Mutations in the guanine nucleotide-binding domains of a yeast Gα protein confer a constitutive or uninducible state to the pheromone response pathway

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Several domains of guanine nucleotide-binding proteins are conserved and form the guanine nucleotide-binding pocket. Mutations in these domains in EF-Tu, ras, and Gos have been shown to result in informative phenotypes. We made several analogous changes in SCG1, which encodes the α subunit of the G protein involved in pheromone response in yeast. The scg1<sup>lys388</sup> and scg1<sup>ala391</sup> mutations resulted in severe growth and cell morphology defects; this phenotype is similar to the null phenotype and results from constitutive activation of the pheromone response pathway. On the basis of the model for the action of the yeast G protein, the effect of these mutations is consistent with the effect of analogous mutations in ras, which result in a transforming phenotype. The scg1<sup>ala322</sup> mutation resulted in pheromone response and mating defects. This effect is similar to the effect of the analogous Gos mutation, which results in a defect in stimulation of adenylate cyclase. The scg1<sup>val59</sup> mutation, which is analogous to the transforming mutation ras<sup>val11</sup>, resulted in multiple effects, including defects in growth, cell morphology, and mating. Some of our results and interpretations are different from previously published results of others for the same mutation in SCG1; specifically, our gene replacement of this mutation resulted in high basal activation of the pheromone response pathway, consistent with a GTPase defect, which was not seen previously with scg1<sup>val59</sup> on a low-copy plasmid. Implications of these phenotypes are discussed.

[Key Words: Pheromone response; regulatory G protein; guanine nucleotide-binding domains; mating]

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Yeast cells of opposite mating type secrete peptide pheromones (α cells secrete α-factor and α cells secrete α-factor) and respond to the pheromone produced by the opposite mating type (for review, see Cross et al. 1988). Response to pheromone involves arrest in the G<sub>1</sub> phase of the cell cycle, morphological changes, and induction of a number of genes, many of which play roles in pheromone response or other aspects of mating. The secretion of and response to the pheromones is necessary for mating of α and α cells to produce the α/α diploid. Pheromone response is mediated by specific receptors (the α-factor receptor, Ste2, and the α-factor receptor, Ste3). Although the pheromones and receptors expressed by the two haploid mating types differ, most or all of the intracellular signaling pathway is shared by α and α cells [Bender and Sprague 1986; Nakayama et al. 1987]. Structural features of the pheromone receptors are similar to features of receptors that are coupled to regulatory G proteins in other systems [Hershkowitz and Marsh 1987].

Guanine nucleotide-binding proteins, including translation elongation factor EF-Tu, ras, components of the secretory pathway, and the α subunits of regulatory G proteins, are involved in a diverse array of functions but share a common mechanism with regard to the action of the guanine nucleotide (for review, see Bourne 1988). These proteins bind guanine nucleotides, undergo replacement of GDP by GTP, and hydrolyze bound GTP to GDP by the action of an intrinsic GTPase. Several domains of these proteins show significant amino acid similarity [Halliday 1984; Dever et al. 1987]. X-ray crystallographic analysis of ras and EF-Tu indicates that these domains form the guanine nucleotide-binding pocket (see Jurnak et al. 1990, Milburn et al. 1990 and references therein). Mutations in these domains can affect biochemical activities and result in interesting phenotypes such as transforming activity for ras [Barbacid 1987] or...
an effect on activation of adenylate cyclase for Gα [Bourne et al. 1981; Miller et al. 1988].

Yeast genes that encode homologs to the α, β, and γ subunits of vertebrate G proteins have been identified (called SCG1 or GPA1, STE4, and STE18, respectively) and shown to be involved in pheromone response and mating [Dietzel and Kurjan 1987a, Miyajima et al. 1987; Nakafuku et al. 1987; Jahng et al. 1988, Whiteway et al. 1989]. Genetic analysis has indicated that α plays a negative role and β plays a positive role in this pathway and that γ acts downstream of α [Dietzel and Kurjan 1987a; Miyajima et al. 1987; Jahng et al. 1988, Nakayama et al. 1988, Whiteway et al. 1989, Cole et al. 1990, Nomoto et al. 1990; Whiteway et al. 1990]. Recent biochemical evidence supports the proposal that GTP binding to this G protein plays a role in this pathway [Blumer and Thorner 1990]. To elucidate the role of SCG1 in pheromone response, we have used site-directed mutagenesis to test the effect of mutations in the putative guanine nucleotide-binding domains on Sgc1 function [Fig. 1].

Results

NKKD mutations result in constitutive activation of the pathway

SCG1 mutants with asparagine to lysine and aspartic acid to alanine changes at positions analogous to ras-transforming mutations at residues 116 and 119 were constructed [Fig. 1]. The mutant genes were first tested on a high-copy plasmid [YEp352] in a MATα scgl1::LEU2 pTRPI-MATα strain. The pTRPI-MATα plasmid is stable in this strain because loss of MATα allows expression of the haploid scgl1::LEU2 null phenotype, a growth defect due to constitutive activation of the pathway [Dietzel and Kurjan 1987a; Miyajima et al. 1987; Nakayama et al. 1988; Jahng et al. 1989]. The wild-type SCG1 gene on YEp352 allows loss of pTRPI-MATα [Table 1]. Strains containing either YEp352–scgl1::Lys388 or YEp352–scgl1::Ala391 did not show loss of pTRPI-MATα [Table 1], indicating that these mutant genes could not complement the scgl1 growth defect.

Gene replacements were made to obtain a/a SCG1/scg1::Lys388 and a/a SCG1/scg1::Ala391 diploids. These diploids showed 2:2 segregation for wild-type and tiny colonies composed of morphologically aberrant cells [Fig.

Table 1. Plasmid loss

| SCG1 gene on plasmid | Trp+ colonies* |
|----------------------|----------------|
| Wild type            | 10,12          |
| Val50                | 17,17,14,19    |
| Ala322               | 4,12,11,16     |
| Lys388               | 0,0,0,0        |
| Ala391               | 0,0,0,0        |

*The starting MATα strains contain the scgl1::LEU2 null mutation, which prevents growth of haploid strains, the pTRPI-MATα plasmid, which allows growth due to the presence of both MATα and MATα 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 pTRPI-MATα. For each strain, several independent trials in which the numbers of Trp+ colonies obtained out of a total of 40 colonies are shown.

Figure 1. Guanine nucleotide-binding domain mutations. The 472-amino-acid presumptive Sgc1 protein is indicated, with the positions of the guanine nucleotide-binding domains shown as solid regions. The sequences of these domains and the mutations constructed are shown.

Figure 2. Tetrad analysis. a/a diploids were sporulated and dissected. (A) Tetrads from +/+ [W303], +/scg1::LEU2 [D111], +/scg1::Lys388, and +/scg1::Ala391 diploids. (B) Tetrads from +/scg1::Val50 diploids from two independent gene replacements of each of two independently mutagenized scg1::Val50 genes.
Wild type 1.0 1.0

Table 2. Mating efficiency

| Strain          | Mating as a* | Mating as a/α |
|-----------------|--------------|---------------|
| Wild type       | 1.0          | 1.0           |
| sst2            | 0.17         | 0.47          |
| scg1 Val50      | 2.4 × 10^-4  | 1.3 × 10^-4   |
| SCG1 Alα322     | 3.1 × 10^-6  | 4.2 × 10^-6   |

*aMating frequencies are relative to the wild-type strains W303-1A [α] and W303-1B [a].

SCG1 guanine nucleotide-binding domain mutants

Glycine to valine substitution results in multiple effects

An SCG1 mutant containing a glycine to valine substitution at the position analogous to the rasVal12-transforming allele (for review, see Barbacid 1987) was constructed [Fig. 1]. This mutant showed multiple phenotypes, some of which differ from results of Miyajima et al. (1989; see Discussion). The α scg1 :: LEU2 strain could lose pTRP1-MATα YEp352-SCG1 Alα322 strain could lose pTRP1-MATα [Table 1], indicating that this mutation gene could complement the scg1 growth defect. The resulting strain was sterile [matting-defective].

SCG1 Alα322 gene replacements showed normal growth and morphology. This mutation resulted in mating defects in both mating types [Fig. 3, Table 2]. The SCG1 Alα322 mutant showed no response to pheromone in the pheromone spotting [Fig. 4] and confrontation assays. Using the pheromone-inducible construct, this mutant showed no detectable β-galactosidase activity in either the presence or absence of α-factor [Fig. 5A]. This mutant, therefore, was totally defective in pheromone response and mating in all assays. The reduced basal level of β-galactosidase activity is consistent with previous results, indicating that there is some basal expression of the pheromone response pathway and that defects in components of this pathway result in a reduced level of basal expression, as well as a defect in response to pheromone (Fields et al. 1988).

Dominance tests were done with a/α and a/α SCG1/SCG1 Alα322 diploids, which showed low levels of mating [Fig. 3]. The a/α SCG1/SCG1 Alα322 diploid showed no detectable response to pheromone in the pheromone-spotting [Fig. 4] and confrontation assays. In the β-galactosidase assays, this diploid showed similar basal expression but a lower level of induction than the homozygous wild type [Fig. 5]. These properties indicate that SCG1 Alα322 is partially dominant to the wild type.

showed higher basal expression than the SCG1/SCG1 homozygote [Fig. 5B, below]. Induced expression levels of the heterozygotes were similar or slightly lower than the induced level of the homozygote. The properties of the three heterozygotes are closer to the properties of SCG1/SCG1 diploids than the haploid mutants; therefore, we refer to these mutations as recessive.

Hinge mutation results in a pheromone response defect

An SCG1 mutant containing a glycine to alanine change at a position analogous to the H21a Gas mutation, which uncouples Gas from the effector (Bourne et al. 1981; Salomon and Bourne 1981; Miller et al. 1988), was constructed [Fig. 1]. The a scg1 :: LEU2 [pTRP1-MATα YEp352-SCG1 Alα322] and null [scg1 :: LEU2; Dietzel and Kurjan 1987a] mutations. The strains shown are a haploids and a/α diploids. The results with a haploids and a/α diploids were similar or slightly lower than the basal level of the homozygote. The properties of the SCG1/SCG1 diploids obtained from gene replacements, however, indicated that the scg1 Val50 mutation resulted in a growth defect [Fig. 2]. Division times, as determined by growth curves, were about three times longer than those of wild type. Some cells from these colonies were large and morphologically aberrant. Both the growth and morphological defects were less severe than for the null mutants.

The scg1 Val50 mutation also affected mating and pheromone response. The a and α scg1 Val50 mutants showed greatly reduced mating [Table 2, Fig. 3]. The pheromone response phenotype of the scg1 Val50 mutant was complicated. By the β-galactosidase induction assay, basal expression was very high, and exposure to pheromone resulted in only a slight increase over this basal level [Fig. 5A]. Confrontation tests were difficult due to the aberrant morphology of many of the cells, even in the absence of pheromone. Monitoring of cells of fairly normal size and morphology showed poor response.

The scg1 Val50 mutants showed an unusual phenotype in the pheromone-spotting assay [Fig. 4], as was also observed by others for mutations at this amino acid (Miyajima et al. 1989, Stone and Reed 1990). The α-factor spot results in a small area of growth inhibition of wild-type cells and a large, clear zone of growth arrest of an sst2 mutant, which is defective in desensitization to phero-
Kurjan et al.

Figure 4. Pheromone-spotting assays. Pheromone response was assayed by spotting 400 ng (top) or 40 ng (bottom) of α-factor in 2 μl on thin lawns of cells and observing inhibition of growth. (A) α haploids. (B) α/α diploids.

Discussion

In the model for the pheromone response pathway (Fig. 6A; Dietzel and Kurjan 1987a; Whiteway et al. 1989), α-GDP interacts with βγ in the absence of pheromone to keep the pathway inactive. After receptor activation by pheromone, guanine nucleotide exchange occurs, resulting in dissociation of α-GTP from βγ, and free βγ activates the pathway. We describe the effects on this pathway of SCG1 mutations in conserved domains implicated in interactions with the guanine nucleotide (Fig. 1).

NKXD domain mutations result in analogous effects as in ras

X-ray crystallography indicates that the NKXD domains of EF-Tu and ras interact with the guanine ring of the nucleotide (for review, see Jurnak et al. 1990, Milburn et al. 1990). Mutations of Asn116 and Asp119 in H-ras result in a transforming phenotype (Barbacid 1987; Der et al. 1988). This phenotype results from decreased guanine nucleotide binding due to an increased dissociation rate. A mutation at the analogous position in EF-Tu resulted
suggested to act as a hinge involved in this conformational change, which is associated with guanine nucleotide exchange [Hingorami and Ho 1987, Bourne et al. 1988]. A glycine to alanine substitution [H21a] in Gas at this conserved residue results in an inability to undergo this conformational change and therefore to dissociate

Figure 5. Expression of a pheromone-inducible β-galactosidase construct. Strains were transformed with plasmid pSL973, which contains the FUS1 pheromone-inducible UAS (upstream activating sequence) upstream of a cycl::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 the 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 bars] -α-Factor; [hatched bars] +α-factor.

Figure 6. Models for pheromone response pathway in wild-type and mutant haploids. [Ph] Pheromone; [R] receptor; [α] Scg1; [βγ] Ste4/Ste18; [E] effector (currently unidentified). Activated forms are spotted. [A] In the wild type, the heterotrimeric G protein is proposed to interact with the activated receptor and undergo replacement of GDP by GTP and a subsequent conformational change, resulting in dissociation of activated α-GTP from βγ. Free βγ then activates the effector and the pheromone response pathway. A GTPase activity intrinsic to the α subunit returns the system to the basal state (not shown). Because there is some basal expression of the pathway [Fields et al. 1988; Errede and Ammeter 1989], a small proportion of the G protein and the effector must be in the activated form even in the absence of pheromone (not shown). [B] In the scg1 Lys388 and scg1 Ala391 mutants, the G protein is in the activated form even in the absence of pheromone, resulting in constitutive activation of the pathway. In disruption mutants, the effect is the same but results from an absence of the α subunit, rather than the presence of the activated form of the α subunit. [C] In the scg1 Ala322 mutation, the G protein cannot be activated, possibly due to a defect in undergoing the conformational change of the α subunit that normally accompanies guanine nucleotide exchange. This defect results in a defect in induction and also reduced basal expression of the pathway. [D] The multiple phenotypes associated with the scg1 Val50 mutation suggest a complicated effect. The pathway is partially activated in the absence of pheromone, indicating that increased levels of the activated form of the G protein are present, possibly due to a GTPase defect. In addition, the pathway shows little additional activation upon exposure to pheromone, suggesting that the unactivated G protein present is inefficient in shifting to the activated form, possibly due to the same defect as in C.

Inefficient protein synthesis and decreased guanine nucleotide binding [Hwang and Miller 1987].

Mutations in the NKXD domain of SCG1 [Asn to Lys388 and Asp to Ala391; Fig. 1] resulted in recessive effects similar to the null phenotype, i.e., growth and morphological defects [Fig. 2]. Such an effect is predicted on the basis of analogous EF-Tu and ras mutants. An increased guanine nucleotide dissociation rate would shift the protein toward the GTP-bound form due to a higher concentration of GTP than GDP in cells [Ditzel-muller et al. 1983, Feig and Cooper 1988]. The activated α-GTP would not associate with βγ, resulting in constitutive activation of the pheromone response pathway [Fig. 6B]. Alternatively, the scg1 Lys388 and scg1 Ala391 proteins may be unstable, resulting in free βγ.

Hinge mutation has a phenotype analogous to the effect in Ras

Activation of ras results in a conformational change [Jumak et al. 1990, Milburn et al. 1990]. The conserved glycine in the DXXG domain of Ga proteins has been
from βγ, resulting in a defect in stimulation of adenylate cyclase [Bourne et al. 1981; Saloman and Bourne 1981; Miller et al. 1988].

The analogous SGC1 Ala322 mutant [Fig. 1] showed partially dominant pheromone response and mating defects [Table 2, Figs. 3–5]. This effect is predicted for a mutant defective in the conformational change, leading to an inability to dissociate from βγ and to activate the pheromone response pathway [Fig. 6C]. Other changes of this glycine residue in SGC1 [to glutamic acid and to arginine] have been shown to eliminate response to pheromone by a cells [Stone and Reed 1990]. The similar effect of three different changes at this position is consistent with the proposal that the glycine residue acts as a hinge. The partially dominant effect of this mutation is discussed elsewhere [Hirsch et al., this issue].

GXXXXGK domain glycine to valine substitution has multiple effects

The glycine to valine change in H-ras Val12 leads to decreased GTPase activity and a transforming phenotype [Barbacid 1987] and results in hyperactivation of adenylate cyclase by yeast Ras Val19 [Broek et al. 1985]. This position is in the conserved GDP phosphate-binding loop, although it does not interact directly with the phosphates [see Jurnak et al. 1990; Milburn et al. 1990].

The analogous Gas Val19 mutant showed decreased GTPase activity both in vitro and in vivo [Graziano and Gilman 1989; Masters et al. 1989]. The predicted increase in stimulation of adenylate cyclase in the presence of GTP was seen in vitro; however, stimulation of adenylate cyclase in vivo was somewhat reduced. The in vivo and in vitro results therefore agree with respect to decreased GTPase activity but show opposite effects with regard to activation of the effector, adenylate cyclase.

The analogous scg1 Val50 mutant showed properties consistent with a GTPase defect [Fig. 6], that is, growth [Fig. 2] and morphological defects and high constitutive expression of a pheromone inducible reporter gene [Fig. 5]. The phenotype was less severe than for null mutants [Fig. 2], indicating that basal activation of the pathway is less extreme than in the complete absence of Scg1. Miyajima et al. [1989] did not see a growth defect associated with this mutation [see below].

A GTPase defect cannot explain other scg1 Val50 phenotypes. This mutant showed a mating defect [Fig. 3; Table 2]. A less severe defect was observed by Miyajima et al. [1989], who proposed that this defect was due to supersensitivity to pheromone, because supersensitive strains show reduced mating [Chan and Otte 1982]. We disagree with this interpretation, because isogenic sst2 mutants show much less severe mating defects than scg1 Val50 mutants [Table 2, Fig. 3]. Our scg1 Val50 mutant showed low gene induction over the very high basal level [Fig. 5], suggesting that the mating decrease may result from a defect in activation of the pathway upon pheromone addition. A final scg1 Val50 phenotype is difficult to interpret; exposure to pheromone resulted in only partial inhibition of growth in the pheromone-spotting assay [Fig. 4; Miyajima et al. 1989], as also seen for a glycine to aspartic acid change at this position [Stone and Reed 1990]. This turbid halo phenotype was suggested to reflect supersensitivity to pheromone combined with induced desensitization. However, we suggest that this phenotype may reflect the defect in induction of the pathway.

The multiple scg1 Val50 phenotypes suggest that the equilibrium between the inactive and active forms is severely disrupted [Fig. 6D]. The basal state is pushed toward the activated form, resulting in constitutive expression of the pathway and a growth defect. Exposure to pheromone barely increases the level of the activated form, resulting in defects in dissociation from βγ and reduced mating. Alternatively, the high basal level of activated protein may result in desensitization and the mating defect [see below].

The in vivo scg1 Val50 and Gas Val19 results [Masters et al. 1989] suggest that this mutation results in effects not seen in ras. Also, wild-type EF-Tu contains a valine at the analogous position, and a valine to glycine change results in decreased GTPase [Jacquet and Parmeggiani 1988], that is, the opposite effect of glycine versus valine as in ras and Gas. The consensus sequence of this domain is quite flexible [GXXXXGK], but more defined consensus sequences are found within families of guanine nucleotide-binding proteins [Halliday 1984, Dever et al. 1987]. The differential effects of mutations in this domain may reflect differences between families of guanine nucleotide-binding proteins with respect to activities associated with this domain or interactions with accessory proteins [e.g., GTPase activating protein, Trahey and McCormick 1987].

Genomic versus plasmid expression

Differences between our results and those of Miyajima et al. [1989] may be due to expression of scg1 Val50 on a plasmid versus as a gene replacement. Unlike the gene replacements, our high-copy plasmid YEp352–scg1 Val50 allowed normal growth and cell morphology. Expression on YEp352 should result in increased levels of both the unactivated and activated forms of mutant protein on the basis of our interpretation of this mutant. The unactivated form would bind βγ [Fig. 6D] and prevent the growth defect resulting from the activated form, as was also seen in SGC1/scg1 Val50 heterozygotes. Miyajima et al. [1989] expressed scg1 Val50 on a centromere plasmid, which should be present at about one copy per cell. A growth defect resulting from scg1 Val50 would provide a strong selection for increased expression of the scg1 Val50 gene to produce higher levels of the inactive form, thus inhibiting the pathway. This selection could result in increased copy number of the pCEN–scg1 Val50 plasmid, thus preventing the growth and morphological defects. Other mutations have produced different phenotypes on a centromere plasmid and as a gene replacement [James and Hall 1990].
Does Scg1–GTP play a role in desensitization?

A role for the activated form of Scg1 in desensitization has been suggested (Miyajima et al. 1989; Stone and Reed 1990). The constitutively activated scg1Val50 protein would desensitize the pathway and therefore cause the mating defect. This proposed role was based on two results [Miyajima et al. 1989]. Slow colony formation was seen with scg1–null [psecg1Val50] cells at a pheromone concentration that prevented growth of wild-type cells, suggesting that scg1Val50 might be more efficient in recovery from pheromone arrest than the wild type. Alternatively, we suggest that this effect may represent the defect in response to pheromone that we have observed. They also reported that scg1Val50 promoted the recovery of sst2 cells, which are defective in desensitization to pheromone (Chan and Otte 1982; Dietz and Kurjan 1987b), suggesting that scg1Val50 resulted in increased desensitization that was independent of SST2. Our results were quite different, in all assays the scg1Val50 mutation was epistatic to sst2 and gave no indication of increased desensitization.

If activated Scg1 [Scg1–GTP] results in desensitization, a strain expressing both wild-type Scg1 and a mutant protein that is in the activated form should be desensitized, resulting in defects in pheromone response and mating. The dips in which are homozygous for mating type and heterozygous for the scg1Lys388 and scg1Ala391 activating mutations should represent such strains. These dips showed increased basal expression of the pathway and only somewhat lower induced levels of expression of a pheromone-inducible construct [Fig. 5] and of mating [Fig. 3] than the wild-type strain. Unless the scg1Lys388 and scg1Ala391 proteins are unstable, these results suggest that the activated form of Scg1 is not involved in desensitization. Further characterization of desensitization and other phenotypes associated with these mutants will require biochemical analysis.

Materials and methods

Strains, DNA, media, and procedures

Strains, plasmids, phage, media, assays of mating and pheromone response, and site-directed mutagenesis were as described in Hirsch et al. (this issue). Mutations were tested in the isogenic yeast strains W303-1A [a], W303-1B [a], and W303 [a/a]. The scg1Val50 [Yep13–SCG1] haploids obtained in this study were mated to sst2 :: lacZ–URA3 strains [Dietz and Kurjan 1987b], streaked to allow loss of Yep13–SCG1, sporulated, and dissected to obtain scg1Val50 sst2 :: lacZ–URA3 segregants. The mutagenesis (Zoller and Smith 1984) used the following oligonucleotides [base changes from the wild-type sequence are underlined]: ECORI, TTAGCATACATACAGAATGTCAG; GVAL, CTATTAGTCCGTTAGTC; NLYS, TTTGGTTT- TAAAGAAAT; DALA, TCTCTGCACAAGCAATTT; and GALA, TCAGAAGGCTCCACCA.

Testing on multicopy plasmids and gene replacements

The mutant genes [called SCG1 m] were first tested on plasmids as described by Hirsch et al. (this issue). Genomic replacements with two independently isolated mutant genes for each SCG1-m mutant were made by subcloning into Ylp535, integrating, and selecting for loss of one of the two resulting genomic copies [Hirsch et al. 1991]. The SCG1Val50 and SCG1Ala382 plasmids were cleaved with Sphi, and the SCG1Lys388 and scg1Ala391 plasmids were partially cleaved with HindIII to integrate the plasmids. On the basis of the replicating plasmid assays, the scg1Lys388 and scg1Ala391 mutations did not allow growth of haploid strains; therefore, the replacements were made in the a [pTRP1–MATa] strain. On the basis of the assays with the replicating plasmids, the scg1Val50 and SCG1Ala382 mutations allowed growth of haploid strains. The SCG1Ala383 replacements were made in wild-type strains. The scg1Val50 integrants were also made in wild-type strains, but due to generation of suppressor mutations with this mutant (J. Kurjan, unpubl.), the integrants were transformed with Yep13–SCG1 before selection on 5-FOA plates [Boeke et al. 1984] for recombination resulting in gene replacements.

Dominance tests

Diploid strains heterozygous for SCG1 [SCG1/SCG1 m] and homozygous for MAT were constructed using pGAL–HO [Jensen and Herskowitz 1984] as described by Hirsch et al. (this issue). To construct the a/a diploids needed for this procedure, the strains were crossed to isogenic wild-type strains. The scg1Lys388 [pTRP1–MATa] and scg1Ala391 [pTRP1–MATa] mutants were mated as described for the scg1Amb45 mutant, and the SCG1Ala382 mutant was mated as described for mating-defective SCG1 mutants [Hirsch et al., this issue]. The strains containing the scg1Val50 replacements also contained Yep13–SCG1, allowing low-level mating; they were crossed to wild-type strains containing pGAL–HO.

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