0.53
Och468	3.1×10^{-6}	1.0×10^{-6}

^aMating frequencies are relative to the wild-type strains W303-1A (α) and W303-1B (α).

ducible reporter construct, the *SCG1^{Och468}* mutant showed lower basal expression than the wild-type strain and no induction by pheromone (Fig. 2). Reduced basal expression of pheromone-inducible genes has been seen previously in mutants defective in components of the pheromone response pathway, indicating that there is some basal activity of the pathway (Fields et al. 1988).

The *SCG1^{Pro467}* mutant showed somewhat less severe effects than the *SCG1^{Och468}* mutant. The mating defect was slightly greater in α cells than in α cells (Table 2). No pheromone response was detectable by pheromone spotting or confrontation assays. In the β -galactosidase assays, both basal and induced levels were lower than in the wild type, but the ratio of the induced to the basal level was similar to the wild type (Fig. 2).

The magnitude of the effects of the *SCG1^{Pro468}* mutation differed greatly in α and α cells. In α cells, the phenotypes were similar to the *SCG1^{Pro467}* phenotypes. Mating was decreased several orders of magnitude (Table 2; Fig. 1), no response to pheromone was seen in the pheromone spotting and confrontation assays, and both basal and induced activities were decreased in the β -galactosidase assay (Fig. 2). In α cells, however, the *SCG1^{Pro468}* mutation resulted in only slightly decreased pheromone response and mating (Table 2; Fig. 1).

Dominance tests were again done in α/α and α/α diploids. The *SCG1^{Pro468}* mutation behaved like the wild type in the α/α *SCG1/SCG1^{Pro468}* diploid, consistent with its haploid phenotype. In mating assays, the *SCG1^{Pro467}* and *SCG1^{Och468}* mutations were partially dominant in α/α diploids, and all three mutations were partially dominant in α/α diploids (Fig. 1). In the pheromone spotting and confrontation assays, α/α diploids heterozygous for all three mutations were defective in pheromone response. In the β -galactosidase assays, the heterozygotes showed reduced induction, and the degree of reduction correlated with the mating decrease seen in the corresponding haploid mutant.

Discussion

To investigate a possible role of the Scg1 carboxyl terminus in receptor interactions, we analyzed the effect of mutations in this domain. In the model for the pheromone response pathway (Dietzel and Kurjan 1987a; Whiteway et al. 1989), the G protein composed of Scg1 (α), Ste4 (β), and Ste18 (γ) interacts with nonhomologous receptors in cells of opposite mating type, the Ste2 (α -factor) receptor in α cells and the Ste3 (α -factor) recep-

tor in α cells. Constructs in which the carboxy-terminal 142 amino acids of Scg1 were replaced by similar regions from rat G α s, G α i2, or G α o resulted in mating defects, consistent with an inability to interact with the pheromone receptors and suggesting that the carboxy-terminal 30% of Scg1 is involved in receptor interactions (Kang et al. 1990). The effects of the mutations described here are consistent with a role for the carboxyl terminus in interactions with the pheromone receptors and suggest that the interactions with the two receptors may have different specificity.

Mutations resulting in reduced mating are consistent with defects in receptor interactions

An arginine-to-proline change 6 amino acids from the carboxyl terminus of G α s uncouples Gs from the β -adrenergic receptor (Haga et al. 1977; Rall and Harris 1987; Sullivan et al. 1987). This position is highly conserved in G α subunits, consisting of an arginine or lysine residue. We tested the effect of the analogous lysine-to-proline change at this position in *SCG1* (*SCG1^{Pro467}*), the same change at the adjacent nonconserved lysine (*SCG1^{Pro468}*), and a truncation of 5 amino acids (*SCG1^{Och468}*).

These three mutants showed phenotypes consistent with a role for the carboxyl terminus in interactions with the pheromone receptors. The *SCG1^{Och468}* mutation resulted in a severe mating defect in both α and α cells (Fig. 1; Table 2). The α *SCG1^{Och468}* mutant showed no detectable pheromone response.

Both lysine-to-proline substitutions resulted in less severe mating and pheromone response defects. The *SCG1^{Pro467}* defects were somewhat more severe in α cells than in α cells (Table 2). This phenotype is similar to the effect of the analogous mutation in G α s (Haga et al. 1977; Rall and Harris 1987; Sullivan et al. 1987), that is, both result in reduced activation of the respective pathways. The *SCG1^{Pro468}* mutant showed similar effects as the *SCG1^{Pro467}* mutant in α cells (Table 2; Figs. 1 and 2). In α cells, however, the *SCG1^{Pro468}* mutation resulted in only mild decreases in pheromone response and mating. The extreme difference in severity of the effects of the *SCG1^{Pro468}* mutation and possibly the more moderate difference of the *SCG1^{Pro467}* mutation in α and α cells suggest that the specificity for the interactions with the two nonhomologous receptors, Ste2 and Ste3, may involve different amino acids or is affected differently by the structure of the Scg1 carboxyl terminus. A glycine-to-aspartic acid change at position 470 also resulted in decreased pheromone response in α cells, but was not tested in α cells, and was suggested to indicate a defect in receptor interactions (Stone and Reed 1990).

A 22-amino-acid truncation results in constitutive activation of the pheromone response pathway

A 22-amino-acid truncation (*scg1^{Amb451}*) resulted in growth and cell morphology defects similar to the null

phenotype (Dietzel and Kurjan 1987a). A possible explanation for this effect is that the *scg1^{Amb451}* protein is unstable. A more interesting possibility is that this truncation results in constitutive guanine nucleotide exchange, as has been seen in G α s constructs with altered carboxyl termini. Expression in CHO cells of a hybrid in which the carboxy-terminal 38 amino acids of G α s was replaced with the carboxy-terminal 36 amino acids of G α i showed constitutive activation of adenylate cyclase due to an increase in the GDP dissociation rate, resulting in increased guanine nucleotide exchange (Woon et al. 1989). A mutant G α s in which 3 amino acids near the carboxyl terminus were mutated to resemble G α i did not show an intrinsic increase in guanine nucleotide exchange but did show an increase when reconstituted into membranes (Freissmuth and Gilman 1989). Adenylate cyclase activity was not increased further by agonist addition. Other possible explanations for this phenotype are that the *scg1^{Amb451}* product is defective in membrane localization or the $\beta\gamma$ interaction. Although there is some evidence suggesting a role for the amino termini of G α subunits in the $\beta\gamma$ interaction (Masters et al. 1986), it has also been suggested that the carboxyl terminus or that both the amino and carboxyl termini, which are predicted to be in close proximity, are involved in this interaction (Hingorami and Ho 1987).

Dominance relationships and implications of the mutant phenotypes on the mechanism of pheromone response

With the exception of one *SCG1* mutant with a mixed phenotype, the mutants described in this study, disruption mutants described previously (Dietzel and Kurjan 1987a), and mutants with alterations of the putative guanine nucleotide-binding domains of Scg1 (Kurjan et al., this issue) fall into two classes with opposite phenotypes. Disruption mutants, the *scg1^{Amb451}* truncation, and two guanine nucleotide-binding domain mutants (*scg1^{Lys388}* and *scg1^{Ala391}*) showed constitutive expression of the pheromone response pathway. The phenotypes of heterozygotes for these mutations were similar to wild-type *SCG1/SCG1* diploids (in diploids homozygous for mating type; Figs. 1 and 2), indicating that these mutations were recessive, although the heterozygotes did show somewhat greater sensitivity to pheromone and increased basal expression of the pheromone response pathway (Fig. 2).

These phenotypes are consistent with the model for pheromone response, in which the G protein exists as a heterotrimer (α -GDP/ $\beta\gamma$) in the absence of pheromone, and free $\beta\gamma$ activates the pathway after exposure to pheromone (Dietzel and Kurjan 1987a; Whiteway et al. 1989). In strains heterozygous for the recessive mutations, wild-type Scg1 would bind $\beta\gamma$ but is present at half the normal level. Mutant Scg1 cannot bind $\beta\gamma$ even in the absence of pheromone, leading to free $\beta\gamma$ and increased basal activation of the pathway. Exposure to pheromone should activate the wild-type Scg1, allowing pheromone response and mating.

The *SCG1^{Och468}*, *SCG1^{Pro467}*, and *SCG1^{Pro468}* (in a cells) mutants and one guanine nucleotide-binding domain mutant (*SCG1^{Ala322}*; Kurjan et al., this issue) showed the opposite effect, decreased pheromone response and mating. The normal growth and morphology of these mutants suggests that the mutant Scg1 proteins can bind $\beta\gamma$ to keep the pathway inactive. The pheromone response and mating defects suggest that these proteins cannot be activated on exposure of cells to pheromone, resulting in a defect in dissociation from $\beta\gamma$. These mutations are partially dominant, again consistent with the model for this pathway (Dietzel and Kurjan 1987a; Whiteway et al. 1989). In strains containing both wild-type and mutant Scg1 of this type, $\beta\gamma$ bound to mutant Scg1 would be sequestered, and activation of the pathway would only free the portion of $\beta\gamma$ bound to wild-type Scg1, resulting in reduced mating and pheromone response.

These phenotypes, that is, the partial dominance of the mutants showing decreased mating and the increased basal activation of strains heterozygous for the recessive mutations, indicate the importance of proper stoichiometry of components of this pathway. Other results have indicated that an increased level of wild-type Scg1 inhibits mating (Kang et al. 1990) and that an increased level of Ste4 results in constitutive activation of the pathway but increased levels of Scg1 as well as Ste4 prevent this constitutive activation (Cole et al. 1990; Nomoto et al. 1990; Whiteway et al. 1990). Together, these results indicate that the pheromone response pathway is extremely sensitive to the balance between levels of the components of this pathway.

Materials and methods

Phage and plasmids

M13*SCG1*/1-7 and M13*SCG1*/5-5 contain the 1.9-kb *EcoRI* fragment of *SCG1* subcloned into M13mp10 in opposite orientations (Dietzel and Kurjan 1987a) and were used for site-directed mutagenesis. The 1.9-kb *EcoRI* fragments containing the wild-type and mutant *SCG1* genes were subcloned into the replicating vector YEpl352, which contains the yeast *URA3* gene and the 2 μ origin of replication (Hill et al. 1986); and the mutant *SCG1* genes were also subcloned into the integrating vector YIp353, which contains the *URA3* gene but no origin of replication. Other plasmids used in this study were p*TRP1*-MAT α , a relatively unstable centromere plasmid (provided by F. Cross) and YEpl3, a *LEU2*-containing multicopy vector. pGAL-*SCG1*, pGAL-*STE4* (pL19, Whiteway et al. 1990; obtained from M. Whiteway), and pGAL-*HO* (Jensen and Herskowitz 1984; obtained from R. Rothstein) are *URA3*-containing centromere plasmids that also contain the *SCG1*, *STE4*, or *HO* genes, respectively, under the control of the *GAL1,10* promoter. pSL973 is a *URA3*-containing centromere plasmid with the *FUS1* upstream activating sequence (UAS) upstream of a *cyc1-lacZ* fusion (provided by G. Sprague).

Strains and media

Gene replacements of *SCG1* were made in the isogenic yeast strains W303-1A (a) and W303-1B (α) with the following genotype: *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100* [pro-

vided by R. Rothstein). The *scg1 :: URA3* and *scg1 :: LEU2* disruption mutations were described previously (Dietzel and Kurjan 1987a). D111 was transformed with *pTRP1-MAT α* , sporulated, and dissected to obtain the *a scg1 :: LEU2* [*pTRP1-MAT α*] strain. Mating assays utilized strains 227 [*a lys1 cry1*] and 70 [*a thr3*]. *Escherichia coli* strain YMC10 was used to propagate plasmids, and strain HB101 [F' Kan^r] was used to grow M13 phage.

Media used were as described previously (Kurjan and Herskowitz 1982), with two exceptions. Expression of genes under the control of the *GAL1,10* promoter utilized media containing 2% galactose instead of 2% glucose. Selection of Ura⁻ strains utilized 5-fluoro-orotic acid (5-FOA) plates, which contain 0.5 mM uracil and 0.88% 5-FOA (Boeke et al. 1984).

Site-directed mutagenesis

Oligonucleotides containing the desired nucleotide differences from the *SCG1* gene were synthesized on an Applied Biosystems model 380A DNA synthesizer by the phosphoramidite method. Mutations of the *SCG1* gene (which will be referred to as *SCG1-m* for simplicity) were constructed in M13SCG1/1-7 or M13SCG1/5-5 by site-directed mutagenesis as described (Zoller and Smith 1984). Oligonucleotides used in the mutagenesis were as follows (base changes from the wild-type sequence are underlined): OCH468, CAAAACCTTAAATAAAGT; AMB451, ACCCAAACCATGTAGTTC; PRO467, GCAAAACCTTCCA AAAAGT, and PRO468, GCAAAACCTTAAACCAAGTGG. The mutated genes were sequenced by Sanger dideoxy sequencing using primers homologous to the *SCG1* sequence to confirm the desired nucleotide changes.

Testing of mutant genes on multicopy plasmids

Because of the possibility of generating random mutations in addition to the directed mutation during site-directed mutagenesis, several independently mutagenized genes were tested for each *SCG1-m* mutation. Mutant genes were subcloned into plasmid YEp352 and transformed into an *a* strain containing the *scg1 :: LEU2* disruption allele and the *pTRP1-MAT α* plasmid. The *a scg1 :: LEU2* [YEp352-*SCG1-m pTRP1-MAT α*] transformants were grown in nonselective medium, and colonies were tested for the presence of the YEp352-*SCG1-m* and *pTRP1-MAT α* plasmids.

Gene replacements

Two independent isolates of each *SCG1-m* mutant gene were used to make genomic replacements of the wild-type *SCG1* gene. The mutant genes were subcloned into the integrating vector Yip353 and digested with *SphI*, which cleaves the plasmid once within the *SCG1* gene. For the *SCG1^{Pro467}*, *SCG1^{Pro468}*, and *SCG1^{Och468}* mutations, which were able to complement the *scg1 :: LEU2* growth defect based on the replicating plasmid assay, the linear DNAs were used to transform *a* (W303-1A) and α (W303-1B) strains. For the *scg1^{Amb451}* mutation, which was unable to complement the *scg1 :: LEU2* growth defect based on the replicating plasmid assays, the linear DNAs were used to transform W303-1A containing *pTRP1-MAT α* . Ura⁺ transformants were tested by Southern analysis to determine that the correct integration event had occurred. These transformants, which contain tandem copies of wild-type and mutant *SCG1* genes separated by plasmid sequences, including *URA3*, were streaked on 5-FOA plates (Boeke et al. 1984) to select for Ura⁻ recombinants in which the *URA3* plasmid had been lost, leaving a single copy of either *SCG1* or *SCG1-m*.

Ura⁻ isolates fell into two classes that displayed either the wild-type phenotype or a mutant phenotype that was similar to the phenotype of strains containing the *SCG1-m* gene on a multicopy plasmid described above. For the *scg1^{Amb451}* mutant, this phenotype was an inability to lose *pTRP1-MAT α* . The resulting mutant strains were tested by Southern analysis to confirm the presence of a single copy of *SCG1* sequences.

Dominance tests

Diploid strains heterozygous for *SCG1* (*SCG1/SCG1-m*) and homozygous for *MAT* were constructed. The first step involved construction of *a/a SCG1/SCG1-m* diploids. Mutants defective in mating were first transformed with *pGAL-STE4*. Because *Ste4* is a positive activator of the pheromone response pathway that acts downstream of *Scg1* (Blinder et al. 1984; Nakayama et al. 1988; Whiteway et al. 1989), overexpression of *Ste4* allows some mating by mating-defective *SCG1-m* mutants. These strains were mated on galactose medium to the isogenic wild-type strain W303-1A or W303-1B containing YEp13 to allow for selection of the diploids. For the *scg1^{Amb451}* mutant, which showed the null phenotype, the *scg1^{Amb451}* [*pTRP1-MAT α*] strains were transformed with *pGAL-SCG1* and mated to W303-1B [YEp13] on galactose medium. As these cells lose *pTRP1-MAT α* , they are able to mate. After loss of the *URA3*-containing plasmid on 5-FOA medium, the *a/a* diploids were sporulated and dissected to confirm 2 : 2 segregation of the *SCG1-m* mutations. These diploids and control diploids W303 (*SCG1/SCG1*) and D111 (*SCG1/scg1 :: LEU2*) were transformed with *pGAL-HO* (Jensen and Herskowitz 1984), grown on galactose medium to induce mating-type interconversion, streaked on glucose medium, and tested for the ability to mate. The resulting *a/a* and *a/a SCG1/SCG1-m* diploids were grown on 5-FOA medium to select for loss of *pGAL-HO* and retested for mating.

Mating and pheromone response assays

Assays for growth arrest resulting from response to pheromone involved spotting 400 and 40 ng of α -factor (in 2 μ l; Sigma) on thin lawns of the *a* strain to be tested and observing inhibition of growth (Dietzel and Kurjan 1987a). Confrontation assays were done by separating 20 unbudded cells by micromanipulation onto a spot of 400 ng (in 2 μ l) of α -factor and following budding and shmoo formation microscopically. Control cells, for example, α cells exposed to α -factor, started budding within several hours and formed small colonies by 24 hr. Wild-type *a* cells arrested as unbudded cells and started undergoing morphological changes (shmooing) after several hours. After 24 hr, the wild-type *a* cells had not formed a colony and the few cells present showed very aberrant morphology. In addition, induction of β -galactosidase activity of the pheromone-inducible reporter gene on pSL973 after exposure to 10^{-7} M α -factor (McCaffrey et al. 1987) was tested. β -Galactosidase assays were done as described previously (Dietzel and Kurjan 1987b).

Qualitative mating tests were done as described previously (Kurjan 1985). For quantitative mating assays (Hartwell 1980), yeast cultures were grown to 1×10^7 to 5×10^7 cells/ml, and 3×10^6 cells of the experimental cultures were mixed with five times as many tester cells, filtered onto sterile filters, and transferred to YEPD plates for 4 hr at 30°C. The cells were washed from the filters into 1 M sorbitol, diluted, and plated. Mating frequency was determined by comparison of numbers of colonies on plates that select for the diploids and plates that allow growth of both haploids and diploids.

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