Identification and Characterization of a Mutation Affecting the Division Arrest Signaling of the Pheromone Response Pathway in *Saccharomyces cerevisiae*

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ABSTRACT

Mating pheromones, **a**- and α-factors, arrest the division of cells of opposite mating types, α and **a** cells, respectively. I have isolated a sterile mutant of *Saccharomyces cerevisiae* that is defective in division arrest in response to α-factor but not defective in morphological changes and agglutinin induction. The mutation was designated *dac2* for *d*ivision *a*rrest *c*ontrol by mating pheromones. The *dac2* mutation was closely linked to *gal1* and was different from the previously identified cell type nonspecific sterile mutations (*ste4*, *ste5*, *ste7*, *ste11*, *ste12*, *ste18* and *dac1*). Although *dac2* cells had no phenotype in the absence of pheromones, they showed morphological alterations and divided continuously in the presence of pheromones. As a result, *dac2* cells had a mating defect. The *dac2* mutation could suppress the lethality caused by the disruption of the *GPA1* gene (previously shown to encode a protein with similarity to the α subunit of mammalian G proteins). In addition, *dac2* cells formed prezygotes with wild-type cells of opposite mating types, although they could not undergo cell fusion. These results suggest that the *DAC2* product may control the signal for G-protein-mediated cell-cycle arrest and indicate that the synchronization of haploid yeast cell cycles by mating pheromones is essential for cell fusion during conjugation.

SEXUAL conjugation in the yeast *Saccharomyces cerevisiae* is initiated by the interaction of oligopeptide pheromones with their target cells (reviewed in SPRAGUE, BLAIR and THORNER 1983; CROSS *et al.* 1988). The **a** cells respond to the α-factor pheromone that is secreted by α cells; the α cells respond to the **a**-factor pheromone secreted by *a* cells. Pheromones bind to the putative receptors on the target cells of opposite mating types to induce arrest of cell division at the G1 phase, production of the cell surface agglutinin, morphological changes, and transcriptional activation of specific genes required for conjugation. Haploid cells activated by mating pheromones of the opposite mating types aggregate, fuse and undergo karyogamy to produce diploid cells (**a**/α cells) (TRUEHEART, BOEKE and FINK 1987; MCCAFFREY *et al.* 1987; CURRAN and CARTER 1986; ROSE, PRICE and FINK 1986).

The yeast mating pheromone response provides a model system for studying hormone action and signal transduction in eukaryotic cells. Genetic dissection of the pheromone response pathway has been attempted by isolating mutants that are unable to arrest cell division in response to α-factor (MACKAY and MANNEY 1974; HARTWELL 1980; WHITEWAY, HOUGAN and THOMAS 1988; FUJIMURA 1989). Out of a set of genes designated *STE*, the *STE2* and *STE3* genes encode the receptors for α-factor and **a**-factor, respectively (JENNESS, BURKHOLDER and HARTWELL 1983; MARSH and HERSKOWITZ 1988; BLUMER, RENEKE and THORNER 1988; HAGEN, MCCAFFREY and SPRAGUE 1986). The *STE2* and *STE3* gene products are predicted to contain seven hydrophobic membrane spanning domains (NAKAYAMA, MIYAJIMA and ARAI 1985; BURKHOLDER and HARTWELL 1985; HAGEN, MCCAFFREY and SPRAGUE 1986). This characteristic resembles the β-adrenergic receptors, rhodopsin, and the muscarinic-cholinergic receptors thought to transduce extracellular signals by interacting with guanine nucleotide binding regulatory proteins (G proteins) (DIXON *et al.* 1986; NATHANS and HOGNESS 1984; KUBO *et al.* 1986; GILMAN 1987).

Recently *S. cerevisiae* has been shown to contain a gene (*GPA1* or *SCG1*) that encodes a protein similar to the α subunit of the mammalian G proteins (NAKAFUKU *et al.* 1987; DIETZEL and KURJAN 1987). Mutations in this gene cause a constitutive arrest of cell division or a specific defect in pheromone responsiveness (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987; JAHNG, FERGUSON and REED 1988; BLINDER, BOUVIER and JENNESS 1989; FUJIMURA 1989). Thus, the *GPA1* product appears to be involved in the pher-

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omone response pathway. Furthermore, the products of the *STE4* and *STE18* genes have been shown to comprise the β and γ subunits of a G protein complex coupled to the mating pheromone receptors. The genetic data suggest that pheromone-receptor binding leads to the dissociation of the α subunit from $\beta\gamma$, and the free $\beta\gamma$ element initiates the pheromone response (WHITEWAY *et al.* 1989).

The products of other *STE* genes (*STE5*, *STE7*, *STE11*, and *STE12*) are also required for pheromone response and mating in both *a* and α cells, although their precise roles in mating or signal transduction are unknown. Since *a* and α cells appear to utilize a common transduction pathway (BENDER and SPRAGUE 1986; NAKAYAMA, MIYAJIMA and ARAI 1987), these mating-type-nonspecific *STE* genes may constitute part of the signal transduction machinery. However, mutations in several *STE* genes confer additional characteristics, which suggests that the products of these genes may not be directly involved in the signal transduction pathway (JENNESS, GOLDMEN and HARTWELL 1987). In fact, several genes (*STE7*, *STE11*, and *STE12*) are shown to be required for expression of the mating-type-specific genes (FIELDS and HERSKOWITZ 1985; FIELDS, CHALEFF and SPRAGUE 1988). The *STE5* gene appears to control a post-receptor-G protein complex signaling function, because the *ste5* mutation does not abolish pheromone binding and suppresses the lethality caused by disruption of the *GPA1* gene (JENNESS, GOLDMEN and HARTWELL 1987; NAKAYAMA *et al.* 1988; BLINDER, BOUVIER and JENNESS 1989).

Products of two genes, *FUS1* and *FUS2*, are required for fusion of haploid cells during conjugation (TRUEHEART, BOEKE and FINK 1987; MCCAFFREY *et al.* 1987). Mutations in these genes lead to an interruption of the mating process at a point just before cytoplasmic fusion; the partition dividing the mating pair remains undissolved after the cells have initially formed a stable "prezygote." The *fus1* and *fus2* mutations have no effect on pheromone responsiveness.

In this study, I isolated a recessive mutant that was specifically defective in division arrest in response to α-factor but not defective in morphological changes. The mutation was designated *dac2* for division arrest control by mating pheromones. The phenotypic characteristics of the *dac2* mutant suggest that the *DAC2* product is involved in the division arrest signaling of the pheromone response pathway and, moreover, indicate that the synchronization of haploid yeast cell cycles by mating pheromones is essential for cell fusion during conjugation.

MATERIALS AND METHODS

Yeast strains, media, and genetic methods: The strains of *S. cerevisiae* used in this study are listed in Table 1. The media used were YEPD rich medium and SD minimal medium (SHERMAN, FINK and HICKS 1983). SD medium was also supplemented with appropriate bases and/or amino acids (SHERMAN, FINK and HICKS 1983). Solid media were prepared with 2.5% agar (Difco). Standard procedures were used for mating, diploid isolation, and tetrad analysis (SHERMAN, FINK and HICKS 1983). Partially purified mating pheromones (**a**-factor and α-factor) were prepared as described previously (FUJIMURA 1989). Synthetic α-factor was purchased from the Peptide Institute (Osaka, Japan).

Mutant isolation: All the procedures were performed as described previously (FUJIMURA 1989). The parent strain A364A (*MAT***a**) was grown overnight in 10 ml YEPD. The cells were washed once in water by centrifugation and then resuspended in 10 ml of 0.05 M potassium phosphate buffer, pH 7.0. Ethylmethane sulfonate (0.3 ml) was added and the mixture agitated with a vortex mixer. The cells were then incubated at 30° for 60 min without further agitation. After mutagenesis, cells were collected by centrifugation, resuspended in 10 ml of 5% sodium thiosulfate and incubated at 23° for 10 min. Aliquots of 0.1 ml of cells suspended in sodium thiosulfate were inoculated into each of 20 tubes containing 10 ml YEPD and grown overnight at 30° until the cell density increased 20-fold. Samples were plated on YEPD plates (pH 4.5, adjusted with 0.1 M citrate buffer) containing partially purified α-factor (about 10^2 units per plate) and incubated at 30° for 2 days. α-Factor-resistant mutants were cloned and tested for zygote formation with the tester strain X2180-1B (*MAT*α). The mutants were further tested for an ability to undergo morphological changes in the presence of synthetic α-factor (5 μM), and to yield prototrophic diploids upon mating to DBY746 (*MAT*α). The mating type specificity of the sterile character was tested by tetrad analysis.

Pheromone response: Three assays were performed as described previously (FUJIMURA 1989).

G1 arrest: Test strains were grown overnight at 30° in YEPD until middle logarithmic phase. Cells were washed and inoculated into 5 ml of YEPD containing synthetic α-factor (1 μM) at a cell density of 2×10^6 cells per ml and incubated at 30° 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.

Morphological changes: Test strains growing exponentially in YEPD were inoculated into 1 ml of YEPD containing synthetic α-factor (10 μM) at a cell density of 10^7 cells per ml. Morphological changes were examined microscopically after 4 hr of incubation at 30°.

Agglutinin induction: Test strains were grown exponentially in YEPD at 30°, and 10^7 cells were inoculated into 400 μl of YEPD containing *a*-factor (1 unit/ml) or synthetic α-factor (0.1 μM) for 2 hr at 30°. 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 type, X2180-1A (*MAT***a**) or X2180-1B (*MAT*α), whose agglutination ability had been enhanced by pretreatment with pheromones for 2 hr at 30°. The agglutination test was scored 2 hr after the strains were gently mixed.

Halo assay for pheromone production and barrier activity: The ability to produce *a*-factor, α-factor and barrier activity was determined as described previously (FUJIMURA 1989). The tester strain XBH8-2C (*MAT*α *sst2*) was used for the **a**-factor assay. The tester strain RC629 (*MAT***a** *sst1*) was used for α-factor and barrier activity assay. About 10^5

TABLE 1

Yeast strains used in this study

Strains	Genotype	Source
A364A	*MAT***a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1*	Yeast Genetics Stock Center
FM2	*MAT***a** *dac2-1*; isogenic to A364A	This work
DBY746	*MATα ura3-52 leu2-3, −112 his3−Δ1 trp1-289*	Yeast Genetics Stock Center
RC629	*MAT***a** *sst1-2 rme1 ade2 ura1 his6 met1 can1 cyh2*	R. CHAN
XBH8-2C	*MATα sst2-4 ura1 his6 cyr1*	J. SCHULTZ
XF25R8	*MAT***a** *ade1 ade2 ura1* / *MATα* + *ade2* + *ura3* + *his7* / + *trp1 leu2* + *lys2 tyr1 gal1* / + + *gal1* + *dac2-1* / +	This work
XF64	*MAT***a** *his3 his7 trp1* / *MATα* + + + *leu2 met1 cyh2* + *gal1* / + *dac2-1* / +	This work
XF78-2A	*MATα ura3-52 trp1-289 can1 GAL*	This work
XF78-23B	*MAT***a** *ura3-52 trp1-289 can1 GAL dac2-1*	This work
XF78-23BU	*MAT***a** *gpa1::URA3*; isogenic to XF78-23B	This work
XF78-24C	*MATα ura3-52 trp1-289 can1 GAL dac2-1*	This work
XF78-24CU	*MATα gpa1::URA3*; isogenic to XF78-24C	This work
XF78-39B	*MAT***a** *ura3-52 trp1-289 can1 GAL*	This work
52A	*MAT***a** *his3-Δ1 his7 met1 GAL*	FUJIMURA (1989)
54D	*MATα his3-Δ1 his7 met1 GAL*	FUJIMURA (1989)
XF91	*MAT***a** *ura3 trp1* / *MATα ura3* + *leu2* / + *met1* / + *his4* + *gal2* / +	This work
XF92	*MAT***a** *ura3 trp1* / *MATα ura3* + *leu2* / + *met1* / + *his4* + *gal2* / + *dac2-1* / +	This work
X2180-1A	*MAT***a** *SUC2 mal1 gal2 CUP1*	Yeast Genetics Stock Center
X2180-1B	*MATα SUC2 mal1 gal2 CUP1*	Yeast Genetics Stock Center

cells of the tester strains were spread onto YEPD (pH 4.5) plates. Halos were visible after 2 days of incubation at 30°.

Quantitative mating test: The mating efficiency was quantitatively determined as described previously (FUJIMURA 1989). Strains to be tested for mating were grown exponentially in YEPD at 30°. Cultures of the mating type tester strains, 52A and 54D, were also prepared by growth overnight in YEPD. Samples of 10^7 cells to be tested were mixed with 10^7 cells of the tester strain, collected by centrifugation, resuspended in 1 ml of YEPD, incubated for 4 hr at 30°, and plated on SD, 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.

Gene disruption: Isolation and restriction enzyme digestion of plasmid DNAs, ligation to form a new recombinant, and transformation of *Escherichia coli* HB101 were performed by standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982).

The plasmid pGPA1-URA3 was constructed to perform one-step gene disruption replacement (ROTHSTEIN 1983). Plasmid pGI1 (NAKAFUKU *et al.* 1987) (kindly provided from Y. KAZIRO and M. NAKAFUKU) was partially digested by *Hin*dIII. The 1.1-kb *Hin*dIII fragment of *URA3* was ligated with the *Hin*dIII-partially cleaved pGI1 to yield pGPA1-URA3. Insertion of *URA3* into the *Hin*dIII site inside the coding region for *GPA1* was confirmed by restriction analysis of pGPA1-URA3.

The *Eco*RI-digested pGPA1-URA3 was introduced into yeast cells by the lithium acetate transformation method (ITO *et al.* 1983). The transformants were selected by uracil prototrophy.

RESULTS

Isolation of *dac2* mutant: In the interest of elucidating the yeast pheromone response pathway, I sought to identify mutants that might be specifically defective in the division arrest signaling of the pheromone response pathway. If the signaling pathway for morphological changes and division arrest diverged at a certain level, mutants that were specifically defective in division arrest signaling should divide continuously in the presence of mating pheromone but still undergo a morphological alteration.

A total of over 2000 mutants resistant to the mating pheromone α-factor were obtained from a mutagenized culture of strain A364A by the procedure described previously (FUJIMURA 1989). All the mutants were sterile and failed to form zygotes with the X2180-1B (*MATα*) tester strain as detected by microscopic observation. These α-factor-resistant mutants were further assayed for their ability to undergo morphological changes in response to synthetic α-factor (5 μM). Only one mutant strain, FM2, could form typical pear-shaped "shmoo" morphologies in the presence of α-factor. The mutant strain FM2 mated inefficiently with DBY746 (*MATα*). The resulting diploid was sporulated. In tetrad analysis of the diploid, about one-half of the spore clones were sterile and sterility was divided equally between prospective **a** and α spores, 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 *dac2* (for division arrest control by mating pheromones).

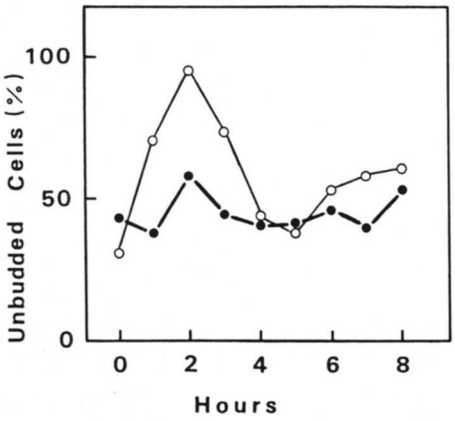

FIGURE 1.—Accumulation of unbudded cells in response to α-factor. Cells were incubated in YEPD medium containing α-factor (1 μM). A364A (*MAT***a** *DAC2*) (○); FM2 (*MAT***a** *dac2-1*) (●).

The recessiveness of the *dac2* mutation was determined as follows. Since no **a**/α diploids respond to mating pheromones, diploids homozygous at the *MAT* locus were constructed from an **a**/α *DAC2/dac2-1* diploid, XF25R8, 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. Five **a**-factor-producing clones and one α-factor-producing clone were obtained among about 500 colonies of the UV-treated XF25R8. All of them responded to the appropriate pheromone to arrest cell division and mated with the tester strain of the opposite mating type, X2180-1A (*MAT***a**) or X2180-1B (*MAT*α). Thus, the *dac2* mutation proved to be recessive.

Phenotypic characteristics of *dac2* mutants: The *dac2* mutant was originally isolated from the *MAT***a** strain A364A as an α-factor-resistant mutant, and other *dac2* strains used were constructed by successive backcrosses to a strain derived from a cross of FM2 with DBY746. The *MAT***a** *dac2* strain FM2 showed α-factor resistance; that is, the cells showed no cell division arrest at the G1 phase in response to α-factor, using the frequency of unbudded cells as an indication of G1 arrest (Figure 1). This α-factor resistance of the *MAT***a** *dac2* strain was confirmed by the halo test. *MAT***a** *sst1 dac2* cells were unable to arrest cell division in response to α-factor, and therefore a clear halo zone was not formed (data not shown). Similarly, *MAT*α *dac2* cells were also unable to arrest cell division at the G1 phase even in the presence of high concentration of **a**-factor (data not shown). As might be predicted from their insensitivity to division arrest by mating pheromones, *dac2* strains had a mating defect (Table 2). However, *MAT***a** *dac2* strains underwent morphological changes in response to α-factor (Figure 2). *MAT*α *dac2* strains also became larger and elongated in response to **a**-factor. Thus, the *dac2* mutant

TABLE 2

Mating efficiencies of *dac2* mutants

Strain	Genotype	Mating efficiency (No. of diploids)	
		With *MAT***a** strain	With *MAT*α strain
XF78-39B	*MAT***a** *DAC2*	<1	2.2×10^5
XF78-23B	*MAT***a** *dac2-1*	<1	1.5×10^3
XF78-2A	*MAT*α *DAC2*	1.6×10^5	<1
XF78-24C	*MAT*α *dac2-1*	2.5×10^2	<1

Efficiency of mating was determined at 30° as described in MATERIALS AND METHODS and is expressed as the number of diploids formed in a mixture of 10^7 cells of each parent.

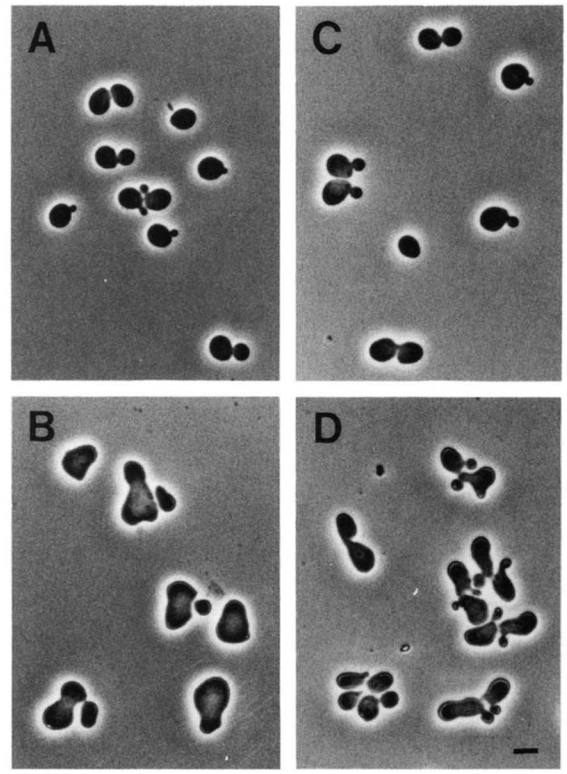

FIGURE 2.—Morphological changes in response to α-factor. Cells were incubated in YEPD medium containing or not α-factor for 4 hr at 30°. (A) A364A (*MAT***a** *DAC2*); (B) A364A (*MAT***a** *DAC2*) with 10 μM α-factor; (C) FM2 (*MAT***a** *dac2-1*); (D) FM2 (*MAT***a** *dac2-1*) with 10 μM α-factor. Bar, 5.0 μm.

is specifically defective in division arrest in response to mating pheromones.

*MAT***a** and *MAT*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 *dac2* mutants inducibly produced **a** and α agglutinins to the extent similar to wild-type strains in the presence of mating pheromones (Table 3). Thus, the *dac2* mutation had no effect on the two pheromone-induced processes, morphological changes and agglutinin induction.

It has been observed previously that mutations in

TABLE 3

Agglutinin induction of *dac2* mutants by mating pheromones

Strain	Genotype	Agglutination intensity[a] −MP	+MP
XF78-39B	*MAT***a** *DAC2*	−	+
XF78-23B	*MAT***a** *dac2-1*	−	+
XF78-23BU	*MAT***a** *dac2-1 gpa1::URA3*	++	++
XF78-2A	*MATα DAC2*	−	+
XF78-24C	*MATα dac2-1*	−	+
XF78-24CU	*MATα dac2-1 gpa1::URA3*	++	++

[a] Agglutination test was carried out as described in MATERIALS AND METHODS. Agglutination intensity was expressed qualitatively as no agglutination (−), mild agglutination (+) or intensive agglutination (++). Cells were untreated (−MP) or treated with mating pheromone (+MP) of the opposite mating type for 2 hr at 30° (0.1 μM α-factor or 1 U of **a**-factor per ml).

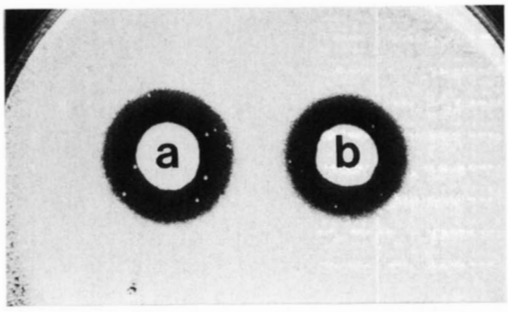

FIGURE 3.—Production of α-factor. Halo test was carried out as described in MATERIALS AND METHODS. Cells of strain RC629 (*MAT***a** *sst1*) were spread onto a YEPD (pH 4.5) plate. (a) XF78-2A (*MATα DAC2*); (b) XF78-24C (*MATα dac2-1*).

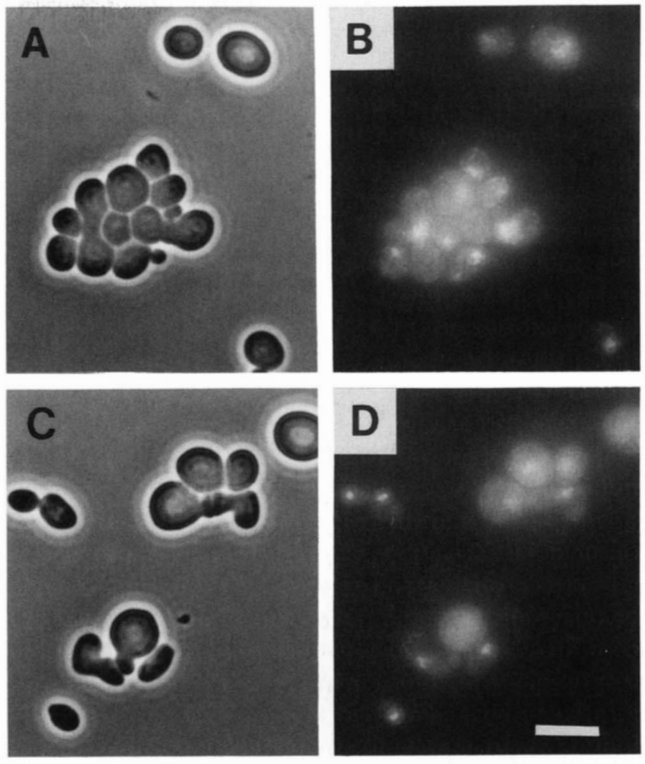

FIGURE 4.—Zygote formation. Cells were allowed to mate for 6 hr in YEPD medium at 30°. Samples were then washed off, fixed in 70% ethanol, and viewed with a Nikon phase-contrast microscope. DAPI staining was performed at the concentration of 0.3 μg/ml. (A, B) *DAC2* (XF78-39B) × *DAC2* (XF78-2A); (C, D) *dac2-1* (XF78-23B) × *DAC2* (XF78-2A). (A, C) Phase-contrast images; (B, D) Fluorescence micrographs of DAPI-stained cells. Bar, 5.0 μm.

five known *STE* genes (*STE4*, *STE5*, *STE7*, *STE11* and *STE12*) lead to an inability of both *MAT***a** and *MATα* strains to respond to mating pheromones and to a reduction of production of two *MAT***a**-specific products, **a**-factor and Barrier protein, and one *MATα*-specific product, α-factor (MACKAY and MANNEY 1974; HARTWELL 1980; HAGEN and SPRAGUE 1984). These phenotypic characteristics suggest that known sterile mutants have a general defect in the expression of *MAT***a**- and *MATα*-specific genes. Therefore, I examined the effect of the *dac2* mutation on the expression of *MAT***a**- and *MATα*-specific genes by the plate halo assay, although the halo assay system is not quantitative for the determination of pheromone production and Barrier activity.

*MAT***a** *dac2* strains produced **a**-factor and Barrier activity at levels comparable to that of wild-type *MAT***a** strains (data not shown). Furthermore, *MATα dac2* strains produced α-factor in a similar amount to wild-type *MATα* strains (Figure 3). Thus, the *dac2* mutation had no significant effect on the constitutive expression of mating-type-specific genes.

Yeast cells must be activated so that they can leave the mitotic cycle and undergo the developmental events required for cell fusion (BUCKING-THROM *et al.* 1973). The synchronization of nuclear cycles of haploid yeast cells by mating pheromones is a prelude to conjugation (HARTWELL 1973). I was interested in the behavior of *dac2* cells in mixed culture with wild-type cells, because *dac2* strains could undergo morphological changes and produce agglutinin in response to mating pheromones. As shown in Figure 4, *dac2* cells formed the prezygotes with wild-type cells (they could not be separated by sonication) but could not complete cell fusion, which presumably accounts for their sterile phenotype. In matings between *DAC2* and *dac2* cells, more than 99% of mating pairs formed such structures in which the mating partners adhered tightly but retained cell wall material that kept them separate. This result leads to a conclusion that division arrest by mating pheromones is essential for cell fusion.

Mapping the *dac2* mutation: Many chromosomal genes are involved in either response to mating pheromones, cell cycle control, cell size control or cell fusion. I decided to map the *DAC2* locus to see if it was different from all previously mapped genes. The *dac2* mutation was initially mapped relative to the centromere marker *trp1*. Asci of strain XF64 were dissected, and complete tetrads that segregated 2:2 for both markers were scored. The data show that the *dac2* mutation mapped to chromosome *II*, 6.1 centi-

TABLE 4

Tetrad analysis of *dac2* mutation

Gene pair[a]	Tetrad types[b] PD	NPD	T	Linkage[c]
dac2-gal1	71	0	22	11.8[d]
trp1-dac2	48	33	12	6.1[e]
trp1-gal1	46	33	14	7.2[e]

[a] Diploid strain XF64 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] Linkage is given incentimorgans (cM).

[d] The distance was calculated by the formula: 100 (T + 6NPD)/2(PD + NPD + T) (PERKINS 1949).

[e] These distances were calculated as one half of the second-division segregation frequency, assuming that the second-division segregation frequency of *trp1* was 55/6060 (MORTIMER and SCHILD 1985).

morgan (cM) from *cen2* and 11.8 cM from *gal1* (Table 4). This position is not coincident with any previously described *ste* gene (see MORTIMER and SCHILD 1985).

Suppression of the *gpa1* mutation by the *dac2* mutation: The *GPA1* (or *SCG1*) gene was shown to have homology to the α subunit of mammalian G proteins, and disruption in this gene leads to haploid-specific growth arrest, apparently by causing a constitutive mating response signal (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987). The order in which two gene products function in a common pathway can often be inferred from the phenotype of the double mutant, providing that the phenotypes of the two single mutants can be distinguished from one another. In fact, the order in which *GPA1* and several *STE* gene products function was inferred. Several sterile mutations (*ste4*, *ste5*, *ste7*, *ste11*, *ste12*, and *ste18*) can suppress the lethality caused by disruption of the *GPA1* gene, indicating that these *STE* gene products should act after *GPA1* (NAKAYAMA *et al.* 1988; WHITEWAY *et al.* 1989; BLINDER, BOUVIER and JENNESS 1989). I have used this approach to determine if the *DAC2* gene product functions after the *GPA1* gene product in the pheromone response pathway.

A linear DNA fragment containing a *URA3* disruption in the *GPA1* gene was introduced into two diploid strains, XF91 and XF92. When a Ura$^+$ transformant of diploid strain XF91 was sporulated and subjected to tetrad analysis, I observed the 2 Ura$^-$ maters: 2 dead spores segregation pattern and could obtain no viable Ura$^+$ (*gpa1*) colonies (Table 5), confirming the observation by others (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987). On the other hand, when a Ura$^+$ transformant of diploid strain XF92 was sporulated and subjected to tetrad analysis, I observed three types of segregation pattern; 2 Ura$^+$ nonmaters: 2 Ura$^-$ maters; 1 Ura$^+$ nonmater: 1 Ura$^-$ nonmater: 1 Ura$^-$ mater: 1 dead spore; 2 Ura$^-$ nonmaters: 2 dead spores. Ura$^+$ (*gpa1*) nonmating haploid spores were recovered at the frequencies expected if *gpa1* is genetically unlinked to *dac2*. No Ura$^+$ (*gpa1*) mating colonies were formed (Table 5). This result indicates that the haploid-specific growth arrest caused by the *gpa1* mutation is completely suppressed by the *dac2* mutation. However, the sterility caused by the *dac2* mutation was not suppressed by the *gpa1* mutation. The *gpa1 dac2* double mutants also had high agglutination ability and showed the morphological alterations even in the absence of mating pheromones (Table 3). These results suggest that the *DAC2* product functions after the *GPA1* product in the pheromone response pathway.

TABLE 5

Suppression of the *gpa1* mutation by *dac2* mutation

Genotype of transformant[a]	Segregation of spore phenotype[b] 4$^+$:0$^-$	3$^+$:1$^-$	2$^+$:2$^-$
*MAT***a** *ura3* *gpa1::URA3* *MAT*α *ura3* +	0	0	22
*MAT***a** *ura3* *dac2-1* *gpa1::URA3* *MAT*α *ura3* + +	8	7	8

[a] Each transformant was constructed by transformation of diploid strains XF91 or XF92 with a linear DNA fragment containing a *URA3* disruption in the *GPA1* gene.

[b] Entries are the number of tetrads showing the viable:dead ratio indicated.

DISCUSSION

I have identified a mutation, *dac2*, that causes **a** and α cells to become resistant to mating pheromones. The *dac2* mutant was selected as a result of the inability of **a** cells to arrest cell division in response to α-factor and was accompanied by the appearance of a sterile phenotype. Since *dac2* mutants could express some pheromone-induced processes (morphological changes and agglutinin induction) when they were exposed to the appropriate pheromone, they seemed to be specifically defective in division arrest induced by mating pheromones.

Many mutants that were unable to arrest cell division in response to mating pheromones (*ste2*, *ste3*, *ste4*, *ste5*, *ste7*, *ste11*, *ste12*, *ste18*, and *dac1*) have been isolated and characterized to dissect the various elements of the pheromone response pathway genetically (MACKAY and MANNEY 1974; MANNEY and WOODS 1976; HARTWELL 1980; WHITEWAY, HOUGAN and THOMAS 1988; FUJIMURA 1989). All of these mutants fail to express any pheromone-induced processes (division arrest, morphological changes, and agglutinin induction) in the presence of mating pheromones; no mutants specifically defective in division arrest have been found so far.

Two lines of evidence suggest that the *dac2* mutation results in a defect in division arrest in response to mating pheromones: the α-factor resistance of the

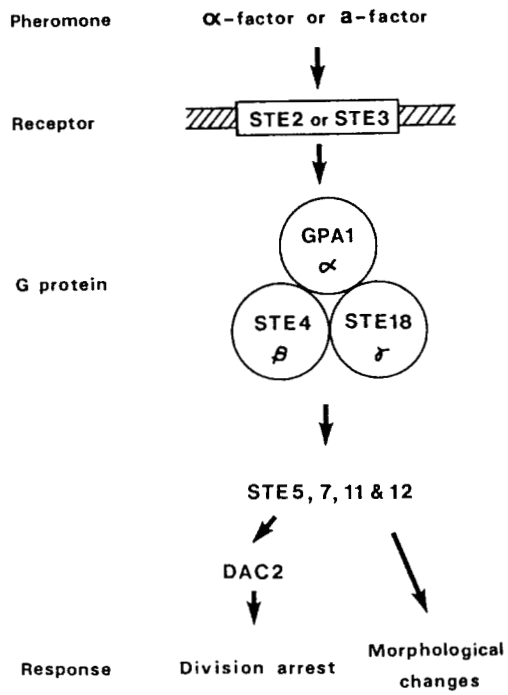

FIGURE 5.—Possible model for division arrest signaling of yeast mating pheromone response. The *STE2* and *STE3* genes encode the receptors for α-factor and *a*-factor; the *GPA1*, *STE4*, and *STE18* genes are thought to be encode Gα, Gβ, and Gγ subunits of a G protein (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987; WHITEWAY *et al.* 1989). The products of the genes *STE5*, *STE7*, *STE11*, and *STE12* are presumed as part of the pheromone response pathway (NAKAYAMA *et al.* 1988; BLINDER, BOUVIER and JENNESS 1989). The signal transduction pathway for division arrest and morphological changes is thought to diverge after the *STE5*, *STE7*, *STE11*, and *STE12* gene products function. The *DAC2* product is presumed to function as a negative signal on cell division and lead to entry into an alternative developmental pathway for mating from mitotic cycle (see text).

mutant (measured by growth of **a** cells in the presence of α-factor and the lack of increase in the frequency of unbudded cells when α-factor was present), and the suppression of the *gpa1* mutation by *dac2*. Thus, the *DAC2* product seems to be involved in the division arrest signaling. Since the *ste5*, *7*, *11*, and *12* mutants are defective in all aspects of the pheromone-induced processes, the *DAC2* product may function after the *STE5*, *STE7*, *STE11*, and *STE12* products in the pheromone response pathway and might be an entity of pheromone-induced arrest signal as illustrated in Figure 5.

On the other hand, the *SIR/MAR* mutants show α-factor resistance because the expression of the normally silent *HML* and *HMR* loci makes the cells have the **a**/α phenotype (RINE and HERSKOWITZ 1987). A mutant that leads to low level expression of the *HML* and *HMR* loci could give the partial defect in mating. In fact, the *ard1* mutant, that was initially interpreted to be defective in pheromone response, later proved to be partially defective for repression of *HMLα* (WHITEWAY *et al.* 1987). However, it seems unlikely that the *dac2* mutation affects the regulation of *HML* and *HMR* loci, because the *dac2* mutants produce amounts of mating pheromones and barrier activity similar to those in the wild-type strains and the *dac2* mutants are normal for agglutinin induction and morphological alterations. However, I have no decisive evidence to rule out this possibility.

Another possibility concerning the *DAC2* gene is that the *DAC2* product may be a normal component of the arrest recovery pathway and therefore the *dac2* mutant may show accelerated recovery from division arrest rather than a defect in division arrest. In this case the *DAC2* product may indirectly regulate the division arrest in the recovery pathway. A definite conclusion on this issue awaits experiments using null alleles of the *DAC2* gene.

Mutation in the *FUS1* or *FUS2* genes blocks the conjugation process at a point after cell contact, preventing cytoplasmic fusion. Since the *fus* mutants normally respond to mating pheromones, they are specifically defective for cell fusion. The *fus1* and *fus2* genes were mapped to chromosomes *III* and *XIII*, respectively (TRUEHEART, BOEKE and FINK 1987). In contrast to the *fus1* and *fus2* mutants, the *dac2* mutants are defective in both division arrest and cell fusion. This behavior of *dac2* mutants suggests that various apparatuses are required to mediate plasmogamy and that the *DAC2* product promotes cell fusion by a mechanism different from the *FUS1* and *FUS2* products. Since the *dac2* mutant shows a definite mating defect compared with *fus1* and *fus2* mutants, the *DAC2* product seems to be more indispensable for cell fusion than the *FUS1* and *FUS2* products.

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