Mating in *Saccharomyces cerevisiae:* The Role of the Pheromone Signal Transduction Pathway in the Chemotropic Response to Pheromone

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ABSTRACT

The mating process in yeast has two distinct aspects. One is the induction and activation of proteins required for cell fusion in response to a pheromone signal; the other is chemotropism, *i.e.*, detection of a pheromone gradient and construction of a fusion site available to the signaling cell. To determine whether components of the signal transduction pathway necessary for transcriptional activation also play a role in chemotropism, we examined strains with null mutations in components of the signal transduction pathway for diploid formation, prezygote formation and the chemotropic process of mating partner discrimination when transcription was induced downstream of the mutation. Cells mutant for components of the mitogen-activated protein (MAP) kinase cascade (*ste5, ste20, ste11, ste7* or *fus3 kss1*) formed diploids at a frequency 1% that of the wild-type control, but formed prezygotes as efficiently as the wild-type control and showed good mating partner discrimination, suggesting that the MAP kinase cascade is not essential for chemotropism. In contrast, cells mutant for the receptor (*ste2*) or the β or γ subunit (*ste4* and *ste18*) of the G protein were extremely defective in both diploid and prezygote formation and discriminated poorly between signaling and nonsignaling mating partners, implying that these components are important for chemotropism.

MANY cells exhibit polarity. In some cases, cells inherit their asymmetry from their progenitor cells. In other cases, asymmetry is induced by surrounding cells or environmental cues. Yeast cells exhibit both classes of polarity. The site of bud emergence on the cell surface is determined by the location of the previous bud site (reviewed in CHANT 1994; HERSKOWITZ *et al.* 1995; PRINGLE *et al.* 1995; DRUBIN and NELSON 1996), while pheromone-induced polarity during mating is determined by external information, a pheromone gradient, and can occur along any axis (reviewed in CHENEVERT 1994). Chemotropism, the ability to reorient cell polarity in response to a gradient, has been demonstrated for individual *MAT***a** cells in α-pheromone gradients (SEGALL 1993; VALTZ *et al.* 1995). In mating mixtures of *MAT***a** cells with equal numbers of *MAT*α partners that produce α-pheromone and mutant *MAT*α cells that do not produce pheromone, the *MAT***a** cells mate almost exclusively with pheromone-producing partners. Thus they exhibit a behavior termed mating partner discrimination (JACKSON and HARTWELL 1990b). In high isotropic concentrations of pheromone where gradients are obscured, cell polarization occurs at the previous bud site, indicating that the polarity determinant for bud formation can be utilized for the mating pathway (MADDEN and SNYDER 1992). Under these conditions, *MAT***a** cells mate randomly with both pheromone-producing and -nonproducing partners via an alternative, default pathway (DORER *et al.* 1995).

A pheromone-response signal transduction pathway is activated during mating. Many of the genes involved in sensing and responding to pheromone have been identified by mutation and molecular analysis (reviewed in KURJAN 1992, 1993; SPRAGUE and THORNER 1992; BARDWELL *et al.* 1994; HERSKOWITZ 1995). *MAT***a** and *MAT*α partners secrete complementary peptide pheromones, **a**- and α-pheromone (KURJAN 1985; MICHAELIS and HERSKOWITZ 1988), that bind to cell surface receptors on the opposite mating type. The pheromone receptors are encoded by *STE2* in *MAT***a** cells (JENNESS *et al.* 1986; BLUMER *et al.* 1988) and *STE3* in *MAT*α cells (NAKAYAMA *et al.* 1985; HAGEN *et al.* 1986). The receptors are members of the seven-transmembrane-segment class (reviewed in DOHLMAN *et al.* 1991) that transmit an external signal by interaction with a guanine-nucleotide-binding protein composed of three subunits: Gα (*GPA1*), Gβ (*STE4*) and Gγ (*STE18*) (WHITEWAY *et al.* 1989; CLARK *et al.* 1993). Binding of pheromone to receptor is postulated to result in exchange of GDP for GTP and dissociation of Gα from G$\beta\gamma$. Null mutations in *GPA1* result in constitutive activation of the signaling pathway implying a negative regulatory role for the Gα subunit (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987; FUJIMARA 1989). The downstream kinase cascade includes a classic mitogen-activated protein kinase

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(MAPK) cascade composed of MEKK-MEK-MAPK components encoded by *STE11* (TEAGUE *et al.* 1986), *STE7* (RHODES *et al.* 1990), and the partially redundant genes *FUS3* and *KSS1* (ELION *et al.* 1991a,b) (reviewed in AMMERER 1994; HERSKOWITZ 1995; LEVIN and ERREDE 1995). Ste5p interacts physically and independently with Ste11p, Ste7p and Fus3p/Kss1p; it is proposed to act as a molecular scaffold to link the kinases with each other (CHOI *et al.* 1994; FERGUSON *et al.* 1994; KRANZ *et al.* 1994; MARCUS *et al.* 1994; PRINTEN and SPRAGUE 1994). Ste5p also associates with Gβ (WHITEWAY *et al.* 1995) although the functional significance of this association is not known.

STE20 encodes a protein kinase (LEBERER *et al.* 1992a,b; RAMER and DAVIS 1993) that appears to act upstream of the MAPK cascade. Ste20p, although it is apparently not in direct association with Ste5p, phosphorylates the MEKK component Ste11p (NEIMAN and HERSKOWITZ 1994; WU *et al.* 1995). Ste11p in turn phosphorylates and activates Ste7p, the MEK component (NEIMAN and HERSKOWITZ 1994), while Ste7p phosphorylates and activates Fus3p and Kss1p, the MAPK components (ERREDE *et al.* 1993). The known substrates of both MAPKs include Ste5p, Ste7p (ZHOU *et al.* 1993; KRANZ *et al.* 1994), and the DNA-binding protein encoded by *STE12* (ELION *et al.* 1993). Ste12p transcriptionally activates many genes involved in mating (DOLAN *et al.* 1989; ERREDE and AMMERER 1989). The MAPK Fus3p also phosphorylates Far1p, a protein required for pheromone-induced G1 arrest (CHANG and HERSKOWITZ 1992; PETER *et al.* 1993; TYERS and FUTCHER 1993; VALDIVIESO *et al.* 1993) and reorientation of polarity (DORER *et al.* 1995). Fus3p also appears to play a role in cell fusion (ELION *et al.* 1990).

It may be that components of the signal transduction cascade are required not only for activation of downstream responses such as transcriptional induction, but are intimately involved in establishment of the fusion site and directional growth in response to pheromone gradients. To examine the possibility that some components of the signal transduction play multiple roles in the mating process, we constructed a series of null mutations for genes in the signal transduction pathway. Because interruption of signal transduction prevents mating, we activated the signaling pathway downstream of the mutation by overexpression of Ste12p, which has been shown to partially suppress the sterility of *ste* mutants (DOLAN and FIELDS 1990). Diploid formation was monitored by the growth of colonies on selective media. Mutants that are unable to form diploids may fuse to form a sonication-resistant structure called a prezygote (TRUEHEART *et al.* 1987). By differentially staining the *MAT***a** and *MAT*α cells with fluorescent dyes before mating (PRINGLE *et al.* 1989), we were able to monitor prezygote formation by signal transduction mutants overexpressing Ste12p.

MATERIALS AND METHODS

Strains and plasmids: The strains used in this study are listed in Table 1. All of the strains are congenic with the strain 381G *MAT***a** *cry1 ade2-1^o his4-580^a lys2^o trp1^a tyr1^o SUP4-3^s* (HARTWELL 1980), with the exception of YEL33-7-3B (LEBERER *et al.* 1992b), which is congenic with W303-1A.

The *ste2-10::LEU2* allele is a deletion of most of the *STE2* gene that is replaced by *LEU2* (BURKHOLDER and HARTWELL 1985; KONOPKA *et al.* 1988). The *gpa1::LEU2* (also called *scg1::LacZ6*) is a null allele in which *GPA1* is disrupted by *LEU2* (DIETZEL and KURJAN 1987; JACKSON *et al.* 1991). The *ste5Δ1::LYS2* allele is a replacement of nearly the entire coding region of *STE5* with *LYS2* (HASSON *et al.* 1994). The *ste11Δ::hisG* allele is a deletion of the *STE11* gene in which an internal *Hin*dIII fragment of the gene is replaced by Salmonella *hisG* repeats (HASSON *et al.* 1994). The *ste7::LEU2* allele encodes disruption of the *STE7* gene with *LEU2* inserted into a *Bgl*II site (HASSON *et al.* 1994). The *ste12::LEU2* allele is a replacement of most of the coding region of *STE12* by *LEU2*. The *fus3Δ::LEU2* (also called *fus3-6::LEU2*) (ELION *et al.* 1990) and *kss1Δ::URA3* (COURCHESNE *et al.* 1989) alleles represent deletions of these genes with insertions of *LEU2* and *URA3*, respectively. The *fus3Δ::LEU2 kss1Δ::ura3^{FOA}* double mutant strain was obtained by selection on 5-fluoroorotic acid (BOEKE *et al.* 1984, 1987). The YEL33-7-3B strain contains the *ste20::TRP1* allele, a *STE20* C-terminal truncation allele in which the open reading frame is disrupted upstream of the putative protein kinase domain (LEBERER *et al.* 1992b).

The *ste4::LEU2* null allele was constructed by inserting *Pst*I-*Xba*I fragment carrying *LEU2* from plasmid pDJ154 into the internal *Bgl*II site of *STE4* (HASSON *et al.* 1994). The *ste18::LEU2* null allele was constructed by inserting a *Hin*dIII-*Pst*I fragment containing *LEU2* from plasmid M68p5 into the *Nsi*I site of *STE18* (WHITEWAY *et al.* 1989) (pDJ154 and M68p5 plasmids were gifts of MALCOLM WHITEWAY). The *ste20Δ::TRP1* allele was constructed by transformation with the *Kpn*I fragment of the pDH104 plasmid (a gift from DOREEN HARCUS) that contains a complete deletion of the *STE20* open reading frame. The *mfα1::URA3D* and *mfα2::LEU2C* plasmids (KURJAN 1985; CAPLAN and KURJAN 1991) that were used for construction of the *mfα1::URA3 mfα2::LEU2* double mutant strains were kindly provided by JANET KURJAN. 8907-4-1b *ura3^{FOA}* (*mfα1::ura3^{FOA} mfα2::LEU2*) was obtained by selection on 5-fluoroorotic acid (BOEKE *et al.* 1984, 1987). P_{GAL1}-*STE12* (also known as pNC252; B. ERREDE, personal communication), a 2μ plasmid containing the *STE12* gene under control of the *GAL1,10* promoter, was a gift from REBECCA CADE.

Media and transformation: Liquid cultures were grown in synthetic complete medium with 2% raffinose or 2% glucose as the carbon source (SHERMAN *et al.* 1981). Strains containing a *URA3* plasmid were grown in synthetic medium lacking uracil. Selective plates contained synthetic medium with 2% agar (Difco Laboratories). The Nobel Agar plates used for the mating assays contained synthetic complete medium with 2% Noble Agar (Sigma). Genetic strain constructions were performed using standard methods (MORTIMER and HAWTHORNE 1969). Lithium acetate transformation was used to introduce plasmids into yeast and to make chromosomal gene replacements (SCHIESTL and GIETZ 1989). All other manipulations were performed using standard molecular methods (SAMBROOK *et al.* 1989).

Mating assays: To measure diploid production of a given *MAT***a** strain with a wild-type partner *MAT*α strain, a quantitative mating assay was used (REID and HARTWELL 1977). We found that to achieve consistent results, fresh stocks had to be prepared from frozen cultures for each experiment. Our protocol was as follows: Cells from frozen stocks were streaked on synthetic plates containing 2% glucose and incubated for

TABLE 1

Strain list

Strain	Genotype	Source or reference
7646-11-1a	*MAT***a** *cry1 lys2 trp1 tyr1 leu2 ura3 cyh2 SUP4 gpa1::LEU2 ste5-3*[a]	JACKSON *et al.* (1991)
7646-19-1a	*MAT***a** *cry1 lys2 trp1 tyr1 leu2 ura3 cyh2 SUP4 ste5-3*[a]	CATHERINE JACKSON
7681-4-1a	*MAT***a** *cry1 lys2 trp1 tyr1 leu2 ura3 cyh2 SUP4*[a]	CATHERINE JACKSON
8906-1-4b	*MATα cry1 ade6 leu2 lys2 trp1 can1 ura3 SUP4*[a]	This study
8907-4-1b *ura3*^{FOA}	*MATα cry1 ade6 his4 leu2 tyr1 ura3 can1 cyh2 SUP4 mfb1::ura3*^{FOA} *mfb2::LEU2*[a]	This study
8942-9-1a	*MAT***a** *cry1 ade6 his4 tyr1 trp1 leu2 ura3 can1 cyh2 SUP4 ste4::LEU2*[a]	This study
8943-32-3a	*MAT***a** *cry1 ade6 his4 tyr1 trp1 leu2 ura3 can1 SUP4 ste18::LEU2*[a]	This study
8960-7-2a	*MAT***a** *cry1 his4 trp1 leu2 lys2 ura3 tyr1 cyh2 SUP4*[a]	This study
8960-21-4a	*MAT***a** *cry1 ade2 his4 leu2 lys2 ura3 trp1 cyh2 SUP4 ste5Δ1::LYS2*[a]	This study
DJ1003A	*MAT***a** *cry1 ade2 ade3 his4 leu2 trp1 ura3 SUP4 ste11Δ::hisG*[a]	HASSON *et al.* (1994)
DJ1023A	*MAT***a** *cry1 ade2 ade3 his4 leu2 trp1 lys2 ura3 SUP4 ste7::LEU2*[a]	HASSON *et al.* (1994)
DJ213-6-3a	*MAT***a** *cry1 ade2 ade3 his4 leu2 trp1 lys2 ura3 SUP4 ste2-10::LEU2*[a]	KONOPKA *et al.* (1988)
12414-tx4a	*MAT***a** *cry1 ade2 his4 lys2 trp1 tyr1 leu2 ura3 SUP4 ste12::LEU2*[a]	This study
W303-1A	*MAT***a** *ade2-1 his3-1,15 trp1-1 leu2-3 ura3-1 can1*	LEBERER *et al.* (1992b)
YEL33-7-3B	*MAT***a** *ade2-1 his3-1,15 trp1-1 leu2-3 ura3-1 can1 ste20::TRP1*	LEBERER *et al.* (1992b)

[a] The alleles in the 381G strains are *ade2-1*[o]; *his3-1,15*; *his4-580*[a] *leu2-3,112*; *lys2*[o]; *SUP4-3*[ts]; *trp1*[a]; *tyr1*[o]; and *ura3-52*.

2 days at 30°. Colonies from this plate were inoculated into synthetic medium containing 2% raffinose and incubated 2–3 days at 30°. These stocks were used to inoculate cultures that were grown overnight at 23° to midlog phase (2×10^6 to 1×10^7). For experiments in which *MAT***a** cells contained P_{GAL1}-*STE12*, concentration of both *MAT***a** and *MATα* cells was adjusted to 2×10^6, galactose was added to a final concentration of 2% and cells were incubated at 23° for 2 hr before staining. For experiments involving *gpa1 ste5-3*, all strains were grown in complete synthetic medium at 34°, adjusted to 2×10^6 cells per ml, and incubated at 23° for 2 hr before mating. *MAT***a** and *MATα* cells were mixed together in 8 ml of medium containing raffinose and galactose and filtered onto 25-mm filters (0.45 μm pore size; Millipore Corporation). The fraction of cells of each mating type was 0.5 and the total number of cells on each filter was 1×10^7. The filters were incubated on synthetic complete medium containing 2% raffinose and 2% galactose and 2% Noble Agar. To stop the mating reaction, the cells from the filter were resuspended in -N liquid medium. The number of diploids formed between *MAT***a** and *MATα* cells was determined by selecting prototrophic colonies on appropriate plates. The numbers of haploid input cells of each mating type were determined by plating each haploid culture on complete medium. The percentage diploids was calculated using the following formula: diploids per input *MAT***a** cells multiplied by 100%.

The discrimination assay measures the mating efficiency of the responder strain with two mating partners in the same mating mixture (JACKSON and HARTWELL 1990b; JACKSON *et al.* 1991). Discrimination assays were performed as described above except that *MAT***a** cells were mixed with equal numbers of wild-type pheromone-producing cells and *mfα1 mfα2* pheromone-nonproducing cells. The haploid strains carried auxotrophic markers so that diploid formation of a *MAT***a** cell with either type of *MATα* cell could be selected. The fraction of diploids formed with the nonsignaling partner divided by the fraction of nonsignaling partners in the mating mix is the randomness index (RI). RI = 1 indicates that the responder cells do not discriminate between the two types of *MATα* cells, while RI = 0 indicates perfect discrimination where all responder cells choose signaling partners. Equal numbers of all three types of cells were used (3.3×10^6) so that the total number of cells in the mating mixture was 1×10^7.

Although the cells were grown under selection, 40–60% of the viable cells had lost the P_{GAL1}-*STE12* plasmid. For this reason, we used a *ste12Δ* strain as the wild-type control rather than a *STE+* strain that could form diploids in the absence of the plasmid. We calculated mating efficiency based on input *MAT***a** cells that contained the plasmid for *STE+* and all mutant strains and counted only diploids that contained the plasmid.

Mating conditions: All of the mating reactions were performed at 23° on synthetic medium supplemented with amino acids, adenine, and uracil. Matings utilizing galactose induction were performed on Nobel Agar plates containing a mixture of 2% raffinose plus 2% galactose for 9 hr. Since the strains of the 381G (HARTWELL 1980) background are unable to metabolize galactose, raffinose was added as the carbon source. Raffinose does not interfere with galactose induction. Matings of *gpa1* mutants were performed on Nobel Agar plates containing 2% glucose incubated for 4.5 hr at 23°. For confusion assays, α-pheromone was added to the medium to a final concentration of 30 μM (DORER *et al.* 1995).

Cell staining: *MAT***a** cells were stained with tetramethylrhodamine 5(and 6)-isothiocyanate (TRITC) and *MATα* cells with fluorescein 5-isothiocyanate (FITC) conjugated to the lectin Concanavalin A (Sigma) (PRINGLE *et al.* 1989). After induction, cells were adjusted to 1×10^7 cells per ml in medium containing raffinose and galactose. ConA-TRITC was added to a final concentration of 0.05 mg/ml or ConA-FITC to a final concentration of 0.1 mg/ml. Cells were incubated 15 min at 30°, centrifuged and washed twice with the same medium to remove unbound lectin.

RESULTS

Experimental rationale: In this report, we examine whether genes that are required for the pheromone signal transduction pathway also play a role in mating efficiency and mating partner discrimination. Figure 1 shows the components of the signal transduction pathway in the order in which they are thought to act (reviewed in CHANT 1994; HERSKOWITZ *et al.* 1995; PRINGLE *et al.* 1995; DRUBIN and NELSON 1996). Since the assays used in the work depend upon mating, components of the signal transduction pathway had to be eliminated

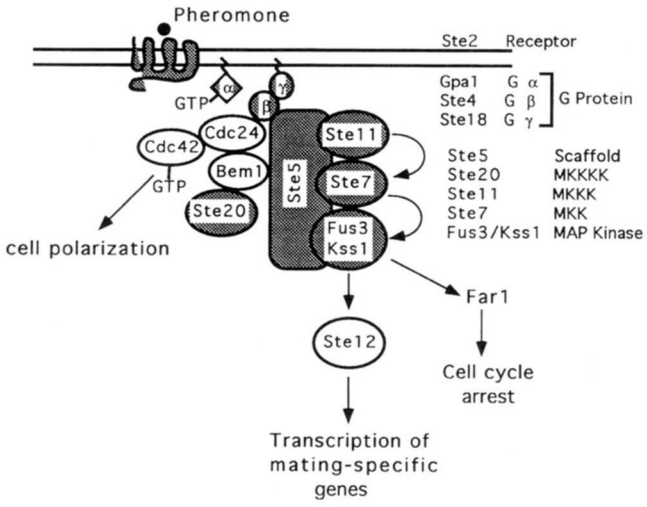

FIGURE 1.—Model of the yeast pheromone signal transduction pathway, showing postulated protein-protein interactions (ELION *et al.* 1993; ERREDE *et al.* 1993; ZIMAN *et al.* 1993; NEIMAN and HERSKOWITZ 1994; LEEUW *et al.* 1995; SIMON *et al.* 1995; WHITEWAY *et al.* 1995; WU *et al.* 1995; ZHAO *et al.* 1995; LYONS *et al.* 1996). The arrows indicate activation steps. The proteins considered in this study are indicated by shading.

without completely eliminating mating itself. To this end, we used *MAT***a** strains with null mutations in various components of the pheromone response pathway transformed with a multicopy plasmid, P_{GAL1}-*STE12,* in which the wild-type *STE12* gene was under the control of the *GAL1* promoter (DOLAN and FIELDS 1990). Ste12p is a downstream target of the kinase cascade that transcriptionally activates mating-specific genes (DOLAN *et al.* 1989; ERREDE and AMMERER 1989). We examined the ability of mutant *MAT***a** strains to form diploids and prezygotes with pheromone-producing and nonproducing *MATα* cells when Ste12p expression was induced by galactose. The ability of mutant cells to respond to pheromone gradients was measured in mating assays in which the *MAT***a** cell was presented with pheromone-producing and pheromone-nonproducing *MATα* cells (discrimination assay). Mating partner discrimination is quantified by calculating the randomness index, RI, the fraction of diploids formed with the nonsignaling partner divided by the fraction of nonsignaling partners in the mating mix (see MATERIALS AND METHODS). In addition to diploid formation, we examined prezygote formation in discrimination assays where only the signaling or the nonsignaling *MATα* cell was fluorescently labeled. In this way we were able to determine whether discrimination at the level of prezygote formation played a role in overall mating partner discrimination. The *ste12Δ* strain containing P_{GAL1}-*STE12* is the appropriate control for the other null mutants and we refer to it as the wild-type control. Overexpression of Ste12p was slightly deleterious to diploid formation, reducing mating efficiency of the *STE+* strain about fourfold (Table 2). The RI was not affected by overexpression of Ste12p.

Components of the signal transduction pathway fall into two classes with regard to mating efficiency and mating partner discrimination: The mating efficiency and mating partner discrimination of the *ste12Δ* strain is similar to a *STE+* strain containing the P_{GAL1}-*STE12* plasmid. The mutants other than *ste12* fell into two classes with respect to their mating efficiency (Table 2). One class, consisting of cells lacking the receptor (*ste2*) or the Gβ or Gγ subunit of the heterotrimeric G protein (*ste4, ste18*), was extremely defective, forming diploids with an efficiency of only 0.002–0.006%. The other class, consisting of cells lacking components of the kinase cascade (*ste5, ste20, ste11, ste7* and *fus3 kss1*), was less defective, mating with an efficiency of ~0.5%. (The MAP kinase genes *FUS3* and *KSS1* are partially redundant in the 381G strain background used here: only the double mutant was examined.) Both classes were defective compared to the wild-type control that formed diploids with an efficiency of 30%.

The two classes of mutants defined by mating efficiency also differed in their mating partner discrimination. A strain lacking receptors (*ste2*) was found to mate randomly (RI = 0.95) in the mating partner discrimination assay. This result is consistent with the previous observation that the *STE2* gene is required for mating partner discrimination in *MAT***a** cells that are constitutively activated through a *gpa1* null mutation (JACKSON *et al.* 1991). Mutations in the β and γ subunits of the G protein (*ste4, ste18*) also result in significant defects in mating partner discrimination (RIs = 0.31 and 0.25) but are less defective than the receptorless mutant, *ste2.* We conclude that the receptor, or some other unidentified components, retains some ability to mediate mating partner discrimination in the absence of the known G protein subunits.

Although the mating efficiency of *ste5, ste20, ste11, ste7* and *fus3 kss1* null mutants are reduced ~60-fold in comparison to wild-type, cells with these mutations discriminate proficiently (RIs $\leq 5.0 \times 10^{-3}$) in the mating partner discrimination assay. Therefore, none of the known components of the MAP kinase cascade that act downstream of the G protein and upstream of the *STE12* transcription factor appears to be required for mating partner discrimination in this experimental regime. However, it should be noted that while cells mutant for components of the kinase cascade mate with the nonsignaling *MATα* cells only on the order of one time in 1000 matings, this is 100-fold less accurate than the wild-type control (RI $\leq 4.6 \times 10^{-5}$).

Prezygote formation by cells null for components of the pheromone response pathway when mating is induced by Ste12p overproduction: The results above suggest that the receptor and the G$\beta\gamma$ subunits of the G protein (*STE2, STE4* and *STE18*) play a more important role in the efficiency with which cells locate a signaling partner than do the components of the linked MAP kinase cascade (*STE5, STE20, STE11, STE7* and

TABLE 2

Sterile mutants fall into two groups with regard to mating efficiency and mating partner discrimination when mating is induced by overexpression of Ste12p

Responder *MAT***a** strain[a]	Plasmid	Mating efficiency[b]	Randomness index[c]
STE+	pP_{GAL1}-*STE12*	44 ± 15	$\leq 3.2 \times 10^{-5} \pm 2.3 \times 10^{-5}$
STE+	Vector	162 ± 36	$\leq 6.2 \times 10^{-6} \pm 3.5 \times 10^{-6}$
ste12	pP_{GAL1}-*STE12*	30 ± 5	$\leq 4.6 \times 10^{-5} \pm 0.7 \times 10^{-5}$
ste12	Vector	$<5.1 \times 10^{-5} \pm 0.7 \times 10^{-5}$	Not calculated
ste2	pP_{GAL1}-*STE12*	0.0024 ± 0.0023	0.95 ± 0.11
ste4	pP_{GAL1}-*STE12*	0.0036 ± 0.0023	0.31 ± 0.04
ste18	pP_{GAL1}-*STE12*	0.0062 ± 0.0025	0.25 ± 0.05
ste5	pP_{GAL1}-*STE12*	0.50 ± 0.02	<0.0019
ste20	pP_{GAL1}-*STE12*	0.53 ± 0.26	<0.0002
ste11	pP_{GAL1}-*STE12*	0.32 ± 0.02	≤0.0045 ± 0.0007
ste7	pP_{GAL1}-*STE12*	0.49 ± 0.30	≤0.0014 ± 0.0007
fus3 kss1	pP_{GAL1}-*STE12*	0.61 ± 0.17	≤0.0016 ± 0.0009

[a] Strains used were 8960-7-2a (*STE+*), 12414-tx4a (*ste12*), DJ213-6-3 (*ste2*), 8942-9-1a (*ste4*), 8943-32-3a (*ste18*), 8960-21-4a (*ste5*), YEL33-7-3B (*ste20*), DJ1003A (*ste11*), DJ1023A (*ste7*), and DJ1021-9-1a (*fus3 kss1*). All *MAT***a** strains were transformed with pP_{GAL1}-*STE12* (pNC252), a multicopy 2μ plasmid carrying *STE12* under control of the *GAL1,10* promoter. The wild-type *MAT*α strain was 8906-1-4b. The pheromoneless *MAT*α strain was 8907-4-1b *ura3*^{FOA}. Numbers are averages of three to seven repetitions.

[b] Mating efficiency is the number of diploids formed with either a wild-type or pheromoneless *MAT*α cell divided by the number of plasmid-containing *MAT***a** present in the mating mixture at the beginning of the experiment multiplied by 100.

[c] Randomness index (RI) is calculated by dividing the fraction of diploids formed between the responder *MAT***a** cell and the pheromoneless *MAT*α cells by the fraction of pheromoneless *MAT*α cells in the mating mixture.

FUS3/KSS1). However, because the mating efficiencies of all the mutants were so low compared to *STE+* or the wild-type control in these experiments, we were concerned about the validity of the conclusions. Suspecting that the sterile mutants might be able to carry out the early stages of mating more effectively than diploid formation, we examined the ability of the mutant cells to form prezygotes, cells that have formed a sonication-resistant wall fusion but have not completed cytoplasmic fusion (TRUEHEART *et al.* 1987). To identify prezygotes unambiguously, we stained the cell walls of the parental cells with fluorescent dyes conjugated to the lectin Concanavalin-A before mating (PRINGLE *et al.* 1989). Mutant *MAT***a** cells were stained with ConA-TRITC, a red dye; the *MAT*α cells with ConA-FITC, a green dye.

Like diploid formation, prezygote formation by the mutants was dependent on Ste12p overexpression. Less than 0.2% prezygotes or zygotes were formed by *ste5, fus3 kss1* or *ste12* cells containing a control vector (0 out of 500, data not shown) compared to 12–17% when they contained P_{GAL1}-*STE12*. The *ste12* wild-type control that contained P_{GAL1}-*STE12* was proficient at forming prezygotes and converting prezygotes to diploids. All of the other *ste* mutant strains formed more prezygotes than diploids with wild-type *MAT*α cells when mating was induced by overexpression of Ste12p (Table 3). Mutants of the phosphorylation cascade (*ste5, ste11, ste7, fus3 kss1* and *ste20*) formed prezygotes at levels nearly equal to the *ste12*Δ wild-type control (8–13% compared to 17%). Prezygote formation was more defective in receptor and G protein mutants (*ste2, ste4* and *ste18*) than in kinase mutants (*ste5, ste11, ste7, fus3 kss1* and *ste20*). *ste2, ste4* and *ste18*, mutants that showed the poorest mating efficiency and mating partner discrimination, were also the most defective in forming prezygotes, exhibiting a frequency of ~1–2% prezygotes.

Mutants also fell into two classes with respect to their ability to convert prezygotes into zygotes. For *ste2, ste4* and *ste18*, the efficiency of completing diploid formation from the prezygote is less than one in 100 (Table 3). For the mutants of the MAP kinase cascade, *ste5, ste20, ste11, ste7* and *fus3/kss1*, about one in 20–50 prezygotes give rise to a diploid colony.

The morphology of the prezygotes formed by the *ste* mutants was unusual compared to zygotes formed by *STE+* cells (Figure 2). Mutant *MAT***a** cells displayed no visible projection and therefore appeared not to undergo directional growth; *MAT*α cells typically had long unstained projections toward the *MAT***a** partner. The nucleus of the mutant *MAT***a** cells remained round and centrally located, in contrast to the *MAT*α nucleus that appeared elongated and had moved into the projection. Prezygotes could be distinguished from incidental contact by disappearance of the red-stained *MAT***a** cell wall at the point of contact. The morphology of the zygotes formed by the *ste12*Δ strain was heterogeneous, with many zygotes indistinguishable from those

TABLE 3

Mating of sterile mutants induced by overexpression of Ste12p results in far more prezygotes than viable diploids

Responder *MAT***a** strain[a]	Mating efficiency (%)[b]	Prezygotes per *MAT***a**[b]	Efficiency of diploid formation[c]
STE+	17.4	62/200 (31)	0.44[d]
ste12 (control)	21.2	33/200 (16.5)	1.0
ste2	<0.003	7/907 (0.8)	<0.003
ste4	<0.001	18/918 (2.0)	<0.000
ste18	0.01	24/924 (2.6)	0.003
ste5	0.19	118/1000 (11.8)	0.012
ste11	0.15	127/1000 (12.7)	0.009
ste7	0.09	55/500 (11.0)	0.006
fus3 kss1	0.32	59/500 (11.8)	0.021
ste20	1.3	42/500 (8.4)	0.12

[a] Strains used were 8960-7-2a (*STE+*), DJ213-6-3 (*ste2*), 8942-9-1a (*ste4*), 8943-32-3a (*ste18*), 8960-21-4a (*ste5*), DJ1003A (*ste11*), DJ1023A (*ste7*), DJ1021-9-1a (*fus3 kss1*), and 12414-tx4a (*ste12*) in the 381G background and YEL33-7-3B (*ste20*) in the W303 background. All *MAT***a** strains were transformed with pP_{GAL1}-*STE12* (pNC252), a multicopy 2μ plasmid carrying *STE12* under control of the *GAL1,10* promoter. The *MAT*α mating partner was 8906-1-4b.

[b] Mating efficiency is the number of diploids formed divided by the number of plasmid-containing *MAT***a** present in the mating mixture at the beginning of the experiment; values in parentheses are percentages. Growth and mating were performed as described in Table 2, except that only pheromone-producing *MAT*α cells were present and 5×10^6 of each cell type were mixed and cells were stained with concanavalin A-TRITC (*MAT***a**) or concanavalin A-FITC (*MAT*α) prior to mixing. Some mutations gave rise to triple fusions of one *MAT***a** cell to two *MAT*α cells. These were counted as two fusion events in the fourth column.

[c] Mating efficiency divided by percentage of prezygotes. All values are normalized to the *ste12* control.

[d] Only diploids that contained the plasmid carrying P_{GAL1}-*STE12* were measured. The ratio of diploids to prezygotes for the *STE+* strain reflects the fact that cells that have lost the plasmid continue to be able to form zygotes.

formed by the *STE+* strain but others resembling the prezygotes formed by the other *ste* mutants.

Mating partner discrimination at the level of prezygote formation when mating is induced by Ste12p overexpression: In the prezygotes described above, the *MAT*α partner cells exhibit long projections toward the *MAT***a** cells and therefore appear to be the more active participants in choosing a mate. The mutant *MAT***a** cells, showing no visible morphological changes, seem to play a passive role, posing the possibility that they do not distinguish between partners at the level of prezygote formation. Thus the differences in mating partner discrimination (Table 2) observed for the different sterile mutants might lie at the level of resolution of prezygotes into diploids. To test this idea, we measured discrimination by prezygote formation rather than diploid formation. For each mutant we carried out two parallel discrimination assays. In one mating mixture, the pheromone-producer was stained; in the parallel mating mixture, the nonproducer was stained. After mating, 50 to 200 *MAT***a** cells involved in fusions were scored as to whether they had fused with stained or unstained *MAT*α partners (Table 4). For all strains, the total zygote formation was similar whether the pheromone-producer or -nonproducer was stained, implying that staining itself did not have a strong effect on the efficiency of zygote formation. Matings with unstained cells could represent either fusions with the unstained partner or with *MAT*α cells that had arisen by budding after the cells were stained and were indeterminate for mating partner discrimination. The *STE+* and wild-type control formed zygotes with the pheromone-nonproducing partner at a frequency of <1% (RI = 0.01 for *STE+* and 0.006 for the wild-type control). The RI for zygote formation was higher than for diploid formation (Table 2, RIs on the order of $\leq 5 \times 10^{-5}$). This implies that pheromone producing cells have an advantage in converting prezygotes to diploids. Cells mutant for the pheromone receptor Ste2p formed prezygotes with stained cells in about the same proportion whether the stained cells in the mating mixture were pheromone-producers (36%) or nonproducers (55%). Cells mutant for *STE4*, the Gβ of the G protein, displayed little or no preference for the pheromone-producing partner (66% with stained nonproducer *vs.* 37% with stained producer). Cells mutant for *STE18*, the Gγ subunit, displayed a weak preference for pheromone-producing partners (78% *vs.* 24% with the stained nonproducer).

Like *STE+* and *ste12*Δ, cells mutant for components of the MAP kinase cascade, *ste5, ste20, ste11, ste7* and *fus3 kss1*, showed strong preferences for prezygote formation with pheromone-producing partners (88–96%) (Table 4). However, these mutants formed prezygotes with stained pheromone-nonproducing partners at a slightly higher frequency than the *STE+* or wild-type control (2–8% compared to <1%). This is 10–100 times more frequently than they formed diploids with nonsignaling cells (Table 2). Therefore, mutation of

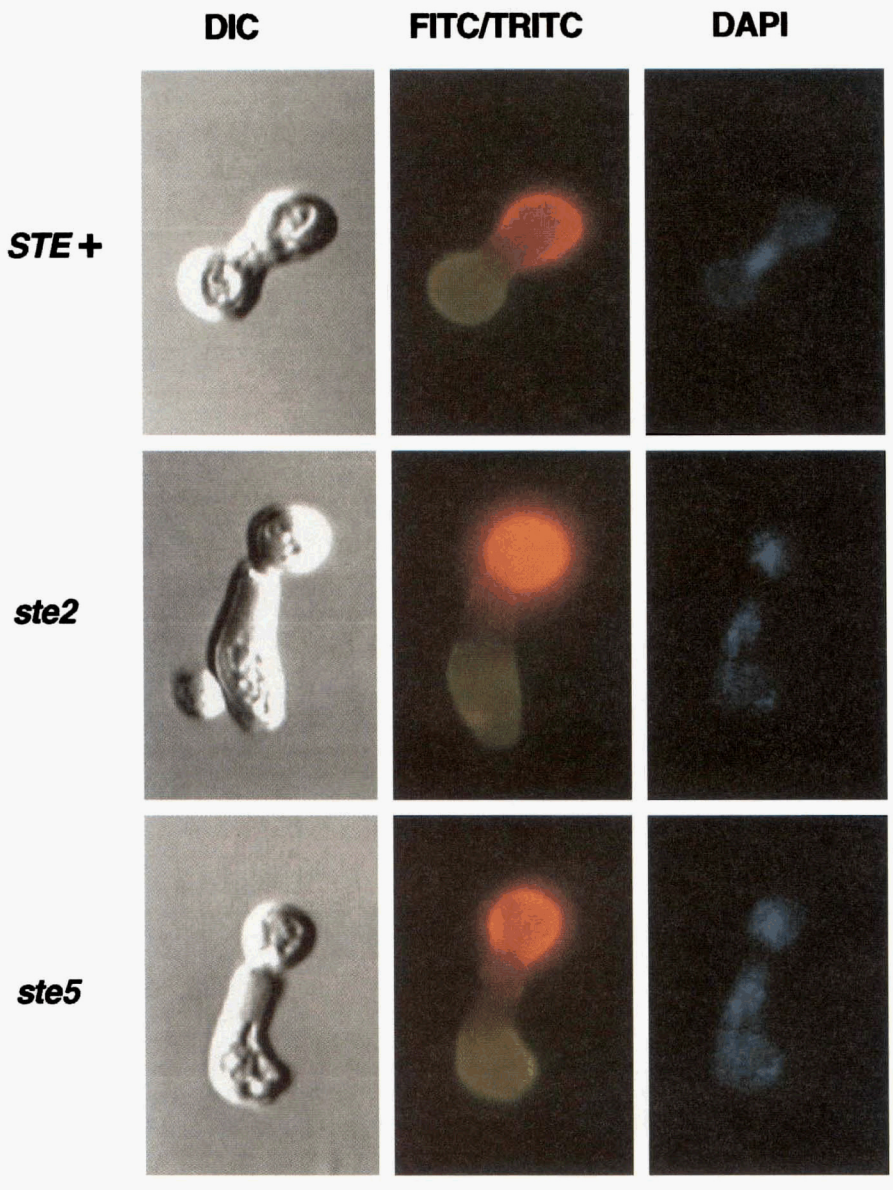

FIGURE 2.—A zygote formed by a *STE*+ cell (top row) and prezygotes formed by *ste2* (middle row) and *ste5* (bottom row) *MAT***a** cells with wild-type *MATα* cells under conditions of Ste12p overexpression. The *MAT***a** cells were stained with TRITC conjugated to the lectin Concanavalin A before mating. The *MATα* cells were stained by FITC conjugated to Concanavalin A before mating. DAPI was included in the mounting medium of the slides. The middle column is a double exposure through red and green filters.

components of the kinase cascade does not abolish the ability of cells to discriminate between pheromone-producing and -nonproducing cells at the level of prezygote formation, but as with wild-type cells discrimination at the level of prezygote formation does not account for all the discrimination in diploid formation. Just as for *STE*+ and *ste12*Δ, some aspect of mating partner discrimination in the kinase mutants must occur after prezygote formation. The fact that the mutants of the kinase cascade form prezygotes with pheromone nonproducing cells at a frequency greater than wild type may contribute to their relative defect in mating partner discrimination compared to wild-type cells (Table 2). The G protein mutants *ste4* and *ste18* also showed a slightly higher level of mating partner discrimination (RIs = 0.31 and 0.25) than observed for prezygote formation (RI = 0.7 and 0.5); it may be that for *ste4* and *ste18* the pheromone-producing partner is better able to complete cell fusion and/or karyogamy after the prezygote has formed. Only the *ste2* mutant seemed completely defective in mating partner discrimination both at the level of prezygote formation and diploid formation.

The confusion assay as a test for chemotropism: *ste4* mutants are defective in chemotropism while *ste5* cells are not: *STE*+ cells mate by a chemotropic response to the pheromone gradient produced by the mating partner. This chemotropic response of the *MAT***a** cells

TABLE 4

Mating partner discrimination for prezygote formation

Responder *MAT***a** strain[a]	Percentage prezygotes[b]	Stained *MATα*[c]	Prezygotes with stained *MATα*[b]
STE+	32	MFB	391/450 (87)
STE+	31	mfb	3/450 (0.7) (RI = 0.01)
			653/700 (93)
ste12	8.9	MFB	2/700 (0.3) (RI =
ste12	9.8	mfb	0.006)
ste2	0.8	MFB	29/80 (36)
ste2	0.2	mfb	46/80 (55) (RI = 1.1)
ste4	0.9	MFB	65/99 (66)
ste4	1.2	mfb	31/83 (37) (RI = 0.7)
ste18	1.4	MFB	39/50 (78)
ste18	2.9	mfb	12/50 (24) (RI = 0.5)
ste20	6.6	MFB	45/51 (88)
ste20	4.8	mfb	4/50 (8) (RI = 0.16)
ste5	12	MFB	241/250 (96)
ste5	10	mfb	7/407 (1.7) (RI = 0.03)
ste7	12	MFB	45/50 (90)
ste7	11	mfb	1/50 (2) (RI = 0.04)
ste11	18	MFB	48/50 (96)
ste11	13	mfb	2/50 (4) (RI = 0.08)
fus3 kss1	7.2	MFB	137/150 (91)
fus3 kss1	6.9	mfb	13/450 (3) (RI = 0.06)

[a] Strains used were 8960-7-2a (*STE+*), DJ213-6-3 (*ste2*), 8942-9-1a (*ste4*), 8943-32-3a (*ste18*), 8960-21-4a (*ste5*), YEL33-7-3B (*ste20*), DJ1003A (*ste11*), DJ1023A (*ste7*), and DJ1021-9-1a (*fus3 kss1*). All *MAT***a** strains were transformed with P_{GAL1}-*STE12* (pNC252), a multicopy 2μ plasmid carrying *STE12* under control of the *GAL1,10* promoter. The wild-type *MATα* strain was 8906-1-4b. The pheromoneless *MATα* strain was 8907-4-1b *ura3*^{FOA}.

[b] Stained *MAT***a** cells were scored for apparent fusion, based on disappearance of the cell wall stain at the point of contact. Fusions were scored as to whether the *MATα* partner was stained or unstained. Numbers are a summation of all experiments; 50 to 200 prezygotes were scored per mating assay. The percentage prezygotes in parentheses is the average of all mating assays.

[c] Cells were prepared as described in Tables 2 and 3, except that the *MATα* cultures were divided and only one aliquot was stained, while the other aliquot was treated in parallel with buffer lacking dye. Two mating mixtures were prepared for each *MAT***a** strain, each with 3.3×10^6 stained *MAT***a** cells. One contained 3.3×10^6 stained pheromone-producing *MATα* cells (MFB) and 3.3×10^6 unstained pheromoneless *MATα* cells. The other contained 3.3×10^6 unstained pheromone-producing *MATα* cells and 3.3×10^6 stained pheromoneless *MATα* (mfb) cells.

can be prevented by adding sufficient exogenous α mating pheromone to saturate its receptors. Under these conditions, *STE+ MAT***a** cells mate at ~10-fold reduced efficiency and fail to discriminate pheromone-producing from -nonproducing *MATα* partners, mating by an alternative pathway called the "default" pathway (DORER *et al.* 1995).

Prezygote formation by the *ste5* mutant containing the high copy *STE12* plasmid decreased by 20–30-fold with addition of α-pheromone (Table 5). Moreover, the mating partner discrimination of *ste5* cells was abolished with added α-pheromone and prezygotes formed randomly with pheromone-producing and -nonproducing partners. These responses are similar to those of a *STE+ MAT***a** cell and the "wild-type" control (*ste12*Δ containing P_{GAL1}-*STE12*), consistent with the hypothesis that *ste5* cells in the absence of added α-pheromone are proficient in carrying out a chemotropic response. In contrast, *ste4* cells overexpressing Ste12p exhibited low prezygote formation that was not significantly decreased by saturating levels of α-pheromone. In the presence and absence of added α pheromone, *ste4* mutant cells failed to discriminate pheromone-producing from -nonproducing *MATα* cells. We conclude that the prezygotes formed by *ste4* cells do not arise through a chemotropic response. Our results imply that Gβ is required for chemotropism but that *ste5,* the scaffold of the MAP kinase cascade, is not required.

The *MAT***a** *ste12*Δ cells from the mating reactions done in the presence of α-pheromone did not form narrow projections typical of cells in saturating pheromone (reviewed in CHEVENERT 1994) (data not shown);

TABLE 5

Confusion assay: a test for chemotropism in *ste4* and *ste5* mutant cells

Responder *MAT***a** strain[a]	α-pheromone[b]	Percentage prezygotes	Stained *MATα*[c]	Prezygotes with stained *MATα*[b]
STE+	−	22	MFB	83/100 (83)
STE+	+	4.0	MFB	48/100 (48)
STE+	−	27	mfb	0/100 (0)
STE+	+	3.5	mfb	38/100 (38)
ste12	−	8.5	MFB	96/100 (69)
ste12	+	2.5	MFB	27/50 (54)
ste12	−	8.5	mfb	0/100 (0)
ste12	+	5.5	mfb	24/50 (48)
ste5	−	6.9	MFB	94/100 (94)
ste5	+	0.2	MFB	5/12 (42)
ste5	−	6.1	mfb	1/100 (1)
ste5	+	0.3	mfb	20/29 (69)
ste4	−	0.4	MFB	15/25 (60)
ste4	+	0.2	MFB	10/22 (45)
ste4	−	0.2	mfb	9/22 (41)
ste4	+	0.2	mfb	19/25 (76)

[a] Strains used were 8960-7-2a (*STE+*), 8942-9-1a (*ste4*) and 8960-21-4a (*ste5*). All *MAT***a** strains were transformed with P_{GALI} *STE12* (pNC252), a multicopy 2μ plasmid carrying *STE12* under control of the *GAL1,10* promoter. The wild-type *MATα* strain was 8906-1-4b. The pheromoneless *MATα* strain was 8907-4-1b ura3^{FOA}.

[b] Stained *MAT***a** cells were scored for apparent fusion, based on disappearance of the cell wall stain at the point of contact. Fusions were scored as to whether the *MATα* partner was stained or unstained. Values in parentheses are percentages.

[c] Cells were prepared as described in Tables 2 and 3, except that the *MATα* cultures were divided and only one aliquot was stained, while the other aliquot was treated in parallel with buffer lacking dye. Four mating mixtures were prepared for each *MAT***a** strain, each with 3.3×10^6 strained *MAT***a** cells. Two contained 3.3×10^6 stained pheromone-producing *MATα* cells (MFB) and 3.3×10^6 unstained pheromoneless *MATα* cells; two contained 3.3×10^6 unstained pheromone-producing *MATα* cells and 3.3×10^6 stained pheromoneless *MATα* cells (mfb). The cell mixtures were collected on a filter and allowed to mate for 9 h at 23° on medium containing both raffinose and galactose, with or without 30 μM α-pheromone.

many of the *STE+* cells did form such projections. This suggests that a high level of Ste12p suppresses projection formation and that only *STE+* cells that had lost the plasmid formed projections. It also suggests that *ste12Δ* cells that had lost the plasmid could no longer respond to pheromone by directional growth.

The role of the Gα subunit in prezygote formation and chemotropism: *GPA1* encodes the Gα subunit of the G protein that is coupled to the pheromone receptor. Unlike the other mutants considered in this study, a *gpa1* deletion results in constitutive activation of the signal transduction pathway (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987). To propagate *gpa1* cells, it is necessary to inactivate the signal transduction cascade to prevent cell cycle arrest in G1. We did this by using a temperature-sensitive mutation in a component of the signal transduction pathway downstream of the G protein, *ste5-3*. The *gpa1 ste5-3* strain could be grown at restrictive temperature for *ste5-3* and shifted to permissive temperature for mating (JACKSON *et al.* 1991). For reasons we do not understand, overexpression of Ste12p in *gpa1 ste5-3* cells suppressed diploid formation by ~30-fold (data not shown). Since overexpression of Ste12p is not needed to activate the pathway when the Gα subunit is absent, we examined *gpa1 ste5-3* cells lacking the plasmid for their ability to mate (Table 6). Since these experiments were performed differently than those of the other mutants, the two data sets are not directly comparable.

The *gpa1 ste5-3* strain displayed a very low mating efficiency in the absence of pheromone (0.57) compared to wild type (250). The RI for the *gpa1 ste5-3* strain was similar to that of the Gβ and Gγ mutants indicating a defect in mating partner discrimination. The mating efficiency was reduced threefold by high exogenous α-pheromone consistent with the idea that roughly 30% of the matings in the absence of exogenous alpha-pheromone were occurring by chemotropism. The reduction in mating by added α-pheromone was less than had been observed for wild type or mutants of the MAP kinase cascade. Thus even though these experiments were performed differently than the others, they support a conclusion for the Gα component that is similar to that for Gβ and Gγ, namely that all three mutants are strongly impaired for chemotropic mating.

DISCUSSION

During mating, *S. cerevisiae* cells of opposite mating type develop projections toward each other, then fuse cell walls and membranes when the projections come

TABLE 6

Confusion assay of *gpa1* cells

Genotype	α-pheromone	Mating efficiency (*MFB*)	Mating efficiency (*mfb*)	Mating efficiency (total)	RI
gpa1 ste5-3	−	0.57 ± 0.26	0.06 ± 0.05	0.64 ± 0.31	0.18 ± 0.34
gpa1 ste5-3	+	0.09 ± 0.10	0.13 ± 0.17	0.22 ± 0.27	1.1 ± 0.1
GPA1 ste5-3	−	250 ± 103	$<4 \times 10^{-5}$	250 ± 103	$<3 \times 10^{-7}$
GPA1 ste5-3	+	9.5 ± 5.5	9.8 ± 4.0	9.5 ± 5.5	1.1 ± 0.1
GPA1 STE5	−	139 ± 8.4	$<9 \times 10^{-5}$	139 ± 8.4	$<3 \times 10^{-6}$
GPA1 STE5	+	7.0 ± 2.1	9.7 ± 2.1	16.7 ± 2.6	1.1 ± 0.2

*MAT***a** strains used in this experiment were 7646-11-1a (*gpa1::LEU2 ste5-3*), 7646-19-1a (*GPA1 ste5-3*) and 7681-4-1a (*GPA1 STE5*). *MATα* strains were 8906-1-4b (*MFα1 MFα2*) and 8907-4-1b *ura3*^{FOA} (mfα1 mfα2). Numbers for *gpa1* cultures are averages of four independent cultures, duplicate mating mixes, in three experiments. Numbers for *GPA1 ste5* and *GPA1 STE5* are averages of three mating mixtures representing two and three independent cultures, respectively. Data from one additional experiment were excluded because the mating efficiencies of all cultures was unusually low.

into contact. A cell must be able to precisely locate its mating partner and correctly localize proteins involved in fusion; otherwise cell lysis might occur. Polarized growth and establishment of a fusion-competent site are chemotropic responses to the mating pheromone secreted by the cells of the opposite mating type (reviewed in KURJAN 1992, 1993; SPRAGUE and THORNER 1992; BARDWELL *et al.* 1994). Components of the signal transduction pathway have been shown to interact physically with each other (WHITEWAY *et al.* 1995) and with proteins involved in establishing cell polarity (CHENEVERT *et al.* 1994; LEEUW *et al.* 1995; ZHAO *et al.* 1995; LYONS *et al.* 1996). This suggests that in addition to their role in activating mating specific proteins, some components of the signal transduction pathway may play a role in the chemotropic response of the cell toward its mating partner.

Another indication that the signal transduction pathway may play an additional role in mating is the phenotype of mutations in the Gα subunit (*gpa1*) of the G protein associated with the pheromone receptor (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987). Null mutations in Gα free the Gβγ subunits to activate the MAP kinase cascade and result in constitutive activation of the signaling pathway (DIETZEL and KURJAN 1987; MIYAJIMA *et al.* 1987). However, *gpa1* mutants do not mate efficiently and they are defective in their ability to distinguish between pheromone-producing and -nonproducing partners (JACKSON *et al.* 1991; Table 6).

To examine the role of signal transduction components in mating, we constructed a series of null mutations of genes in the signal transduction pathway. To enable mating to occur in these mutants, we activated the pathway downstream by overexpressing Ste12p, a transcriptional activator of mating-specific proteins (DOLAN and FIELDS 1990). There are several potential caveats to this approach. First, overexpression of the *STE12* gene may abnormally activate certain functions due to an excess of functional Ste12p. Alternatively, since Ste12p is not posttranslationally modified due to a block in the signal transduction cascade, certain functions may be inadequately induced. Finally, proteins other that Ste12p may normally be activated by the signal transduction cascade, for example, Far1p, a protein required for cell cycle arrest (CHANG and HERSKOWITZ 1992; PETER *et al.* 1992; TYERS and FUTCHER 1993; VALDIVIESO *et al.* 1993); these functions would be deficient in our experiments. However, the control strain used for these experiments (a *ste12Δ* mutant overexpressing Ste12p from a plasmid) displayed nearly normal mating efficiency and mating partner discrimination. Therefore, we conclude that the effects produced by eliminating various members of the signal transduction pathway reflect the roles of these genes in the normal mating process.

All the experiments described here are quantitative mating assays in which mutant *MAT***a** responder cells were exposed to wild-type *MATα* partners (REID and HARTWELL 1977) or to equal numbers of wild-type *MATα* cells and mutant *MATα* cells unable to produce α-pheromone (JACKSON and HARTWELL 1990a,b). The total mating efficiency in both assays reflects the ability of the mutant *MAT***a** cell to form a fusion-competent site. The ability of cells to preferentially form diploids with pheromone-producing cells in the discrimination assay reflects their ability to detect and respond to mating pheromone. A wild-type cell shows a strong preference to mate with pheromone-producing cells, forming a diploid with a nonsignalling cell in less than one in 10^4 matings (JACKSON and HARTWELL 1990a,b). In addition, we monitored the ability to form prezygotes by differentially labeling the cell walls of the haploid parents with fluorescent dyes before mating (PRINGLE *et al.* 1989). Prezygotes form in mating mixes when cells can initiate mating but cannot complete cell fusion (TRUEHEART *et al.* 1987). By labeling either the pheromone-producing *MATα* or -nonproducing *MATα* partner in a mating partner discrimination assay, we could determine whether the mating advantage of pheromone-producing cells occurred at the level of prezygote

formation or of resolution of the prezygote to the diploid. As an additional test of whether mating of the mutant *MAT***a** was dependent on the ability to sense and respond to a pheromone gradient, we challenged cells to mate in the presence of a saturating isotropic concentration of α-pheromone (JACKSON *et al.* 1991). The mating efficiency of wild-type cells is reduced 10- to 20-fold by high exogenous α-pheromone while the mating efficiency of mutants that cannot respond to a pheromone gradient is unaffected (DORER *et al.* 1995).

We found that the components of the MAP kinase cascade, *STE5, STE11, STE7* and *FUS3/KSS1* were as proficient in forming prezygotes as the wild-type control (8–13% compared to 17%). Although the efficiency of diploid formation for these mutants was only ~1% that of wild-type *MAT***a** cells, their ability to discriminate between pheromone-producing and -nonproducing partners as measured by diploid formation was strong (on the order of RI = 10^{-3} for *ste5, ste20, ste11, ste7* and *fus3 kss1* compared to RI = 10^{-5} for *ste12*Δ and *STE*+). At the level of prezygote formation, *ste5, ste20, ste11, ste7* and *fus3 kss1* mutants were between 12 and 50 times more likely to form prezygotes with pheromone-producing cells than with pheromone-nonproducing cells. This implies that these mutants were able to carry out mating partner discrimination both at the level of prezygote formation and at the level of resolution of prezygotes into viable diploids. For *ste5* mutants, the presence of saturating amounts of α-pheromone in the medium reduced the efficiency of prezygote formation 20-fold and abolished the preference for fusion with a signaling partner rather than a nonsignaling partner. This result implies that the discrimination displayed by the *ste5* mutant in prezygote formation depends on its ability to sense the pheromone gradient. Thus Ste5p and probably other downstream components of the signal transduction pathway are not required for chemotropism. Consistent with these results, THORBURN *et al.* (1994) showed that the MAP kinases involved in phenylephrine-induced muscle cell hypertrophy are required for transcriptional induction of gene expression but not for the organization of the actin into contractile units.

In contrast to mutations in the downstream components of the signal transduction pathway, null mutations in the β and γ subunits of the G protein (*ste4* and *ste18*) have a profound affect on all aspects of mating. The efficiency of forming prezygotes was one-tenth that of *ste5* and the other downstream sterile mutants, indicating that G$\beta\gamma$ was far more important for creation of a fusion site than members of the kinase cascade. The ability to convert prezygotes to diploids was also more deficient for *ste4* and *ste18* mutants than for mutants with defects in the MAP kinase cascade. Among those few diploids that formed, mating partner discrimination was poor (RI = 0.31 and 0.25 for *ste4* and *ste18*). *ste18* mutants may show a small amount of discrimination between signaling and nonsignaling partners at the level of prezygotes, but *ste4* mutants show little or none. The weak mating partner discrimination in diploid formation by *ste4* mutants probably reflects some advantage of pheromone-producing partners in converting prezygotes to diploid zygotes, as was seen for mutants of the kinase cascade. In *ste4* cells, prezygote formation was unaffected by the presence of saturating levels of α-pheromone, consistent with the idea that the few prezygotes that form do not arise from the chemotropic mating pathway. Therefore, the G$\beta\gamma$ complex appears to be required for efficient chemotropism as well as activation of the MAP kinase cascade. From other systems it is known that the G$\beta\gamma$ subunits may have multiple downstream effectors as targets (BIRNBAUMER 1992). Perhaps G$\beta\gamma$, liberated from Gα at the site of maximal pheromone binding by the receptor, creates a site for nucleation of other components involved in cell fusion. Candidates for Gβ effectors could include the Cdc24p (ZHAO *et al.* 1995) and Akr1p (KAO *et al.* 1996; PRYCIAK and HARTWELL 1996), which are required for both bud emergence and pheromone-induced morphogenesis in mitotic growth (SLOAT and PRINGLE 1978; FIELD and SCHEKMAN 1980; SLOAT *et al.* 1981; ADAMS *et al.* 1990) and which have been shown to associate with G$\beta\gamma$ in the two-hybrid system (ZHAO *et al.* 1995). Cdc24p appears to act in a protein complex with two other proteins that are also required for both types of cell polarization, Cdc42p (ZIMAN *et al.* 1993; SIMON *et al.* 1995; ZHAO *et al.* 1995) and Bem1p (CHENEVERT *et al.* 1992, 1994; PETERSON *et al.* 1994). Bem1p, in turn, has been shown to associate with Ste5p, Ste20p, actin (LEEUW *et al.* 1995) and Far1p (LYONS *et al.* 1996). In at least one other system, the Gβ subunit has been shown to regulate Ca^{2+} channels (HERLITZE *et al.* 1996). In yeast, a role for calcium influx during cell fusion is supported by finding that a putative calcium-binding protein Mid2p is induced in response to pheromone (IIDA *et al.* 1990; IIDA *et al.* 1994; ONO *et al.* 1994). Although not directly comparable, our results with cells mutant for Gα are consistent with a role for the heterotrimeric G protein in the chemotrophic response to α-pheromone.

Of the mutants examined, only the α-pheromone receptor mutant (*ste2*) was completely defective in mating partner discrimination in diploid formation. This implies that the receptor is the only essential component in this aspect of the response to pheromone. Receptorless cells were also the most defective in prezygote formation, forming prezygotes <1% of the time.

The morphology of the prezygotes formed by *MAT***a** cells mutant for components of the signal transduction pathway indicate that polarized growth is defective in all mutants tested. Prezygotes formed by *ste5* and the other downstream mutants that appeared capable of a chemotropic response were indistinguishable from those formed by *ste2, ste4* and *ste18* that were not chemoresponsive. Mutant *MAT***a** partners in prezygotes re-

mained round and their nucleus did not migrate toward the site of fusion. This is in striking contrast to the corresponding *MAT*α partner that typically formed long projections toward the mutant *MAT***a** cell and whose nucleus often elongated and moved into the mating projection. One possible explanation is that Fus3p and or Kss1p are required for polarized cell growth. Consistent with this idea, Ste5p has been shown to form a complex with actin, a component of the cytoskeleton, and Bem1p, a protein required for the development of cell polarity in bud emergence (LEEUW *et al.* 1995). Since Ste5p, Ste7p, Ste11p, and Fus3p/Kss1p are thought to act in a complex to activate Ste12p, it may be that the active complex is required for polarized cell growth. Another possibility is that overexpression of Ste12p inhibits polarized growth. The fact that the *ste12*Δ strain with P_{GAL1}-*STE12* did not form projections in a high isotropic concentration of α-pheromone favors this explanation. In any case, directional growth does not appear to be required for mating partner discrimination under the mating conditions used in these experiments.

In summary, the chemoresponse of yeast can be visualized as a series of steps. The region of the cell surface of the responding cell that experiences the highest concentration of pheromone is selected as the site of fusion. Site selection might involve recruitment of components required for docking of the responding cell, or recruitment of components involved in pheromone secretion so that the growth of the responding cell would be targeted to a specific site, or both. The results described above suggest that free Gβγ complex of the G protein is intimately involved in establishing a fusion site in response to a pheromone gradient. Mutation of downstream members of the signal transduction pathway, *i.e.*, the MAP kinases, does not prevent efficient formation of a fusion site in response to an α-pheromone gradient. However, polarized growth toward a potential mating partner may require the MAP kinases and without them, conversion of prezygotes to zygotes is highly defective.

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