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The Yeast G-Protein Homolog Is Involved in the Mating Pheromone Signal Transduction System†

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I have isolated a new type of sterile mutant of *Saccharomyces cerevisiae*, carrying a single mutant allele, designated *dac1*, which was mapped near the centromere on chromosome VIII. The *dac1* mutation caused specific defects in the pheromone responsiveness of both a and α cells and did not seem to be associated with any pleiotropic phenotypes. Thus, in contrast to the *ste4*, *ste5*, *ste7*, *ste11*, and *ste12* mutations, the *dac1* mutation had no significant effect on such constitutive functions of haploid cells as pheromone production and α-factor destruction. The characteristics of this phenotype suggest that the *DAC1* gene encodes a component of the pheromone response pathway common to both a and α cells. Introduction of the *GPA1* gene encoding an *S. cerevisiae* homolog of the α subunit of mammalian guanine nucleotide-binding regulatory proteins (G proteins) into sterile *dac1* mutants resulted in restoration of pheromone responsiveness and mating competence to both a and α cells. These results suggest that the *dac1* mutation is an allele of the *GPA1* gene and thus provide genetic evidence that the yeast G protein homolog is directly involved in the mating pheromone signal transduction pathway.

Sexual conjugation in the yeast *Saccharomyces cerevisiae* is initiated by the interaction of oligopeptide pheromones with their target cells (reviewed in reference 45). The α-factor pheromone is secreted by haploid cells of the α mating type (α cells) and acts on haploid cells of the **a** mating type (**a** cells). Similarly, **a** cells produce **a**-factor, to which α cells respond. The binding of **a**- and α-factors with their putative receptors on the cell surface of α and **a** cells seems to induce distinct physiological responses in cells of the opposite mating type. These include production of cell surface agglutinin (10, 36, 50), arrest of the cell cycle at the G1 phase (4, 49), and induction of morphologically altered cells called shmoos. In addition, the transcript levels from a number of genes, including the **a**- and α-factor receptor genes, are stimulated by exposure to the opposite pheromone (16, 17, 28, 32, 48). Cells stimulated by the appropriate pheromone aggregate and then fuse to produce diploid cells (**a**/α cells) (18, 28, 35, 48).

The response of **a** and α cells to pheromones requires at least seven genes (*STE2*, *STE3*, *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*) (19, 25). Recent evidence has suggested that the *STE2* and *STE3* genes encode the cell surface receptors for α-factor and **a**-factor, respectively (5, 15, 22, 32). Mutations in five other genes (*STE4*, *-5*, *-7*, *-11*, and *-12*) cause **a** and α cells to become unresponsive to pheromones and also lead to partial inhibition of other mating functions (**a**- and α-factor production and α-factor destruction). In fact, a mutation in the *STE12* gene is known to reduce levels of transcripts from other genes required for mating (*STE2*, *STE3*, *MFa1*, *MFa2*, *MFα1*, *MFα2*) (11), and *ste4* strains show a reduced level of the *STE2* transcript (17). Thus, it is probable that these gene products affect the response pathway indirectly (23). No component has yet been shown to be directly involved in the pheromone response pathway. The *SST2* gene product may be a component of the desensitization process (6, 8). Recent evidence suggests that the pheromone response pathways in the two mating types converge at a point after the pheromone-receptor interaction (2, 33).

G proteins are a family of guanine nucleotide-binding regulatory proteins that couple a wide array of membrane receptors to biochemical effector systems (14). Receptors either stimulate the adenylate cyclase via Gs (the stimulatory G protein) or inhibit it *via* Gi (the inhibitory G protein), whereas transducin (Gt) is involved in coupling retinal rhodopsin to cyclic GMP phosphodiesterase in the rod outer segment. Another G protein, Go, found predominantly in the brain, may be involved in neuronal responses. Recently, Nakafuku et al. (31) and Dietzel and Kurjan (7) isolated the *GPA1* (also called *SCG1*) gene from *S. cerevisiae* and found that it was homologous to cDNAs for mammalian G protein α subunits. Gene disruption experiments (7, 29) indicate that the *GPA1* gene is expressed haploid specifically and may be involved in the pheromone signal transduction pathway.

In this study, I isolated a new type of sterile mutant which was specifically defective in pheromone responsiveness in both **a** and α cells. This mutation was designated *dac1*, for division arrest control by mating pheromones. The *dac1* mutation appeared to have little effect on the constitutive expression of genes required for mating. Here, I describe the characterization of the *dac1* mutant and show that *DAC1* is allelic to *GPA1* and therefore encodes a yeast G protein homolog and that this G protein is directly involved in the pheromone signal transduction pathway.

MATERIALS AND METHODS

Yeast strains, media, genetic methods, and transformation. The strains of *Saccharomyces cerevisiae* used in this study are listed in Table 1. The media used were YEPD (rich medium) (same as YPD [40]), SD (minimal medium) (40) and SD-URA (selective medium) (SD supplemented with ade-

† This article is dedicated to the late Professor Naohiko Yanagishima, who passed away on 28 March 1987, in memory of his contributions to the study of mating reaction in *Saccharomyces cerevisiae*.

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TABLE 1. Yeast strains

Strain	Genotype	Source
X2180-1A	**a** *SUC2 mal gal2 CUP1*	YGSC[a]
X2180-1B	α *SUC2 mal gal2 CUP1*	YGSC
DBY746	α *ura3-52 leu2-3,112 his3-Δ1 trp1-289*	YGSC
SR665-1	α *cdc39-1 met2 tyr1 cyh2 gal1*	YGSC
RC629	**a** *sst1-2 rme1 ade2 ura1 his6 met1 can1 cyh2 GAL*	R. Chan
A364A	**a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1*	YGSC
FS12	**a** *dac1-1*; isogenic to A364A	This work
XF56-4C	**a** *ura3-52 leu2-3,112 his4-519 arg4 gal2*	This work
XF56-21B	**a** *ura3-52 leu2-3,112 his4-519 arg4 gal2 dac1-1*	This work
XF56-36A	α *ura3-52 leu2-3,112 his4-519 arg4 gal2 dac1-1*	This work
XF56-41C	α *ura3-52 leu2-3,112 his4-519 arg4 gal2*	This work
XF63	**a**/α *leu2-3,112/+ his4-519/+ +/his6 +/trp1 +/arg4 dac1-1/+*	This work
52A	**a** *his3-Δ1 his7 met1 GAL*	This work
54D	α *his3-Δ1 his7 met1 GAL*	This work
XF71-5A	α *ura3-52 his6 trp1 gal2*	This work

[a] YGSC, Yeast Genetic Stock Center.

nine, L-leucine, L-histidine, L-arginine, L-methionine, L-tyrosine, L-tryptophan, L-lysine, L-phenylalanine, and L-threonine). Solid media were prepared with 2.5% agar (Difco).

Standard procedures were used for mating, diploid isolation, and tetrad analysis (40).

Yeast transformation was performed by the method of Ito et al. (20). For plasmid integration by homologous recombination, YIp5-GPA1 was digested with *Hin*dIII and used directly for transformation as described by Orr-Weaver et al. (34).

Mating pheromones. Partially purified **a**-factor was prepared as described by Betz et al. (3). Partially purified α-factor was prepared as described by Duntze et al. (9). Synthetic α-factor was purchased from the Peptide Institute (Osaka, Japan). The lowest concentration inducing shmoo formation was determined to be 1 U/ml.

Mutant isolation. All the procedures were performed according to the methods of Hartwell (19) and Manney and Woods (27). An overnight culture of the parent strain (A364A) was mutagenized with ethyl methanesulfonate to 50% survival, diluted into numerous tubes containing YEPD medium, and grown overnight at 30°C until the cell density reached a 20-fold increase. Samples were plated on YEPD plates (pH 4.5, adjusted with 0.1 M citrate buffer) containing partially purified α-factor (about 10^2 U per plate) and incubated at 30°C for 2 days. α-Factor-resistant mutants were cloned and tested for zygote formation with tester strains, X2180-1A (**a**) and X2180-1B (α). The mutants were further tested for **a**-factor production by the halo test and found to mate rarely with DBY746 (α) by prototroph recovery. The mating type specificity of the sterile character was tested by tetrad analysis.

Plasmids. DNA manipulations and transformation and growth of *Escherichia coli* HB101 were performed as described by Maniatis et al. (26). To construct a centromere plasmid carrying the *GPA1* gene, a 5.3-kilobase (kb) *Xho*I fragment from pMN10 (kindly provided by Y. Kaziro and M. Nakafuku) (31) was inserted into YCp19 (kindly provided by S. Harashima) (42) that had been cleaved with *Sal*I, yielding YCp19-GPA1.

To construct an integrating plasmid carrying the *GPA1* gene, a 1.9-kb *Eco*RI fragment from pGI1 (kindly provided by Y. Kaziro and M. Nakafuku) (31) was inserted into YIp5 (43) that had been cleaved with *Eco*RI, yielding YIp5-GPA1. The *Hin*dIII site in YIp5 was first destroyed, making the *Hin*dIII site in the *GPA1* gene unique.

Halo assay for pheromone production and barrier activity. For the **a**-factor assay, about 10^4 cells of tester strain SR665-1 (α *cdc39*) were spread onto a YEPD (pH 4.5) plate, and cells of the **a** strains to be tested were spotted on the plate. Halos were zones of growth inhibition that were clearly visible after 3 days of incubation at 35°C.

For the α-factor assay, an analogous test, using inhibition of RC629(**a** *sst1*), was performed as described for the **a**-factor test, except that about 10^5 cells per plate were used. Halos were visible after 2 days of incubation at 30°C.

The width of the clear zone provides a qualitative measure of pheromone activity.

Barrier activity was detected by interference in α-factor-produced halo zones by a streak of cells of **a** strains as described before (41). The barrier activity of the **a** cells was also tested as described previously (12).

Pheromone response. (i) G1 arrest. Test strains were grown overnight at 30°C in YEPD or SD-URA medium. Cells were washed and inoculated into 5 ml of YEPD or SD-URA medium containing **a**-factor (10 U/ml) or α-factor (1 μM) at a cell density of 2×10^6 cells per ml and incubated at 30°C on a reciprocal shaker. Samples (0.2 ml) were removed at various intervals and mixed with an equal volume of 10% formaldehyde. The percentage of unbudded cells in each sample was measured microscopically after brief sonication.

(ii) Morphological changes. Plasmid-bearing strains grown exponentially in SD-URA medium were inoculated into 1 ml of SD-URA medium containing **a**-factor (10 U/ml) or α-factor (1 or 5 μM) at a cell density of 10^7 cells per ml. Morphological changes were examined microscopically after 5 h of incubation at 30°C.

(iii) Agglutinin induction. Plasmid-bearing strains were grown exponentially in SD-URA medium at 30°C, and 10^7 cells were incubated in 400 μl of SD-URA medium containing **a**-factor (1 U/ml) or α-factor (0.1 μM) for 3 h at 30°C. Induction was stopped by the addition of cycloheximide (100 μg/ml). **a** or α agglutinin was detected by gently mixing the treated cells with tester strains of opposite mating types, X2180-1A (**a**) or X2180-1B (α), whose agglutination ability had been enhanced by pretreatment with pheromones for 2 h at 30°C. Unless **a** or α agglutinin was induced in pheromone-treated cells to be tested, agglutination never occurred between the pheromone-treated cells and tester strains of the opposite mating type. The agglutination test was scored 2 h after the strains were gently mixed.

Quantitative mating tests. Plasmid-bearing strains to be tested for mating were grown overnight in SD-URA medium. Cultures of the mating type tester strains, 52A and 54D, were prepared by growth overnight in YEPD medium. Approximately 2×10^6 cells to be tested and 10^8 cells of the tester strain were mixed, incubated in YEPD medium for 4 h at 30°C, and plated on SD plates, on which only diploids formed by mating of the two strains could grow. All cultures were plated singly on SD plates to assay the reversion of auxotrophic markers; no prototrophs were observed.

RESULTS

Isolation of *dac1* mutant. A total of 247 mutants resistant to the mating pheromone α-factor were obtained from a mutagenized culture of strain A364A. All the mutants were sterile and failed to form zygotes with the X2180-1B (α) tester strain under microscopic observation. For many known sterile mutants, defects in the pheromone response are usually concomitant with partial loss of other **a**-specific functions, **a**-factor production and barrier activity (α-factor destruction). The α-factor-resistant mutants obtained were assayed for **a**-factor production to ascertain whether the defect in the pheromone response was accompanied by a decrease in **a**-factor production. Of 247 mutants isolated, 178 produced no detectable amount of **a**-factor, while 69 produced **a**-factor to various degrees, although most of them secreted less **a**-factor than the parent strain, A364A. Only five mutants secreted an amount of **a**-factor similar to that of A364A. These five mutants mated inefficiently with DBY746 (α), and the resulting diploids were sporulated. In tetrad analysis of the four diploids, no α spores produced sterile clones, whereas about one-half of the **a** spores produced sterile clones. These results indicated that the mutations in these four sterile mutants were not linked to the *MAT* locus and that they were expressed specifically in **a** cells. This is the expected behavior of an *ste2* mutation. In one cross, 11 asci were analyzed, and it was found that one-half of the **a** and α spores produced sterile clones, indicating that the sterile mutation was due to a defect in a single gene, unlinked to the *MAT* locus and expressed nonspecifically in both **a** and α cells. This mutation was designated *dac1* (for division arrest control by mating pheromones).

The recessiveness of the *dac1* mutation was determined as follows. Since no **a**/α diploids respond to mating pheromone, diploids homozygous at the *MAT* locus were constructed from an **a**/α *DAC1*/*dac1* diploid, XF63, by UV-induced mitotic recombination and by testing their ability to respond to the appropriate pheromone. Diploids homozygous at the *MAT* locus (**a**/**a** or α/α cells) were easily detected as **a**-factor-producing or α-factor-producing clones, respectively, by the halo test. Three **a**-factor-producing clones and eight α-factor-producing clones were obtained among about 1,000 colonies of the UV-treated XF63. All of them responded to **a**- or α-factor pheromone and mated with the tester strain of the opposite mating type, X2180-1A (**a**) or X2180-1B (α) (data not shown). Thus, the *dac1* mutation proved to be recessive.

Pheromone responsiveness of *dac1* mutants. The *dac1* mutant was originally isolated from the **a** strain A364A as an α-factor-resistant mutant, and other *dac1* strains used were constructed by successive backcrosses to a strain derived from a cross of FS12 with DBY746. The **a** *dac1* strains showed unresponsiveness to α-factor; that is, they showed no cell division arrest at the G1 phase in response to α-factor (Fig. 1A). This α-factor resistance of **a** *dac1* mutants was confirmed by the halo test (data not shown). **a** *sst1 dac1* cells were unable to arrest cell division in response to α-factor, and therefore a clear halo zone was not formed, although the lawn strain harbored the *sst1* mutation, which enables **a** cells to become supersensitive to α-factor. Similarly, an α *dac1* strain was unable to arrest cell division at the G1 phase even in the presence of a high concentration of **a**-factor (Fig. 1B).

After the division arrest of **a** or α cells by pheromone, the cells continue protein and RNA synthesis (46); they become larger and elongated (the resulting cells are called shmoos). The *dac1* mutant cultures did not produce such shmoos (data not shown), although a sufficient amount of mating pheromone was added to induce shmoo formation in the wild-type strains.

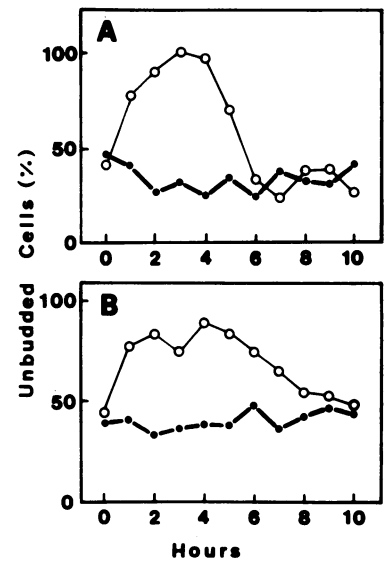

FIG. 1. Accumulation of unbudded cells in response to mating pheromone. (A) **a** cells were incubated in YEPD medium containing α-factor (1 μM). A364A (**a** *DAC1*) (○), FS12 (**a** *dac1-1*) (●). (B) α cells were incubated in YEPD medium containing partially purified **a**-factor (10 U/ml). XF56-41C (α *DAC1*) (○), XF56-36A (α *dac1-1*) (●).

a and α strains are known to inducibly produce cell surface agglutinins in the presence of mating pheromones. The agglutinins are specific for the cells of the opposite mating type and essential for cell-cell recognition between **a** and α cells. The *dac1* mutants no longer inducibly produced agglutinin even after exposure to a high concentration of pheromones (see below).

Expression of mating type-specific genes in *dac1* mutants. It has been observed previously that mutations in five known *STE* genes (*STE4*, *STE5*, *STE7*, *STE11*, and *STE12*) lead to an inability of both **a** and α cells to respond to pheromones and to a reduction of production of two **a**-specific products, **a**-factor and barrier activity, and one α-specific product, α-factor (16, 19, 25). These phenotypic characteristics suggest that known sterile mutants have a general defect in the expression of **a**- and α-specific genes. Therefore, I examined the effect of the *dac1* mutation on the expression of **a**- and α-specific genes by the plate halo assay, although the halo assay system has quantitative limitations for the determination of pheromone production and barrier activity.

An **a** *dac1* mutant was found to form a halo of approximately the same size as a wild-type **a** strain, suggesting that it produces **a**-factor at a similar level (Fig. 2A). In this experiment, it was found that there was a difference in cell growth at 35°C between the *dac1* mutant and a wild-type strain (Fig. 2A). In an α-factor halo test, the α *dac1* mutant produced a clear halo zone in the surrounding lawn of **a** *sst1* cells, which are supersensitive to α-factor, suggesting that they produced α-factor in a similar amount to a wild-type α strain (Fig. 2B). Barrier activity was assayed by patching the strains to be tested next to a wild-type α strain on a lawn of **a** *sst1* cells (Fig. 2C). The growth of the *sst1* lawn was inhibited by the α-factor produced by the wild-type α strain. However, each patch of **a** *dac1* cells protected the *sst1* lawn from growth inhibition, indicating that **a** *dac1* mutants pro-

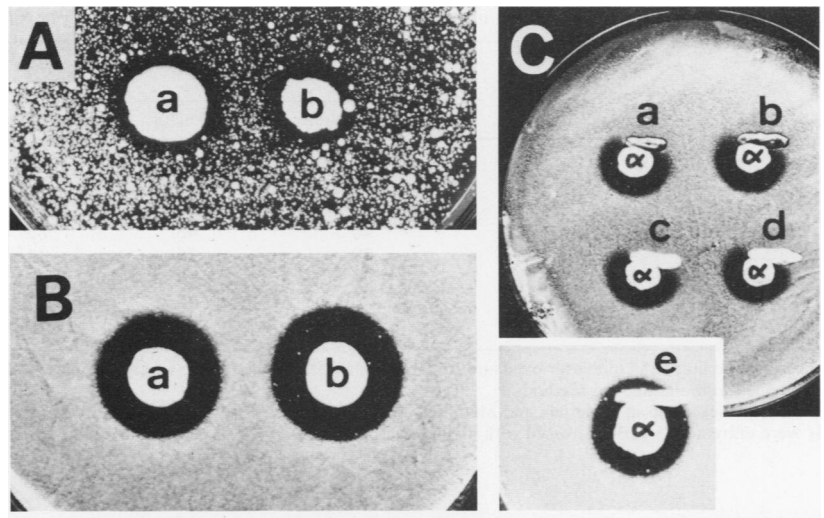

FIG. 2. Production of a-factor, α-factor, and barrier. Halo assays were carried out as described in Materials and Methods. (A) a-Factor halo assay. Cells of strain SR665-1 (α *cdc39*) were spread onto a YEPD (pH 4.5) plate. (a) A364A (a *DAC1*); (b) FS12 (a *dac1-1*). (B) α-Factor halo assay. Cells of strain RC629 (a *sst1*) were spread onto a YEPD (pH 4.5) plate. (a) XF56-41C (α *DAC1*); (b) XF56-36A (α *dac1-1*). (C) Barrier assay. Cells of strain RC629 were spread onto a YEPD (pH 4.5) plate before the a strains to be tested were streaked. Streaks of barrier-producing cells disturbed the halo zone which was formed by inhibition of the growth of the tester strain RC629 by the diffusing α-factor. (a) A364A (a *DAC1*); (b) FS12 (a *dac1-1*); (c) XF56-4C (a *DAC1*); (d) XF56-21B (a *dac1-1*); (e) RC629 (a *sst1*). α, α-Factor-producing strain X2180-1B.

duced barrier activity comparable to that of wild-type a strains.

Mapping the *dac1* mutation. Many chromosomal genes are involved in either sporulation, response to pheromones, or cell cycle control. I decided to map the *DAC1* locus to see if it was different from all previously mapped genes. The *dac1* mutation was initially mapped relative to the centromere marker *trp1*. Asci of strain XF63 were dissected, and complete tetrads that segregated 2:2 for both markers were scored. The data show that the *dac1* mutation mapped to chromosome VIII, 2.1 centimorgans (cM) from *cen8* and 10.4 cM from *arg4* (Table 2). Surprisingly, this locus seemed to be coincident with the *GPA1* locus previously determined by Miyajima et al. (29).

***dac1* is an allele of *GPA1*.** The results of tetrad analysis presented above suggested that the *dac1* mutation is an allele of the *GPA1* gene, which encodes a yeast homolog of the α subunit of mammalian G proteins. Disruption of the *GPA1* gene results in cell division arrest at the G1 phase and morphological changes in haploid cells. Therefore, this yeast G protein is thought to be involved in the pheromone response pathway. To establish whether the *dac1* mutation is indeed an allele of the *GPA1* gene, transformation experiments were performed to introduce the *GPA1* gene into *dac1* mutants. I used a centromere plasmid, YCp19-GPA1, and an integrative plasmid, YIp5-GPA1, for these experiments. The ability of the plasmid-bearing strains to respond to a-factor or to α-factor was evaluated by the following four assays.

TABLE 2. Tetrad analysis

Gene pair[a]	No. of asci[b]			Map distance (cM)	Linkage relationship[c]
	PD	NPD	T		
dac1-arg4	38	0	10	10.4	Direct
dac1-trp1	25	21	2	2.1	Centromere
trp1-arg4	17	20	11	11.4	Centromere

[a] Diploid strain XF63 was sporulated, and asci were dissected and analyzed for each gene pair.

[b] PD, Parental ditype; NPD, nonparental ditype; T, tetratype. Only those asci showing 2:2 segregation for both markers were included.

[c] The genetic markers are linked to one another on the same chromosome (direct) or linked to centromeres on different chromosomes (centromere).

First, I measured the fraction of *dac1* cells in the G1 phase of the cell cycle following pheromone treatment. Introduction of the *GPA1* gene allowed the *dac1* mutants to show cell division arrest in the G1 phase in response to the appropriate pheromone (Table 3). The ability of the *dac1* mutants to arrest cell division in response to pheromones was further assessed by the halo test (data not shown). In the surrounding lawn of an a *sst1 dac1* strain bearing plasmid YCp19-GPA1, a clear halo zone was formed by a patch of α cells, which produced α-factor.

Second, I observed morphological changes in *dac1* mutants following pheromone treatment. YCp19-GPA1-bearing strains showed morphological changes in response to pheromones, but not YCp19-bearing strains (data not shown).

Third, I tested the ability of *dac1* mutants to produce agglutinin in response to pheromones. Introduction of the *GPA1* gene allowed the *dac1* mutants to produce agglutinin in the presence of pheromones (Table 3). Thus, the *GPA1* gene complemented the *dac1* mutation.

Fourth, as a final assessment of the ability of the *GPA1* gene to complement the *dac1* mutation, it was found that the *GPA1* gene could restore mating competence to *dac1* mutants, as expected from the above experiments (Table 4).

Moreover, integration of the 1.9-kb *Eco*RI fragment carrying the *GPA1* gene could also complement the *dac1* phenotype (Tables 3 and 4). I determined the integration site of the *GPA1* fragment. A Ura^+ transformant of strain XF56-21B carrying the integrated plasmid was crossed to a wild-type strain, and tetrad analysis was done. The integrated *URA3* marker mapped 11.5 cM from *arg4* on the right arm of chromosome VIII, consistent with the *dac1* locus (Table 5). All 39 tetrads showed a 2 Ura^+ mater:2 Ura^- mater segregation pattern (Table 5). Therefore, the inte-

TABLE 3. Cell division arrest and agglutinin induction of *dac1* mutants by mating pheromone

Strain	Genotype	Plasmid	% Unbudded cells[a]			Agglutination intensity[b]	
			No pheromone	a-Factor	α-Factor	−MP	+MP
XF56-4C	a *DAC1*	YCp19	43	48	93	−	+
XF56-21B	a *dac1-1*	YCp19	47	41	45	−	−
		YCp19-GPA1	41	44	83	−	+
		YIp5-GPA1 (integrated)	40	43	89	−	+
XF56-41C	α *DAC1*	YCp19	39	76	44	−	+
XF56-36A	α *dac1-1*	YCp19	41	43	38	−	−
		YCp19-GPA1	40	78	47	−	+
		YIp5-GPA1 (integrated)	38	81	41	−	+

[a] The percentage of unbudded cells in a culture 180 min after exposure to pheromone is expressed as the average of three independent experiments. All the procedures were carried out as described in Materials and Methods.

[b] Agglutination test was carried out as described in Materials and Methods. Agglutination intensity was expressed qualitatively as no agglutination (−) or intensive agglutination (+). Cells were untreated (−MP) or treated with mating pheromone (+MP) of the opposite mating type for 3 h at 30°C (0.1 μM α-factor or 1 U of a-factor per ml).

grated *URA3* gene mapped to the *dac1* locus, indicating that the *dac1* mutation is an allele of the *GPA1* gene.

DISCUSSION

I have identified a mutation, *dac1*, which causes **a** and α cells to become unresponsive to mating pheromones. The *dac1* mutant was selected as a result of the inability of **a** cells to arrest cell division in response to α-factor and the appearance of a sterile phenotype. Since *dac1* mutants failed to express various pheromone-mediated processes (cell division arrest, morphological changes, and agglutinin induction) when they were exposed to the appropriate pheromone, they seemed to be defective for some regulatory element common to all three pheromone-mediated processes.

Mutations which prevent **a** and α cells from responding to mating pheromones have been useful for dissecting the various elements of the pheromone response pathway (19, 25). Biochemical and genetic studies with many sterile mutants have supported the idea that the *STE2* and *STE3* genes encode the structural components of α- and **a**-factor receptors, respectively (5, 15, 19, 22, 25, 32). However, the molecular functions of the other *STE* gene products are unknown, although Teague et al. have suggested that the *STE7* gene product is a protein kinase (44). Because cell division arrest by both **a**-factor and α-factor appears to utilize a common pathway (2, 33), the mating type-nonspecific genes necessary for response to pheromones may be part of the machinery that is common to **a** and α cells. This simple model for the roles of the *STE* gene products does not appear to fit with the fact that the *ste4*, *ste5*, *ste7*, *ste11*, and *ste12* mutations are associated with pleiotropic phenotypes (16, 19), although this fact does not rule out their participation in the machinery of the pheromone response pathway.

Dietzel and Kurjan (7) and Miyajima et al. (29), on the basis of their experimental results, have proposed that a yeast G protein homolog encoded by the *GPA1* gene is involved in the pheromone response pathway. Their evidence is strictly indirect, but the observations are suggestive. The model for the role of the yeast G protein homolog in the response pathway predicts that it is possible to obtain mutations in the *GPA1* gene that prevent its functioning in pheromone signal transduction; such mutants should be sterile. This paper has demonstrated this to be true. The fact that integration of a 1.9-kb *GPA1* fragment could complement the *dac1* phenotype is conclusive evidence that *dac1* is an allele of the *GPA1* gene. On the other hand, temperature-sensitive *gpa1* mutations (initially designated *cdc70*) have been identified which allow conjugation by cells lacking a pheromone receptor (21). At the restrictive temperature, the *gpa1* mutation causes cell cycle arrest at the G1 phase, deposition of mating type-specific cell surface agglutinins, and induction of pheromone-specific transcripts in the absence of mating pheromones. Taking account of the result of gene disruption experiments as well (7, 29), putative β-γ subunits or equivalent(s) rather than the *GPA1*-encoded α subunit probably stimulate an as yet unidentified effector for the propagation of the pheromone-induced signal, as suggested previously (7, 21). If this model is true, the deletion mutants in the β and γ subunit-encoding genes should exhibit a pheromone response-negative phenotype. Alternatively, the α-β-γ complex may inhibit the effector. On the other

TABLE 4. Mating efficiencies of *dac1* mutants[a]

Strain	Genotype	Plasmid	Mating efficiency (no. of diploids)	
			With a strain	With α strain
XF56-4C	a *DAC1*	YCp19	<1	8.8×10^4
XF56-21B	a *dac1-1*	YCp19	<1	<1
		YCp19-GPA1	<1	3.5×10^4
		YIp5-GPA1 (integrated)	<1	1.7×10^5
XF56-41C	α *DAC1*	YCp19	2.3×10^5	<1
XF56-36A	α *dac1-1*	YCp19	<1	<1
		YCp19-GPA1	1.1×10^5	<1
		YIp5-GPA1 (integrated)	1.5×10^5	<1

[a] Efficiency of mating was determined at 30°C as described in Materials and Methods and is expressed as the number of diploids formed.

TABLE 5. Mapping of *GPA1[URA3]* integrant

Gene pair[a]	No. of asci[b]			Map distance (cM)
	PD	NPD	T	
GPA1[URA3]-arg4	30	0	9	11.5
GPA1[URA3]-dac1	39	0	0	Allelic

[a] A Ura$^+$ transformant of XF56-21B (**a**) in which *GPA1[URA3]* was integrated at the chromosomal locus homologous to the insert was crossed to XF71-5A, and tetrad analysis was done for each pair.
[b] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

hand, the *dac1* mutants have a phenotype opposite to that of *gpa1* mutants. Since the *dac1* mutants have a response-negative phenotype, the *dac1* mutation perhaps affects the dissociation of the α-β-γ complex.

The finding that the *dac1* mutation affects both **a** and α cells indicates that the same G protein homolog is part of the signal transduction system in both cell types. In view of the fact that the two receptor-pheromone interactions are interchangeable (2, 33), the two response pathways probably converge at the level of the yeast G protein. In this study, I could not ascertain whether the two receptors interact directly with the G protein or whether additional proteins are involved. Baffi et al. (1) and Moore (30) suggested that the signal transduction pathway for morphological changes and agglutination induction diverged at the level of the receptors. However, subsequent studies have indicated that *STE2* and *STE3* gene products are the only receptors for α-factor and **a**-factor on the cell surface of target **a** cells and α cells, respectively. The present study suggests that the yeast G protein mediates all the signals for cellular responses, including transcriptional activation, elicited by pheromones from specific receptors to the intracellular effector system(s).

In contrast to *ras*-related genes (*RAS1*, *RAS2*, *YPT1*, and *SEC4*) (24, 37, 38, 39, 47), the *GPA1* gene shows haploid-specific expression and is not involved in sporulation (7, 29). In fact, a *dac1/dac1* diploid strain could undergo sporulation (data not shown). In addition, the *GPA1* gene product does not seem to play an essential role in secretion or to be required for the expression of the cell type-specific genes, because *dac1* mutants produced amounts of mating pheromones and barrier activity similar to those in the wild-type strains.

It was recently shown that in *Schizosaccharomyces pombe*, its single known *RAS* homolog, *RAS1*, plays a quite different role in the life cycle (13). It is essential for mating but not for vegetative growth or the cyclic AMP pathway. The *RAS1* gene product of *S. pombe* also may be involved in pheromone signal transduction, although there are some significant differences between the *GPA1* gene of *S. cerevisiae* and the *RAS1* gene of *S. pombe*.

Biochemical and genetic analysis of the *dac1* mutation remains for the identification of additional components—in particular, the effector(s)—of the pheromone response pathway.

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