Saccharomyces cerevisiae Cells Execute a Default Pathway to Select a Mate in the Absence of Pheromone Gradients

Russell Dorer, Peter M. Pryciak, and Leland H. Hartwell

Department of Genetics, University of Washington, Seattle, Washington 98195-7360

Abstract. During conjugation, haploid *S. cerevisiae* cells find one another by polarizing their growth toward each other along gradients of pheromone (chemotropism). We demonstrate that yeast cells exhibit a second mating behavior: when their receptors are saturated with pheromone, wild-type **a** cells execute a default pathway and select a mate at random. These matings are less efficient than chemotropic matings, are induced by the same dose of pheromone that induces shmoo formation, and appear to use a site near the incipient bud site for polarization. We show that the SPA2 gene is specifically required for the default pathway: spa2Δ mutants cannot mate if pheromone concentrations are high and gradients are absent, but can mate if gradients are present. ste2Δ, sst2Δ, and far1Δ mutants are chemotropism-defective and therefore must choose a mate by using a default pathway; consistent with this deduction, these strains require SPA2 to mate. In addition, our results suggest that far1 mutants are chemotropism-defective because their mating polarity is fixed at the incipient bud site, suggesting that the FAR1 gene is required for inhibiting the use of the incipient bud site during chemotropic mating. These observations reveal a molecular relationship between the mating and budding polarity pathways.

Haploid *Saccharomyces cerevisiae* cells communicate with each other during conjugation by secreting small peptide pheromones. MAT**a** cells secrete **a**-factor and MATα cells secrete α-factor. These pheromones cause yeast cells to differentiate into cells that are competent for mating (for reviews see Kurjan, 1992; Sprague and Thorner, 1992). Pheromone binds to a seven-pass *trans*membrane receptor and activates a heterotrimeric G-protein, which activates a MAP kinase cascade (for reviews see Bardwell et al., 1994; Herskowitz, 1995). This signal transduction cascade causes cells to arrest in the G1 phase of the cell cycle and to induce the expression of genes that are important for mating. In addition, pheromones act as chemoattractants. Cells find one another in space by polarizing their growth toward each other along gradients of pheromone (Jackson and Hartwell, 1990*a,b*), a behavior that involves polarized changes in the actin cytoskeleton and in secretion (for a review see Chenevert, 1994). For example, an **a** cell (and perhaps an α cell), produces a projection that orients along a gradient of pheromone (Segall, 1993); pheromone receptors and the heterotrimeric G protein play integral roles in this chemotropic growth (Jackson et al., 1991; Schrick, 1994). Mating partners touch at the tips of their projections, fuse cell walls, and then membranes, and finally nuclei (Byers and Goetsch, 1975; Byers, 1981).

Please address all correspondence to L. H. Hartwell, Department of Genetics, University of Washington, Seattle, Washington 98195-7360. Tel.: (206) 543-6583. Fax: (206) 543-0754.

Jackson and Hartwell (1990*b*) devised a discrimination assay that indirectly measures the ability of cells to orient their growth along gradients of pheromone. When wild-type **a** cells are given a choice between wild-type α cells and pheromoneless α cells, **a** cells discriminate between these two partners and mate with a pheromoneless α cell partner only once in 10^5 matings. One might expect that the ability of a cell to orient would be required for mating; however, mutants that are defective at orienting are fertile. For example, cells that are deleted for the pheromone receptor are capable of mating at a reduced efficiency if the signal transduction pathway is activated downstream (Jahng et al., 1988; Dolan and Fields, 1990; Whiteway et al., 1990; Jackson et al., 1991; Stevenson et al., 1992; Hasson et al., 1994; Schrick, 1994); these receptorless cells mate with the pheromoneless cell partner as frequently as they mate with the wild-type partner in the discrimination assay (Jackson et al., 1991; Schrick, 1994). Similarly, cells containing the *sst2-1* mutation, a mutation that causes supersensitivity to pheromone (Chan and Otte, 1982; Dietzl and Kurjan, 1987), are defective both at discriminating mating partners (Jackson and Hartwell, 1990*b*; Schrick, 1994) and at orienting a projection along an artificial gradient of pheromone (Segall, 1993). To explain the fertility of these mutants, Jackson and Hartwell (1990*b*) proposed that when cells cannot sense pheromone gradients, they

© The Rockefeller University Press, 0021-9525/95/11/845/17 $2.00

execute a default pathway in which they select a mating partner at random.

The existence of a default pathway may explain why wild-type **a** cells form a shmoo in the presence of an isotropic concentration of α-pheromone (Jackson and Hartwell, 1990*b*). The shmoo is morphologically similar to a cell containing a gradient-induced projection (Lipke et al., 1976; Tkacz and MacKay, 1979; Baba et al., 1989; Segall, 1993), but the shmoo tip is formed at a predetermined site on the cell surface, near the site where the last bud was formed (Madden and Snyder, 1992). Similarly, haploid **a** and α cells position their buds near the last site where they budded (for reviews see Drubin, 1991; Madden et al., 1992; Chant, 1994). This budding pattern is under the control of the *RSR1*, *BUD2*, *BUD3*, *BUD4*, *BUD5*, *AXL1*, neck filament protein genes, and others (Bender and Pringle 1989; Chant and Herskowitz, 1991; Chant et al., 1991; Fugita et al., 1994; for a review see Chant, 1994), and, in isotropic α-factor, the position of the shmoo tip is also dependent on at least four of these budding pattern genes (*RSR1*, *BUD2*, *BUD3*, and *BUD4* [Madden and Snyder, 1992]). These observations suggest that the shmoo tip is positioned at the incipient bud site. In this paper we demonstrate that wild-type **a** cells mate at a reduced efficiency in the presence of high, isotropic concentrations of α-pheromone, and we propose that under these conditions cells execute a default pathway in which they mate by initially producing a projection near the incipient bud site.

The *SPA2* gene is required for shmoo formation in high, isotropic pheromone concentrations (Gehrung and Snyder, 1990), and the Spa2 protein localizes to sites of cell growth, including the shmoo tip (Snyder et al., 1991). In this report we demonstrate that *SPA2* is required for matings performed in an isotropic environment of α-pheromone, but that *SPA2* is not required for matings performed under conditions where pheromone gradients are present. In contrast, we show that the *SST2* gene is important for oriented growth because it prevents cells from executing the default pathway when a wild-type partner is present. In addition, our results suggest that the *FAR1* gene inhibits the use of the incipient bud site for mating and permits oriented growth along a pheromone gradient. We present a model in which two sets of genes define two pathways for mating partner selection, default and chemotropism: *SPA2* is required for default; pheromone receptors, *SST2*, and *FAR1* are required for chemotropism.

Materials and Methods

Strains, Plasmids, and Media

The strains used in this study are listed in Table I and are isogenic or congenic with the strain 381G *MATa cry1 ade2-1° his4-580^a^ lys2^o^ trp1^a^ tyr1^o^ SUP4-3^ts^* (Hartwell, 1980), unless otherwise indicated. Strains containing deletions of *FAR1* were constructed by transformation (using the lithium acetate method [Gietz and Schiestl, 1991]) with the plasmid pFC13 (Chang and Herskowitz, 1990) that was digested with NotI. Strains containing deletions of *STE2* were constructed by transformation with BamHI-digested pKSU (a gift from J. Konopka), a plasmid containing a substitution of *STE2* sequences with the *URA3* gene. Strains containing deletions of *SST2* were constructed by transforming with pBC14 (cut with NheI), a gift from H. Dohlman and J. Thorner that contains the *SST2* gene deleted for a 2.3-kb HpaI–HpaI fragment, adjacent to the *URA3* gene in the vector YIP5; colonies were selected on synthetic media lacking uracil, and prototrophs were replica plated to synthetic media containing 5-FOA to obtain popouts of pBC14; these colonies were then tested for the presence or absence of a deletion of *SST2*. Deletions of *SST2*, *STE2*, and *FAR1* were confirmed by PCR analysis of genomic DNA using oligonucleotides that flank the deleted regions of the wild-type genes (data not shown). Plasmid p21-1 (a gift from M. Whiteway) contains a galactose-inducible *STE4^Hpl^* allele (Whiteway et al., 1994) in the vector pRS313 (Sikorski and Hieter, 1989). 7611-4*mfa1mfa2* was created by sequential gene replacement using pSM86 (*mfa1::LEU2*) and pSM35 (*mfa2::URA3*) (Michaelis and Herskowitz, 1988) and confirmed by Southern hybridization. *FUS1-lacZ* fusions were integrated at the *FUS1* locus by transformation with plasmid pSB286 or pFL-LYS that had been linearized with SphI; pFL-LYS is a derivative of the integrating *FUS1-lacZ (URA3)* plasmid pSB286 (Chang and Herskowitz, 1990) in which the *URA3* gene has been replaced with a fragment containing the *LYS2* gene. The strain containing the *ste6-81HM* mutation contains a mutation that affects the *STE6* gene, based on the observations that this strain secretes less pheromone and is complemented by a centromere-containing plasmid containing the wild-type *STE6* gene (data not shown). Strains containing the *far1-16D* allele contain a mutation in the *FAR1* gene, based on the observations that the mutation is complemented by a centromere-containing plasmid that contains the wild-type *FAR1* gene and by a *FAR1* strain, and the mutation is not complemented by a strain containing a *far1Δ* mutation (data not shown). Liquid and solid media were described previously (Jackson and Hartwell, 1990*a*).

Responses to Pheromone

For β-galactosidase assays, mating filters were suspended directly in sterile Z-buffer (Miller, 1972), and one-half was permeabilized and assayed for β-galactosidase activity as previously described (Miller, 1972; Trueheart et al., 1987). Units of β-galactosidase activity were calculated as (1,000 × OD_{420} of reaction)/ (OD_{660} of responding cell culture × volume of responding cell culture × minutes of assay). The OD_{660} was determined immediately before cells were mixed for the mating reaction. Arrest and shmoo formation were measured by counting the numbers of unbudded cells, budded cells, and shmoos in a sample that were sonicated and fixed in 3.7% formaldehyde; 200 cells were counted in each sample. Shmoos were scored as those cells with pointed projections; unbudded cells were round or oval. Halo assays were performed as described in Konopka et al. (1988). For the shmoo site selection assay (Fig. 2), strain 7611-4 was grown to a density of 5×10^6 cells/ml; the cells were sonicated, and then 10^5 cells were spread on a YEPD plate containing 4 μM α-factor. Patterns were scored as described in the text after a 2–3-h incubation at 30°C; 400 cell pairs were analyzed.

Mating Assays

Discrimination assays were performed as in Jackson et al. (1991) and quantitative matings were performed as in Hartwell (1980). In the default mating assay, **a** and α-pheromoneless cells were mated quantitatively as in Hartwell (1980), except that 3×10^6 cells of each mating partner were mixed together, filtered onto 25-mm filters (0.45 μm pore size; Millipore Corp., Bedford, MA), and placed onto Noble agar plates containing the indicated concentration of α-factor; these plates were prepared by adding α-factor to warm liquid agar just before pouring the plates. Pheromone from the plate passes from the agar, through the filter, and into the mating mix on top of the filter. Because pheromone must diffuse through the filter and because the **a** cells on the filter secrete the Bar1p protease (MacKay et al., 1988), the concentration of pheromone that the cells experience is probably less than the concentration of pheromone on the plate. In the pheromone confusion assay, discrimination assays were performed as described (Jackson et al., 1991), except the Noble agar plates contained the indicated concentrations of α-factor. The mating efficiency was calculated as: 100% × (the number of diploids formed/ the smallest number of input haploids of one mating type); efficiencies greater than 100% occur if haploids divide before mating or if diploids divide after mating.

Assay of Budding Pattern

The budding patterns of microcolonies on agar were observed as in Chant and Herskowitz (1991), except that cultures were sonicated and spread on agar plates containing YEPD media (Hartwell, 1967) at a density of 10^5 cells per plate. Plates containing α-factor were prepared by adding α-factor to warm agar immediately before pouring the plates. After the indi-

Table I. Yeast Strains Used in This Study

Strain	Genotype	Source
PT-1*	a hom3 ilv1 can1	Hartwell Laboratory
PT-2*	α hom3 ilv1 can1	Hartwell Laboratory
3284-12*	α ade3 lys2^{o} tyr1^{o} trp1^{a} can1 cyh2 SUP4-3^{ts}	Hartwell Laboratory
4213-67*	α ade3 lys2^{o} tyr1^{o} trp1^{a} can1 cyh2 sst2-1 SUP4-3^{ts}	Hartwell Laboratory
3284-12iFL*	α ade3 lys2^{o} tyr1^{o} trp1^{a} can1 cyh2 FUS1::FUS1-lacZ(LYS2) SUP4-3^{ts}	This study
4213-67iFL*	α ade3 lys2^{o} tyr1^{o} trp1^{a} can1 cyh2 sst2-1 FUS1::FUS1-lacZ(LYS2) SUP4-3^{ts}	This study
7609-1-4*	a cry1 ade2-1° lys2^{o} tyr1^{o} cyh2 SUP4-3^{ts}	Hartwell Laboratory
7609-6-4*	α cry1 lys2^{o} tyr1^{o} ura2 SUP4-3^{ts}	Hartwell Laboratory
7611-2*	α cry1 his4-580^{a} lys2^{o} trp1^{a} ura3-52 leu2-3,112 cyh2 SUP4-3^{ts}	Hartwell Laboratory
7611-4*	a cry1 his4-580^{a} lys2^{o} tyr1^{o} ura3-52 leu2-3,112 cyh2 SUP4-3^{ts}	Hartwell Laboratory
7611-4iFL*	isogenic with 7611-4, except FUS1::FUS1-LacZ(URA3)	This study
7611-4mfa1mfa2*	isogenic with 7611-4, except mfa1::LEU2 mfa2::URA3	This study
7612-8-2*	a cry1 ade2-1° lys2^{o} tyr1^{o} ura2 cyh2 sst2-1 SUP4-3^{ts}	Hartwell Laboratory
7623-16-3*	a cry1 ade2-1° his4-580^{a} lys2^{o} trp1^{a} ura3-52 leu2-3,112 cyh2 SUP4-3^{ts}	Hartwell Laboratory
7623-16-3iFL*	isogenic with 7623-16-3, except FUS1::FUS1-LacZ(LYS2)	This study
7647-20-1*	α cry1 lys2^{o} trp1^{a}leu2-3,112 ura3-52 SUP4^{ts}	Hartwell Laboratory
7680-8-1iFL*	a cry1 his4-580^{a} lys2^{o} trp1^{a} tyr1^{o} ura3-52 sst2-1 cyh2 SUP4 FUS1::FUS1-LacZ(URA3)	This study
ste6-81HM*	isogenic with 7623-16-3, except ste6-81HM	This study
8940-4-3*	a cry1 ade6 his4-580^{a} lys2^{o} trp1^{a} tyr1^{o} ura3-52 cyh2 far1Δ::URA3 SUP4-3^{ts}	Kathrin Schrick
8940-6-3*	a cry1 ade6 his4-580^{a} lys2^{o} trp1^{a} tyr1^{o} ura3-52 cyh2 SUP4-3^{ts}	Kathrin Schrick
8941-1-4*	a cry1 ade6 his4-580^{a} trp1^{a} tyr1^{o} ura3-52 leu2-3,112 mfα1::URA3D mfα2::LEU2C	Kathrin Schrick
8941-12-2*	α cry1 ade6 his4-580^{a} trp1^{a} ura3-52 leu2-3,112 mfα1::URA3D mfα2::LEU2C can1 cyh3 SUP4-3^{ts}	Kathrin Schrick
8998-4-2*	a cry1 his3 lys2^{o} ura3-52 cyh2 SUP4-3^{ts}	Kathrin Schrick
8998-4-3*	a cry1 ade6 his3 lys2^{o} tyr1^{o} ura3-52 leu2-3,112 SUP4-3^{ts}	Kathrin Schrick
10703*	a/α ade2/ADE2 cry1/cry1 his4-580^{a}/his4-580^{a} lys2^{o}/lys2^{o} trp1^{a}/trp1^{a} ura3-52/ura3-52 leu2-3,112/leu2-3,112 cyh2/cyh2 SUP4-3^{ts}/SUP4-3^{ts}	This study
10815-14-4*	a cry1 lys2^{o} trp1^{a} tyr1^{o} ura3-52 leu2-3,112 cyh2 SUP4-3^{ts} far1-16D	This study
10843-9-2*	α cry1 his3 ura3-52 trp1^{a} leu2-3,112 cyh2 mfα1::URA3D mfα2::LEU2C SUP4-3^{ts}	This study
Y601‡	α spa2-Δ3::URA3 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200	Gehrung and Snyder, 1990
Y603‡	α SPA2 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200	Gehrung and Snyder, 1990
Y604‡	a SPA2 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200	Gehrung and Snyder, 1990
Y609‡	a spa2-Δ2::TRP1 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200	Gehrung and Snyder, 1990
Y604far1Δ‡	isogenic with Y604, except far1Δ::URA3	This study
Y609far1Δ‡	isogenic with Y609, except far1Δ::URA3	This study
Y604ste2Δ‡	isogenic with Y604, except ste2Δ::URA3	This study
Y609ste2Δ‡	isogenic with Y609, except ste2Δ::URA3	This study
Y604sst2Δ‡	isogenic with Y604, except sst2Δ	This study
Y609sst2Δ‡	isogenic with Y609, except sst2Δ	This study
W303-1B§	α ade2-1 trp1-1 leu2-3,112 ura3-1 his3-11,15 can1	Kurjan, 1985
mfα1::LEU2C§	α ade2-1 trp1-1 leu2-3,112 ura3-1 his3-11,15 can1 mfα1::LEU2C MFα2	Kurjan, 1985
mfα1mfα2§	α ade2-1 trp1-1 leu2-3,112 ura3-1 his3-11,15 can1 mfα1::LEU2C mfα2::URA3D	Kurjan, 1985

*381G strain background.
‡S288C strain background.
§W303 strain background.

cated incubation time, microcolonies were viewed at 400× magnification (Nikon Labophot-2 microscope), and four cell microcolonies were scored as described (Chant and Herskowitz, 1991). The percentage of microcolonies containing three or four cells was determined at 100× magnification.

Results

MATa Cells Execute a Default Mating Pathway in the Presence of High, Isotropic Pheromone Concentrations

Jackson and Hartwell (1990*b*) proposed that there is a default pathway that **a** cells use to choose a mate when they cannot sense pheromone gradients. Therefore, in this paper we adopt this definition of the default pathway: matings that occur by the default pathway are those that occur without sensing pheromone gradients. For clarity, we define a chemotropic mating pathway as the mating pathway by which wild-type cells mate when they sense pheromone gradients and orient their morphogenesis along the gradient toward a mate.

We designed the following experiments to study the default pathway in wild-type **a** cells. Cells that are deleted for both of the α-pheromone structural genes, *MFα1* and *MFα2*, produce no pheromone and are sterile (Kurjan, 1985; Table II; Fig. 1). Therefore, if a wild-type **a** cell is presented with this pheromoneless α cell as its sole mating partner and synthetic α-pheromone is added exogenously to the mating mix, then all matings must occur in the absence of α-pheromone gradients and by the default pathway. We refer to this assay as the default mating assay. We measured the mating behavior of **a** cells over a large range of exogenous pheromone concentrations, and since the experimental conditions cause the actual concentration of pheromone experienced by the **a** cells to be less than the concentration in the media (see Materials and Methods),

we included pheromone concentrations that were many fold higher than necessary to saturate pheromone receptors (Jenness et al., 1983, 1986). We found that the maximum inducible mating efficiency of **a** cells with pheromoneless α cells was ~17-fold lower than the efficiency with which two wild-type cells mated in the absence of exogenous pheromone (9.0% and 150%, respectively ([Fig. 1 *A*; Table II]). Therefore, high levels of exogenous pheromone can suppress the sterility of pheromoneless α cells, but cannot restore the efficiency of these matings to a wild-type level. Consistent with these results, Marcus et al. (1991) showed that the addition of **a**-factor could only partially suppress the sterility of **a** cells deleted for both **a**-factor structural genes.

These results suggest that matings that occur by the default pathway are less efficient than those that occur by the chemotropic pathway. In support of this suggestion, we found that wild-type **a** and α cells mated 19-fold less efficiently in the presence of a high α-factor concentration (Table II), and Marcus et al. (1991) found that exogenous **a**-factor inhibited the mating efficiency of wild-type **a** and α cells. To determine whether these matings occurred at a reduced efficiency because **a** cells were unable to use the chemotropic pathway and instead mated by the default pathway, we measured the behavior of **a** cells in a discrimination assay (Jackson and Hartwell, 1990*b*) to which we added increasing amounts of α-factor. In this assay, a wild-type **a** cell is presented with a choice between equal numbers of wild-type α cells and pheromoneless α cells. The two α cells carry different auxotrophic markers so that matings with each α cell can be scored independently. A randomness index is calculated as the fraction of diploids formed with the pheromoneless α cell, divided by the fraction of α cells that were pheromoneless in the mating reaction. When a wild-type **a** cell mates almost exclusively with the wild-type α cell partner, the randomness index is low. When the **a** cell mates equally often with both α cell mating partners, the randomness index is 1.0.

We found that as increasing amounts of α-factor were added to a discrimination assay, the mating efficiency decreased by about sevenfold (160–23% [Table II; Fig. 1 *A*]), roughly to the mating efficiency of an **a** cell mated with an α-pheromoneless cell as the sole mating partner (9.0%) and an **a** cell mated with a wild-type α cell as the sole partner in the presence of a high pheromone concentration (7.8% [Table II]). In addition, we found that the dose dependence for the increase in mating efficiency to pheromoneless cells in the discrimination assay was identical to the dose dependence displayed for matings with pheromoneless cells when they were the sole mating partner (Fig. 1 *A*). Moreover, as the fraction of cells mating at random in the discrimination assay increased, the total mating efficiency decreased (Fig. 1 *A*); the efficiency of these matings did not change once the randomness index reached 1.0 and the majority of the cells mated by the default pathway (Fig. 1 *A*). Therefore, we conclude that the induction of the default pathway inhibits the use of the chemotropic mating pathway and that the default pathway is a less efficient mating pathway than the chemotropic pathway. This lower mating efficiency reflects how important it is that both partners mate by the chemotropic pathway, since the inability of just one partner to use the chemotropic pathway impairs mating efficiency.

Default Mating Requires Saturation of the Pheromone Response Pathway

Since the execution of the default pathway depended on the dose of pheromone (Fig. 1 *A*), we asked to what degree the signal transduction pathway was activated when default matings were induced. *FUS1* expression is induced by as much as 1,000-fold in the presence of α-factor (Trueheart et al., 1987; McCaffrey et al., 1987; Fig. 1, *B* and *C*), so the level of *FUS1* induction reflects the level of activity in the signal transduction pathway. Therefore, we measured the β-galactosidase activity expressed from a *FUS1-lacZ* construct in the **a** cell in the above experiments. When an **a** cell is maximally induced for *FUS1-lacZ* expression, we interpret this as indirect evidence that the signal transduction pathway is saturated. We found that for **a** cells in matings with pheromoneless cells alone or in the discrimination assay, the levels of *FUS1-lacZ* induction reached a maximum upon the addition of 25 μM α-factor (Fig. 1 *B*), the same concentration at which the mating efficiency of **a** cells with pheromoneless α cells reached a maximum (Fig. 1 *A*). Therefore, we suggest that the default pathway is activated when the pheromone response pathway is saturated by high doses of pheromone. Furthermore, notice that the efficiency of default matings increased by five orders of magnitude with just a 10-fold increase in pheromone concentration, whereas *FUS1-lacZ* induction increased more gradually as the pheromone

*Table II. Mating Behavior of Wild-Type **a** Cells in the Presence of Exogenous α-factor*

α Cell partner(s)*	α-factor‡	Mating efficiency§	Randomness index‖	Fold inhibition by α-factor¶
MFα1MFα2	−	150 ± 16 (3)	NA	NA
MFα1MFα2	+	7.8 ± 0.50 (3)	NA	19
mfα1mfα2	−	$<3.8 \times 10^{-3}$ (3)	NA	NA
mfα1mfα2	+	9.0 ± 1.8 (3)	NA	NA
MFα1MFα2, mfα1mfα2	−	160 ± 16 (3)	$9.0 \times 10^{-6} \pm 3.5 \times 10^{-6}$ (3)	NA
MFα1MFα2, mfα1mfα2	+	23 ± 3.6 (3)	1.1 ± 0.10 (3)	7.0

*The α strains used were 8941-12-2 (lines 3-6) and 7609-6-4 (lines 1, 2, 5, and 6); all matings were with a wild-type **a** strain (7611-4). Equal numbers of cell partners were used in all matings.

‡Matings were allowed to occur for 3 h at 30°C on Noble Agar plates in the absence (−) or in the presence (+) of 20 μM α-factor. The number of experiments performed is indicated in parentheses.

§Percent of input haploid cells that formed diploids.

‖Calculated as the fraction of diploids formed between the **a** cells and the α-pheromoneless cells, and divided by the fraction of α cells that were α-pheromoneless in the mating reaction.

¶The total mating efficiency of the **a** strain in the absence of α-factor, divided by the mating efficiency of the **a** strain in the presence of α-factor.

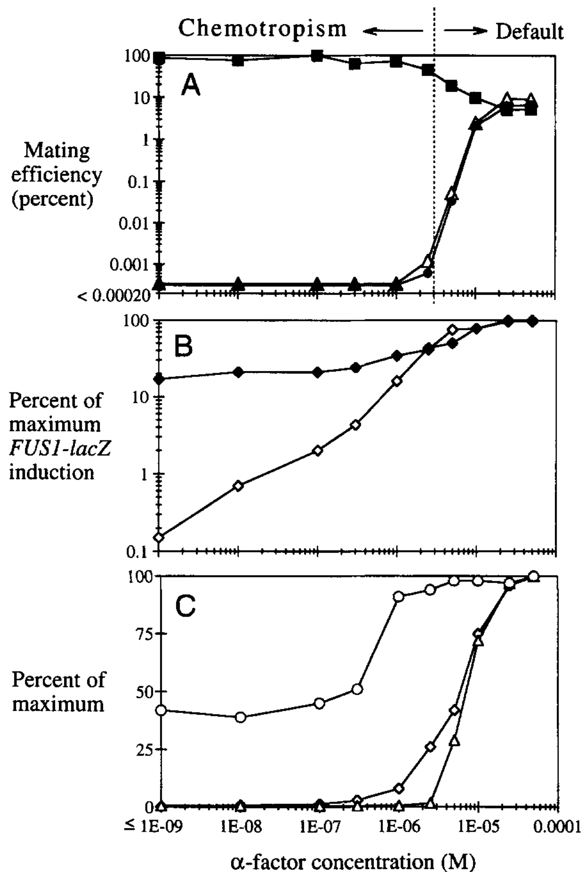


Figure 1. The behaviors of wild-type cells executing the default pathway. (*A* and *B*) Wild-type **a** cells were mated either with pheromoneless α cells alone (*open symbols*) or with both wild-type α cells and pheromoneless α cells in a discrimination assay (*closed symbols*) on Noble agar plates containing the indicated pheromone concentrations for 2.5 h at 30°C (see Materials and Methods). (*A*) The mating efficiency with wild-type *a* cells (*squares*) and with pheromoneless α cells (*circles*) were measured in the discrimination assay; the mating efficiency with α-pheromoneless cells as the sole mating partner were measured under the same conditions (*triangles*). (*B*) The level of *FUS1-lacZ* expression in the **a** cells was measured in each quantitative mating. Data are presented as a percentage of the maximum; the maximum β-galactosidase activity in **a** cells was 74 units in matings with pheromoneless cells alone and 96 units in the discrimination assay. (*C*) An **a** cell was treated exactly the same as in the mating assays, except that no α cell partners were present. The percentage of cells that arrested as unbudded cells (*circles*) or shmoos (*triangles*) was measured; diamonds represent the level of *FUS1-lacZ* expression in the **a** cell. The maximum percentage of cells that were unbudded and shmoos were 100 and 92%, respectively; the maximum β-galactosidase activity was 24 units. The experiments in *A*, *B*, and *C* were all performed in parallel; these data represent the average of duplicate experiments. The **a** strain used was 7623-16-3iFL, and the α strains used were 8941-1-4 and 8998-4-3.

concentration increased (compare Fig. 1, *A* and *B*). This dramatic increase in mating efficiency may reflect an important event that is induced during default matings; perhaps there are cooperative effects or oligomers formed among components that induce default matings.

In addition, these data suggest that in a wild-type mating reaction, **a** cells receive a subsaturating level of signal, a condition that is required for a cell to detect a gradient of pheromone surrounding the cell; in a saturating concentration of exogenous pheromone, **a** cells expressed about sixfold more *FUS1-lacZ* than when all pheromone was supplied by the MATα cells in the discrimination assay (Fig. 1 *B*). This subsaturating level of expression is not due to a small fraction of **a** cells undergoing mating and giving high expression levels in the absence of exogenous pheromone, because in the absence of pheromone the majority of cells mate, and it is not due to an inhibition of *FUS1-lacZ* expression after cell fusion occurs because *FUS1-lacZ* expression in MATα cells is identical when either MAT**a** cells or *matΔ* cells are used as partners (data not shown).

Shmoo Formation Correlates with the Execution of the Default Pathway

When **a** cells are exposed to high concentrations of exogenous pheromone, they form a shmoo (for a review see Chenevert, 1994) by producing a projection on one side of their cell. Madden and Snyder (1992) showed that these projections are located adjacent to the last bud site on the cell. They stained shmoos with calcofluor, which incorporates into the cell wall, and observed that bud scars were predominantly in the third of the cell where the shmoo tip was located. We have made a similar observation in the 381G strain background. We found that when **a** cells were grown to mid-logrhythmic phase, and then spread on a YEPD plate containing a high concentration of α-factor (4 μM), cells that were past START at the time of plating arrested in the G1 phase as a pair of mother and daughter cells. After 2–3 h, the pair produced shmoos, the tips of which were positioned next to their last bud site; for 86% of the cell pairs, both cells produced projections adjacent to their last bud site (class I, Fig. 2), while in only 0.25% of the cell pairs both cells formed projections opposite their last bud site (class III, Fig. 2). This pattern is similar to the pattern of bud site selection that occurs if no α-factor is present in the media (Chant and Herskowitz, 1991; Table X).

We hypothesized that these projections are formed when **a** cells execute the default mating pathway. Therefore, we compared shmoo formation with the induction of default matings in **a** cells in response to increasing concentrations of added α-pheromone (Fig. 1 *C*). Consistent with the observations of Moore (1983), we observed that **a** cells require higher concentrations of pheromone to form shmoos than they require to arrest as unbudded cells; at 1 μM pheromone ~90% of the cells were unbudded, and less than 1% of the cells formed shmoos (Fig. 1 *C*). We found that shmoo formation increased dramatically (from 1.5–89% of the cell population) between 2.5 μM and 25 μM (Fig. 1 *C*), the same range over which default mating efficiency increased dramatically in parallel experiments (Fig. 1 *A*). In addition, the point of maximal shmoo formation correlated with the point at which the pheromone response pathway was saturated, as measured by *FUS1-lacZ* induction (Fig. 1 *C*); consistent with this deduction, shmoo formation and receptor saturation have similar dose-response profiles (Moore, 1983; Jenness et al., 1983, 1986). Since shmoo formation and default mating efficiency also have similar dose-response profiles, we suggest that when

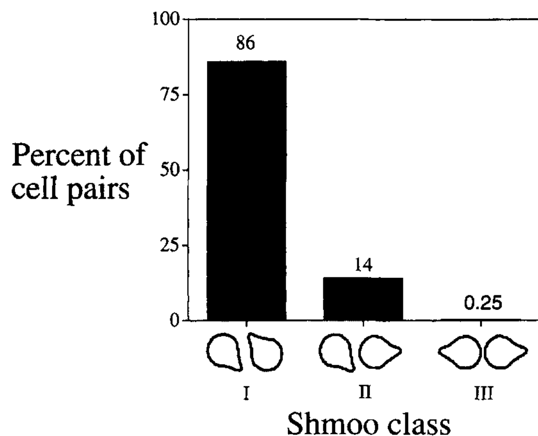


Figure 2. MAT**a** cells produce shmoos adjacent to their last budding site in the presence of a high concentration of α-factor. Cells were grown to mid-logrhythmic phase, sonicated, and spread on a YEPD plate containing 4 μM α-factor (see Materials and Methods). After a 2.5-h incubation at 30°C, the position of the shmoo tips in the mother and daughter cell pairs was scored accordingly; data are the percentage of 400 cell pairs scored that were in each class. In class I pairs, both shmoo tips are positioned in the half of each cell that is adjacent to the other cell in the pair. In class III, each shmoo tip is positioned in the half of the cell that is opposite the other cell in the pair; in class II one shmoo tip is in the half of the cell adjacent to the other cell in the pair (as in class I), but the second shmoo tip is in the half opposite the other cell (as in class III).

a cells receive saturating levels of pheromone they execute the default pathway and form a shmoo.

The SPA2 Gene Is Specifically Required for the Default Mating Pathway

Given these observations, we hypothesized that mutants that are defective for shmoo formation might be defective for the ability to mate with pheromoneless cells at high pheromone concentrations. Therefore, we tested cells deleted for the *SPA2* gene in the default mating assay, since these mutants fail to form pear-shaped shmoos at high pheromone concentrations and instead become oval, spherical, or peanut-shaped, depending on the strain background and allele (Gehrung and Snyder, 1990; Yorihuzi and Ohsumi, 1994; Chenevert et al., 1994).

We found that MAT**a** *spa2Δ* cells mated with an ~3,000-fold lower efficiency in the default mating assay than an isogenic wild-type control strain (about 0.003% and 10%, respectively [Table III]). This mating defect was specific to default-inducing conditions: when no pheromone was added to the mating mix, an **a** *spa2Δ* strain mated about as efficiently as an isogenic wild-type control strain mated either with *SPA2* α cells (50% and 112%, respectively) or with *spa2Δ* α cells (60% and 124%, respectively) under the conditions of this mating assay, where **a** and α cell partners were mated at high cell densities (Table III; Materials and Methods). These data for matings with wild-type cells are consistent with the observations of Gehrung and Snyder (1990), who showed that *SPA2* is not required for matings with wild-type cells when cell densities are high. Therefore, *SPA2* is specifically required for the default mating

Table III. SPA2 Is Required for Matings with α-pheromoneless Cells

a Strain*	α Strain‡	a-factor§	Mating efficiency‖
			%
SPA2	*mfα1mfα2*¶	+	7.9
			12
spa2-Δ2::TRP1	*mfα1mfα2*¶	+	0.002
			0.004
SPA2	*SPA2*¶	−	112
			112
spa2-Δ2::TRP1	*SPA2*¶	−	54
			46
SPA2¶	*SPA2*	−	138
			114
SPA2¶	*spa2-Δ3::URA3*	−	127
			121
spa2-Δ2::TRP1	*spa2-Δ3::URA3*	−	62
			58

*The **a** strains used were Y604 (lines 1 and 3), Y609 (lines 2, 4, and 7), and PT-1 (lines 5 and 6).
‡The α strains used were 8941-12-2 (lines 1 and 2), PT-2 (lines 3 and 4), Y603, and Y601 (lines 6 and 7).
§Matings were allowed to occur for 3 h at 30°C on Noble Agar plates in the absence (−) or in the presence (+) of 10 μM α-factor.
‖See footnote §, Table II.
¶381G strains. All others are S288C.

pathway in **a** cells, since *SPA2* is not required for chemotropic matings.

In addition, we tested cells deleted for *SPA2* in the discrimination assay, either in the presence or in the absence of exogenous α-factor (Fig. 3). We found that in the presence of increasing concentrations of α-factor, the total mating efficiency of *spa2Δ* cells was reduced dramatically, from 81% (no α-factor added) to 0.073% (100 μM α-factor), a 1,100-fold reduction. In contrast, the presence of 100 μM α-factor caused only an eightfold reduction in the mating efficiency of the isogenic wild-type control strain (from 160% to 21%). Therefore, the addition of exogenous α-factor to the discrimination assay dramatically inhibited the ability of *spa2Δ* cells to mate with wild-type cells, demonstrating that *SPA2* is required for all default matings induced in the presence of high, isotropic α-factor concentrations, not just matings with α-pheromoneless cells. Moreover, unlike the *SPA2* control cells, *spa2Δ* cells showed only about a 16-fold increase in mating efficiency with the α-pheromoneless cell partner (from 0.0011% to 0.018%) compared with an 11,000-fold increase for the wild-type control (from 0.0011% to 12%). It is also interesting to note that as more pheromone was added to the discrimination assay above a concentration of 10 μM, the total mating efficiency of the *spa2Δ* cells decreased significantly, from 4.4% to 0.073% (60-fold), and the efficiency of the wild-type control decreased only slightly, from 35% to 21% (1.7-fold). Since this assay is a population assay, the continued decrease in mating efficiency in the *spa2Δ* mutant population is most likely explained by suggesting that with the increasing concentration of pheromone, an increasing percentage of the *spa2Δ* cells had their pheromone receptors saturated by pheromone; in other words, even in the presence of a high exogenous pheromone concentration, some **a** cells in the population could sense a gradient of pheromone produced by an opposite mate and

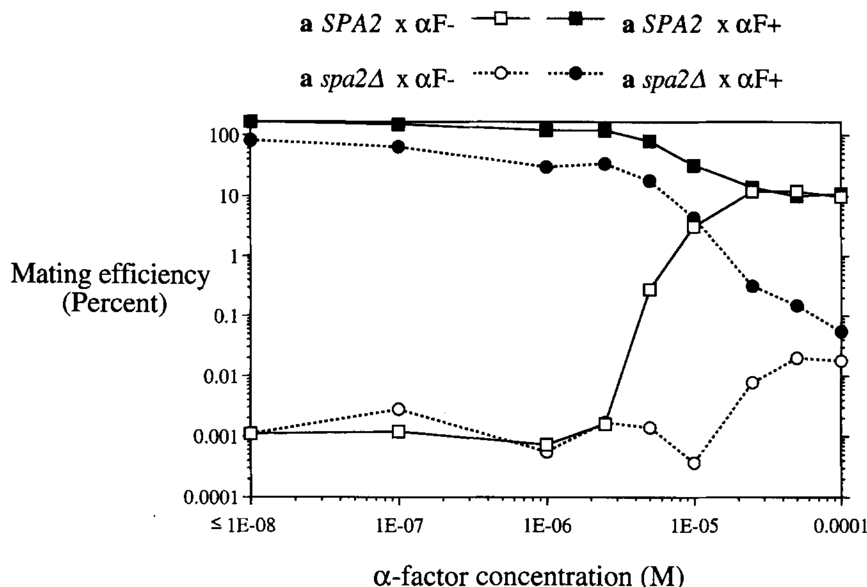


Figure 3. *SPA2* is specifically required for the default mating pathway. *spa2Δ* mutants (*circles*) and *SPA2* cells (*squares*) were each mated in discrimination assays in the presence of the indicated concentrations of α-factor on Noble Agar plates for 3.0 h at 30°C. The mating efficiencies with the wild-type α cell partner (*closed symbols*) and with the pheromoneless α cell partner (*open symbols*) we calculated for each mating assay (see Materials and Methods).

mate by growing along that gradient. At the highest pheromone concentration, 100 μM, most of the *spa2Δ* **a** cells were surrounded by saturating pheromone, and as a result very few gradients remained in the population; since the cells could neither sense a gradient of pheromone because of the high pheromone concentrations, nor execute the default pathway due to the deletion of *SPA2,* very few cells mated (only 0.073%).

The SST2 Gene Is Important for Chemotropism but Is Not Required for the Default Mating Pathway

In contrast to *SPA2*, the *SST2* gene is important for chemotropism. Both *sst2-1* **a** and *sst2-1* α strains, containing null mutations in the *SST2* gene, are 100-fold more sensitive to pheromone than wild-type **a** and α strains (Chan and Otte, 1982; Dietzel and Kurjan, 1987). The following three observations suggest that *SST2* is important for chemotropism: (*a*) both **a** and α *sst2-1* mutants mate randomly in the discrimination assay (Jackson and Hartwell, 1990*b*; Schrick, 1994); (*b*) *sst2-1* mutants mate with wild-type cells ~10-fold less efficiently than *SST2* cells (Jackson and Hartwell, 1990*b;* Table IV [18 ± 3.6% and 190 ± 32%, respectively]); and (*c*) *sst2-1* cells show a defect in orienting growth along pheromone gradients that wild-type cells can detect (Segall, 1993). Despite this role for *SST2* in chemotropism, we found that *SST2* is not required for the default mating pathway, since we observed that *sst2-1* mutants mated as efficiently as *SST2* cells mated with pheromoneless cells in the presence of 20 μM α-factor (3.5 ± 0.59% and 2.0 ± 0.21%, respectively [Table IV]). Therefore, the chemotropic and default mating pathways are genetically distinct, since these mating pathways show reciprocal requirements for the *SST2* and *SPA2* genes.

An sst2-1 a Strain Executes the Default Pathway to Mate with a Wild-Type Partner

Although *SST2* is important for chemotropism, two pieces of evidence suggest that it is not absolutely required for chemotropism, but instead alters the range of pheromone concentrations over which cells can orient. First, Segall (1993) demonstrated that while *sst2-1* mutants are defective at orienting their growth along pheromone gradients that wild-type cells can use to orient, they are capable of orienting their growth if the concentration in the gradient is very low. Second, we found that both the low mating efficiency and the discrimination defect of *sst2-1* α cells could be suppressed if the **a** cell partner produced very low levels of pheromone. During a screen for MAT**a** mutants that mate poorly with an *sst2-1* α strain (Dorer, R., and L. H. Hartwell, unpublished observations), we found mutant **a** cells that mate better than wild-type **a** cells with α *sst2-1* cells. Most of these **a** cells contain hypomorphic mutations in *STE6* (data not shown), the gene encoding the **a**-factor transporter (for a review see Michaelis, 1993), and they secrete less pheromone (unpublished observations). One of these mutants, *ste6-81HM,* partially suppressed both the low mating efficiency and the discrimination defect of α *sst2-1* cells (Table V); when the pheromone-producing **a** strain contained the *ste6-81HM* mutation the randomness index for the *sst2-1* strain was 0.014 ± 0.0016 and the mating efficiency was 67%, compared to a 29-fold higher randomness index (0.41 ± 0.058) and a 2.4-fold lower efficiency (28 ± 1.2%) when the **a** strain was *STE6.* In contrast, the *ste6-81HM* **a** strain mated poorly with a wild-type α strain (0.85 ± 0.69%, compared with 180 ± 20% for

Table IV. sst2-1 Mutants in the Default Assay

a Strain*	α-factor‡	α Strain§	Mating efficiency‖
			%
SST2	−	*MFα1 MFα2*	190 ± 32 (3)
sst2-1	−	*MFα1MFα2*	18 ± 3.6 (3)
SST2	+	*mfα1mfα2*	3.5 ± 0.59 (3)
sst2-1	+	*mfα1mfα2*	2.0 ± 0.21 (3)

*The **a** strains used were 7609-1-4 (*SST2*) and 7612-8-2 (*sst2-1*).
‡Matings were allowed to occur for 3 h at 30°C on Noble Agar plates in the absence (lines 1 and 2) or in the presence (lines 3 and 4) of 20 μM α-factor.
§The α strains used were 7611-2 (lines 1 and 2) and 8941-12-2 (lines 3 and 4).
‖See footnote §, Table II.

Table V. The Mating Defects of an sst2–1 α Strain Are Suppressed by Mating with an **a** *Strain Containing a Mutation in STE6*

α-Strain*	Pheromone-producing **a** strain‡	Total mating efficiency§	Randomness index‖
		%	
SST2	*STE6*	180 ± 20 (3)	$5.9 \times 10^{-6} \pm 3.4 \times 10^{-6}$ (3)
SST2	*ste6-81HM*	0.85 ± 0.69 (3)	$<9.1 \times 10^{-4} \pm 6.9 \times 10^{-5}$ (3)
sst2-1	*STE*	28 ± 1.2 (3)	0.41 ± 0.058 (3)
sst2-1	*ste6-81HM*	67 ± 0 (3)	0.014 ± 0.0016 (3)

*The α strains used were 3284-12 (*SST2*) and 4213-67 (*sst2-1*).
‡The pheromone producing **a** strains used in the discrimination assay are isogenic with 7623-16-3. All assays used 7611-4*mfa1mfa2* as the pheromoneless **a** strain.
§The percentage of **a** cells that formed diploids with either the α wild-type or the α-pheromoneless strain partners. The mean and standard error of the number of independent observations shown in parentheses are indicated.
‖See footnote ‖, Table II.

a *STE6* **a** strain), consistent with a pheromone secretion defect. Therefore, these results demonstrate that *SST2* is only required for discriminating mating partners when one of its partners produces wild-type levels of pheromone.

In addition, we measured the level of *FUS1-lacZ* expression in *sst2-1* **a** cells in mating mixes with wild-type α cells and found that the level of *FUS1-lacZ* expression could not be increased by adding exogenous α-pheromone, suggesting that for *sst2-1* cells the pheromone response pathway is saturated when wild-type mating partners are present (Table VI). Since wild-type **a** cells activate the default pathway when the signal transduction pathway is saturated, these data suggest that a wild-type mating partner causes *sst2-1* **a** cells to execute the default pathway. Consistent with this hypothesis, we found that α *sst2-1* cells are maximally induced for *FUS1-lacZ* expression when their mating partner is a wild-type **a** cell, but not when their partner contains the *ste6-81HM* mutation (Table VI). This hypothesis also predicts that the deletion of *SPA2* in an *sst2Δ* strain should prevent an *sst2Δ* mutant from completing any matings by the default pathway. We predicted that as a result the mating efficiency of the *spa2Δsst2Δ* double mutant would be reduced while the apparent discrimination defect would be suppressed. Indeed, the mating efficiency of the *sst2Δspa2Δ* strain was 43-fold lower than the *sst2Δ* strain (0.74 ± 0.41% compared to 32 ± 3.0%, respectively [Table VII]). In addition, while the *sst2Δ* strain discriminated poorly between wild-type α cells and pheromoneless α cells (randomness index of 0.51 ± 0.083), the *sst2Δspa2Δ* **a** strain discriminated mating partners very well and preferred to mate with the wild-type α strain in the discrimination assay (randomness index of 0.0088 ± 0.0021 [Table VII]). Therefore, while most members of a population of *sst2Δ* **a** cells mate by the default pathway, most *sst2Δspa2Δ* **a** cells cannot mate because they cannot complete the default pathway. The small percentage of *sst2Δspa2Δ* **a** cells that mate are those that do not execute the default pathway because of the incomplete chemotropic defect caused by the *sst2Δ* mutation; as a result, the rare *sst2Δspa2Δ* cells that mate, do so solely with the wild-type α cell in the discrimination assay.

As a control we showed that the deletion of *SPA2* does not alter the supersensitivity of *sst2Δ* **a** cells to α-factor, as judged by halo assay (Fig. 4). Both *spa2Δ* **a** cells and wild-type **a** cells displayed identical sensitivity to pheromone, and both *sst2Δ* **a** cells and *sst2Δspa2Δ* **a** cells displayed identical supersensitivity.

SPA2 Acts Downstream of STE4 in the Default Mating Pathway

Since high levels of pheromone initiate the default mating pathway, some component(s) of the mating signal transduction pathway must respond to high levels of signal and execute the default pathway. The *STE2* gene encodes the receptor for α-factor and is required for sensing pheromone gradients (Jackson et al., 1991; Schrick, 1994). The *GPA1*, *STE4*, and *STE18* genes encode the α, β, and γ components of the heterotrimeric G-protein, respectively. Genetic data support a model in which the α subunit negatively regulates the ability of the βγ complex to initiate the pheromone response (for reviews see Marsh et al., 1991;

Table VI. FUS1-lacZ Induction Levels in Mating Mixes

Responding cell type*	Inducing cell‡	α-factor§	FUS1-lacZ in responding cell (percent of maximum response)‖	
			SST2	*sst2-1*
a	*mfα1mfα2*	−	0.66 ± 0.25 (8)	3.9 ± 1.6 (8)
a	*mfα1MFα2*	−	8.5 ± 1.4 (8)	102 ± 4.5 (8)
a	*MFα1MFα2*	−	18 ± 4.1 (8)	95 ± 6.8 (8)
a	*MFα1MFα2*	+	60 ± 8.1 (8)	98 ± 9.0 (8)
α	*mfa1mfa2*	−	0.59 ± 0.18 (4)	9.6 ± 3.4 (4)
α	*ste6-81HM*	−	2.8 ± 0.33 (4)	66 ± 3.4 (4)
α	*MFa1MFa2*	−	38 ± 0.87 (4)	100 ± 5.0 (4)

*The strain containing the *FUS1-lacZ* construct. The strains used were 7611-4iFL (**a** *SST2*), 7680-8-1iFL (**a** *sst2-1*), 3484-12iFL (α *SST2*), and 4213-67iFL (α *sst2-1*).
‡The mating partner of the responding cell. The strains used, in order from top to bottom, were *mfα1mfα2*, *mfα1::LEU2C*, W303-1B, 7647-20-1, 7611-4*mfa1mfa2*, *ste6-81HM*, and 7623-16-3. The responding strain was mated with this inducing cell for 90–150 min on Noble agar plates at 30°C.
§Cells were mated in either the presence (+) or absence (−) of 20 μM α-factor.
‖The percentage of the maximum level of *FUS1-lacZ* induction observed. The maximum level of β-galactosidase activity was 60.5 units for **a** cells and 144 units for α cells.

Table VII. A spa2Δ Mutation Displays Synthetic Sterility with an sst2Δ Mutation and Suppresses the Discrimination Defect Caused by sst2Δ Mutation

a Strain*	Total mating efficiency‡	Randomness index§
	%	
SST2 SPA2	120 ± 13 (4)	$<5.2 \times 10^{-6} \pm 1.4 \times 10^{-6}$ (4)
sst2Δ SPA2	32 ± 3.0 (4)	0.51 ± 0.083 (4)
SST2 spa2Δ	120 ± 17 (4)	$<8.1 \times 10^{-6} \pm 1.4 \times 10^{-6}$ (4)
sst2Δ spa2Δ	0.74 ± 0.14 (4)	0.0088 ± 0.0021 (4)

*The **a** strains used in this study were, in order from top to bottom, Y604, Y604*sst2Δ*, Y609, and Y609*sst2Δ*. The α strains used were 8998-4-2 and 8941-12-2.
‡See footnote §, Table V.
§See footnote ‖, Table II.

Sprague and Thorner, 1992; Kurjan, 1992). Consistent with this model, overexpression of *STE4* can suppress the mating defect of receptorless cells (Whiteway et al., 1990). We wished to determine whether overexpression of *STE4* causes *ste2Δ* cells to mate by a process that requires *SPA2*. We found that the deletion of *SPA2* decreased the mating efficiency of a *ste2Δ* mutant by 15-fold when matings were induced with pheromoneless cells by overexpressing an activated *STE4* allele, $STE4^{Hpl}$ (0.078 ± 0.013% and 1.2 ± 0.10% for *ste2Δspa2Δ* and *ste2Δ* mutants, respectively [Table VIII]). In addition, pheromoneless matings that were induced by $STE4^{Hpl}$ in a *STE2* background were inhibited by ~31-fold by the deletion of *SPA2* (0.37 ± 0.016% and 0.012 ± 0.0012% for *SPA2* and *spa2Δ*, respectively). These low mating efficiencies of *spa2Δ* $STE4^{Hpl}$ mutants are similar to the mating efficiencies of *spa2Δ* mutants mated in the presence of saturating pheromone (Fig. 3), or mated with pheromoneless cells in an *sst2Δ* background (Table VII). Therefore, $STE4^{Hpl}$ requires *SPA2* to activate default mating events, suggesting that *SPA2* acts downstream of *STE4* in the default pathway. In addition, notice that the deletion of *STE2* does not inhibit a cell's ability to mate with pheromoneless cells (0.37% and 1.2% for *STE2* and *ste2Δ*, respectively), suggesting that beyond their role in activating *STE4* during mating pheromone receptors are not absolutely required for matings that occur by default. Consistent with this conclusion, receptorless cells are capable of shmoo formation if the pheromone-response pathway is activated downstream (Jahng et al., 1988; Clark and Sprague, 1989).

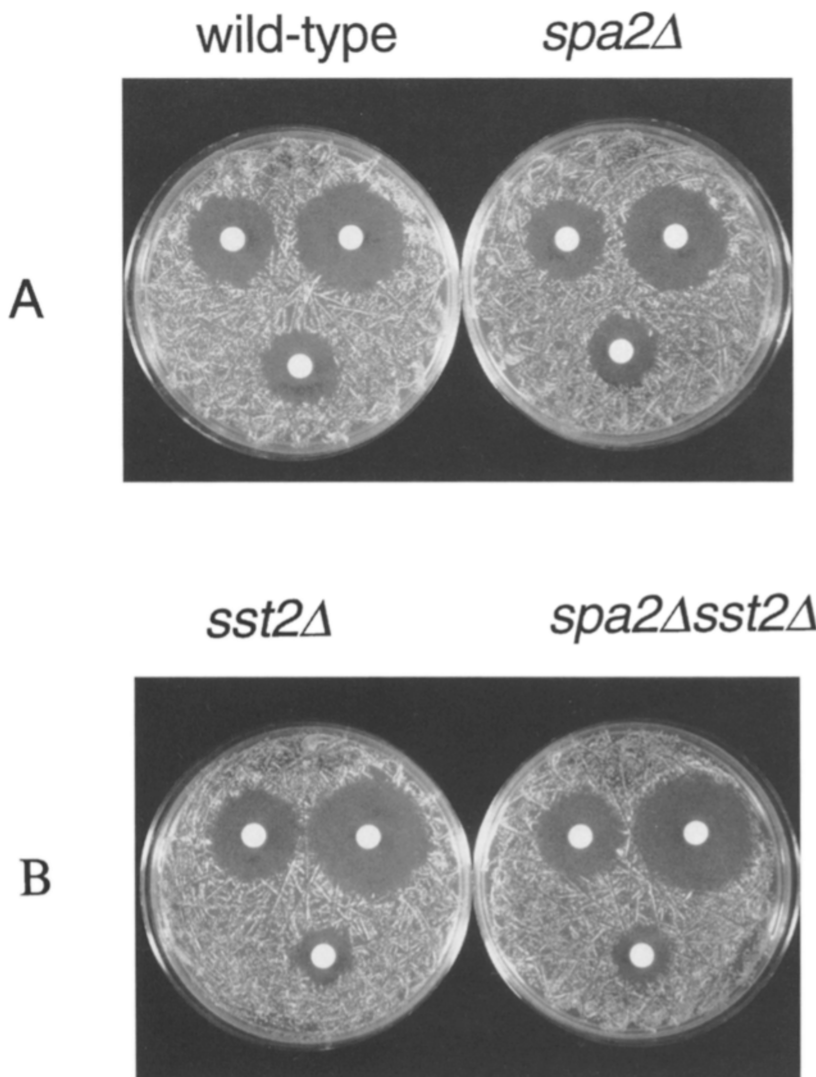


Figure 4. The sensitivity of mutants to pheromone was judged by halo assay. Sterile filter discs containing α-pheromone were placed on agar plates spread with 10^5 cells of the indicated **a** strain. The size of the halo (*clear region*) reflects the sensitivity of the strain to pheromone-induced cell cycle arrest. (*A*) Y604 (*SPA2*) and Y609 (*spa2Δ*) were tested with filters containing 1 mM, 100 μM, and 50 μM α-factor (*counterclockwise from upper right on plate*). (*B*) Y604*sst2Δ* and Y609*sst2Δ* **a** strains were tested with 10 μM, 1 μM, and 100 nM α-factor (*counterclockwise from upper right*).

Table VIII. STE4^{Hpl} Requires SPA2 and Not STE2 to Induce Matings with Pheromoneless α Cells

a Strain*	Mating efficiency‡
	%
SPA2 STE2 + pRS313	<0.00038 (3)
SPA2 STE2 + pGAL-STE4^{Hpl}	0.37 ± 0.016 (3)
spa2-Δ2::TRP1 STE2 + pRS313	<0.00038 (3)
spa2-Δ2::TRP1 STE2 + PGAL-STE4^{Hpl}	0.012 ± 0.0012 (3)
SPA2 ste2Δ + pGAL-STE4^{Hpl}	1.2 ± 0.10 (3)
spa2-Δ2::TRP1 ste2Δ + pGAL-STE4^{Hpl}	0.078 ± 0.013 (3)

*The **a** strains used were Y604 (lines 1 and 2), Y609 (lines 3 and 4), Y604*ste2Δ*, and Y609*ste2Δ;* these strains contained either pRS313 or p21-1(pGAL-*STE4^{Hpl}*), as indicated. All matings were with 8941-12-2 as the α cell partner.
‡The percentage of **a** cells that formed diploids with α-pheromoneless cells. Matings were allowed to occur for 5 h at 30°C on Noble Agar plates containing raffinose and galactose as carbon sources. No α-factor was added to these matings.

The Pheromone Confusion Assay

The observation that high concentrations of exogenous pheromone inhibit the efficiency of chemotropic matings (Tables II and III; Fig. 1 *A*; Marcus et al., 1991) suggested to us that the degree of inhibition should provide a measure of the ability of a cell to sense gradients and orient its growth. In this pheromone confusion assay, chemotropism-defective mutants should mate as efficiently with wild-type cells when gradients of pheromone are present as they do when they must mate by the default pathway in isotropic, high pheromone concentrations; in both conditions chemotropism-defective mutants are unable to sense gradients of pheromone and must find a mate by a mechanism that is independent of gradient information. For example, an *sst2-1* mutant mates nearly as well with wild-type cells in the absence of exogenous pheromone as it does in the presence of exogenous pheromone (11 ± 1.4% and 6.3 ± 0.78%, respectively [Table IX]) and fails to discriminate mating partners under both conditions (randomness indices of 0.63 ± 0.098 and 0.89 ± 0.087, respectively). Under these same conditions a wild-type **a** cell experiences a 6.2-fold inhibition of mating by exogenous pheromone (Table IX), and fails to discriminate only in the presence of pheromone (randomness indices of 0.58 ± 0.20 and $6.7 \times 10^{-6} \pm 4.6 \times 10^{-6}$, respectively [Table IX]).

A far1Δ a Strain Is Defective in the Pheromone Confusion Assay

While *FAR1* was identified because it is required for cell cycle arrest in response to pheromone, it must have an additional function in mating, since mutant alleles of *FAR1* exist that confer an Arrest+ Mating− phenotype (Chang and Herskowitz, 1990; Chang, 1991). This mating defect exists in *far1* mutants despite a wild-type transcriptional response to exogenous pheromone, no defect in karyogamy, and wild-type shmoo formation (Chang and Herskowitz, 1990; Chang, 1991). Therefore, to explain their mating defect, Chang (1991) suggested that *far1* mutants may have a defect in chemotropism. In support of this hypothesis, MAT**a** *far1*-mutants are sterile when mated with MATα *far1*-mutants (Chang and Herskowitz, 1990), a phenotype expected if both mating partners are incapable of finding a mate by chemotropism.

Therefore, we tested *far1Δ* mutants both in the pheromone confusion assay and the discrimination assay. While the addition of 20 μM α-pheromone inhibited the mating efficiency of the control *FAR1* **a** strain by about eightfold (160 ± 25% to 20 ± 2.5% [Table IX]), *far1Δ* **a** cells mated as efficiently in the presence of pheromone as they did in its absence (3.2 ± 0.21% and 3.3 ± 0.55%, respectively [Table IX]). Since the mating efficiency of *far1Δ* **a** cells was not inhibited by the loss of pheromone gradients, we conclude that *far1Δ* **a** cells do not use the information present in pheromone gradients to more efficiently locate a partner and that *far1Δ* cells are chemotropism-defective. In support of this hypothesis, we found that an *sst2-1far1Δ* double mutant mated as efficiently as a *far1Δ* single mutant (data not shown).

However, *far1Δ* cells are not discrimination-defective (randomness index of $5.6 \times 10^{-4} \pm 3.4 \times 10^{-4}$ [Table IX]; Chenevert, 1994; Schrick, 1994). This ability to discriminate pheromone producing from nonproducing mating partners is not due to an inability of *far1Δ* cells to mate

Table IX. sst2-1 Mutants and far1Δ Mutants Are Defective in the Pheromone Confusion Assay

a Strain*	α-factor‡	Total mating efficiency§	Randomness index‖	Fold inhibition by α-factor¶
		%		
SST2	−	160 ± 25 (6)	$6.7 \times 10^{-6} \pm 4.6 \times 10^{-6}$ (6)	
SST2	+	26 ± 6.1 (6)	0.58 ± 0.20 (6)	6.2
sst2-1	−	11 ± 1.4 (3)	0.63 ± 0.098 (3)	
sst2-1	+	6.3 ± 0.78 (3)	0.89 ± 0.087 (3)	1.7
FAR1	−	160 ± 25 (3)	$8.6 \times 10^{-6} \pm 8.6 \times 10^{-6}$ (3)	
FAR1	+	20 ± 2.5 (3)	0.73 ± 0.021 (3)	8.0
far1Δ	−	3.3 ± 0.55 (3)	$5.6 \times 10^{-4} \pm 3.4 \times 10^{-4}$ (3)	
far1Δ	+	3.2 ± 0.21 (3)	0.67 ± 0.16 (3)	1.0

*The **a** strains used were 7609-1-4 (*SST2*), 7612-8-2 (*sst2-1*), 8940-6-3 (*FAR1*), and 8940-4-3 (*far1Δ*); the wild-type α strains used were 7611-2 (lines 1-4) and 7609-6-4 (lines 5-8), and the pheromoneless α strains were 8941-12-2 (lines 1-4) and 10843-9-2 (lines 5-8).
‡Matings were for 3 h at 30°C on Noble Agar plates in the absence (−) or in the presence (+) of 20 μM α-factor.
§See footnote §, Table V.
‖See footnote ‖, Table II.
¶Calculated as the total mating efficiency of the **a** strain in the absence of pheromone, and divided by the mating efficiency of the **a** strain in the presence of pheromone.

with pheromoneless cells, since the addition of pheromone causes *far1Δ* cells to mate randomly in the discrimination assay (randomness index of 0.67 ± 0.16 [Table IX]; see Discussion for an explanation of these observations).

far1 Mutants Display a Fixed Polarity

We wished to test the hypothesis (Chang, 1991) that *far1* mutants have their conjugation site fixed at the incipient bud site. We made use of the fact that when **a** cells are briefly exposed to low levels of α-factor, their axial budding pattern is randomized (Madden and Snyder, 1992). These low levels of pheromone may mimic the chemotropic situation because receptors are not saturated for pheromone binding, and, as a result, cells depolarize their actin distribution and erase the axial bud site (Madden and Snyder, 1992). We reasoned that if *far1* mutants have their conjugation site fixed at the incipient bud site, then the Far1 protein may be the molecular eraser of the axial bud site. In this model *far1* mutants should not randomize their budding patterns in response to low levels of pheromone and should still bud axially.

To perform this experiment we used an allele of *FAR1*, *far1-16D*, that is Arrest+ Mating− (isolated in a screen for MAT**a** mutants that mate poorly with MATα *sst2-1* cells [Dorer, R., unpublished data]). Cells carrying this mutation are not defective in shmoo formation (data not shown) or cell cycle arrest in response to pheromone (Fig. 5), but show about a 10-fold decrease in mating efficiency and a defect in the pheromone confusion assay (data not shown).

In the absence of pheromone, both wild-type cells and *far1-16D* mutants bud at axial sites (Table X), as indicated by the budding patterns of four cell microcolonies (scored as in Chant and Herskowitz, 1991). Madden and Snyder (1992) treated **a** cells in liquid culture with a brief exposure to α-factor and observed a mild randomization of budding pattern after the pheromone was removed; however, not all cells in the culture arrested during the α-factor exposure. To observe a stronger effect, we wanted to ensure that we scored only those cells that in the past had been exposed to pheromone and delayed in the cell cycle. Therefore, we exposed cells to pheromone on YEPD plates and allowed them to adapt to the presence of the pheromone and resume cell division (see Materials and Methods). The presence of 6 nM pheromone caused an ~2-h delay in the appearance of 3 or 4 cell microcolonies (Fig. 5), ensuring that the colonies that we scored after the delay had been affected by pheromone. Higher concentrations of pheromone (10 nM) caused cell shape changes and a significantly longer arrest period (data not shown). We observed that wild-type **a** cells switched from an axial to a bipolar-budding pattern in the presence of 6 nM pheromone, with 59% of the cells budding in a class I pattern and 5.7% in a class II pattern, data indicative of the bipolar-budding pattern seen in diploids (Table X; Chant and Herskowitz, 1991); for example, a diploid displayed 48% class I and 9.0% class II (Table X).

In contrast to wild-type cells, *far1-16D* mutants budded predominantly at axial sites in the presence of 6 nM pheromone (73% class IV [Table X]), even though the cells we scored delayed in the cell cycle in response to α-factor (Fig. 5). The small percentage of cells that budded in a class I pattern (6.8%) were most likely a result of the fact that the *far1-16D* mutation is hypomorphic; this allele still retains partial mating function of the *FAR1* gene (data not shown). In addition, a *far1Δ* strain budded at axial sites in the presence of 0 M, 6 nM, and 4 μM α-factor (Table X).

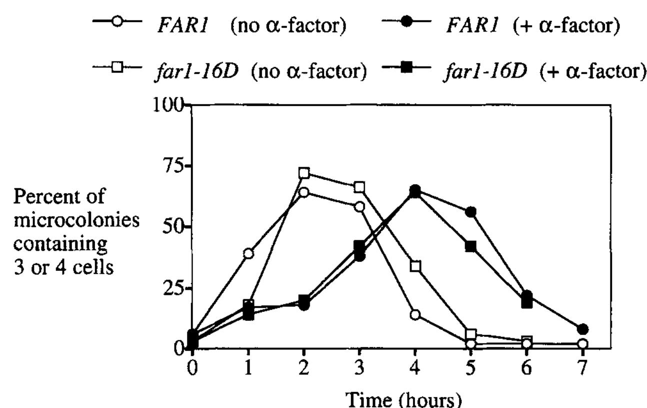


Figure 5. The recovery of **a** cells from low levels of pheromone. YEPD plates were spread with 10^5 cells that were either *FAR1* (7611-4 [*circles*]) or *far1-16D* (10815-14-4 [*squares*]), and the percentage of microcolonies that contained either 3 or 4 cells was determined at the indicated number of hours after plating. Plates were incubated at 30°C. Plates contained either no α-factor (*open symbols*) or 6 nM α-factor (*closed symbols*).

Table X. FAR1 Is Required for the Randomization of Budding Patterns That Is Induced by Low Levels of α-factor

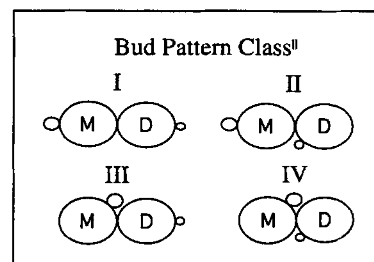
Strains*	Hours after planning‡	α-factor§	Bud pattern class‖ I	II	III	IV	Predominant pattern
			%				
a *FAR1*	2.2–2.4	−	0.5	<0.25	11	88	axial
a *FAR1*	4.6–5.0	+	59	5.7	17	18	bipolar
a *far1-16D*	2.3–2.7	−	0.2	0.75	12	87	axial
a *far1-16D*	4.6–4.9	+	6.8	4.5	16	73	axial
a *far1Δ*	2.3–2.5	−	0.3	0.3	14	85	axial
a *far1Δ*	2.2–2.7	+	<0.3	1.7	7.3	91	axial
a *far1Δ*	3.2–3.7	4 μM	1.0	2.7	11	85	axial
a/α FAR1	2.2–2.4	−	48	9.0	16	28	bipolar

*The **a** strains used were 7611-4 (*FAR1*), 10815-14-4 (*far1-16D*), 8940-4-3 (*far1Δ*), and 10703 (*a/αFAR1*).

‡Four cell microcolonies were scored, the indicated number of hours after cells were plated on YEPD plates. All plates were incubated at 30°C.

§Cells were incubated on YEPD plates either in the presence (+) or absence (−) of 6 nM α-factor.

‖The percentage of four cell microcolonies that displayed the indicated budding pattern class. Patterns were scored as in Chant and Herskowitz (1991). Class I is characteristic of a bipolar pattern and class IV, an axial pattern. Between 300 and 400 microcolonies were scored for each line above.



Therefore, we conclude that *far1* mutants have their polarity fixed at the incipient bud site.

Deletions of FAR1 and SPA2 Are Synthetic Sterile

Since cells form projections near the incipient bud site under conditions in which they execute the default pathway (Madden and Snyder, 1992; Figs. 1 *C* and 2), *far1* mutants may have their conjugation site fixed at the default mating site. As a result, in contrast to wild-type cells, *far1Δ* cells should require *SPA2* to mate with a wild-type cell partner. Consistent with this prediction, we found that a *far1Δspa2Δ* double mutant mated with wild-type α cells at an efficiency that was 1,000-fold lower than the efficiency of the *far1Δ* single mutant (~0.002% and 2.0%, respectively [Table XI]). These data suggest that *far1Δ* mutants mate either by executing the default pathway or by using some components of the default pathway in order to mate.

Discussion

Default Pathway for Mating Partner Selection

Wild-type yeast cells grow toward a mating partner by sensing gradients of pheromone and orienting their actin cytoskeleton and secretion toward the site of highest pheromone concentration on the cell surface (Jackson and Hartwell, 1990*a*,*b*; Segall, 1993). In this paper, we investigate the ability of cells to mate in the absence of pheromone gradients and characterize a novel mating pathway: when yeast cells are exposed to high, isotropic pheromone concentrations and the pheromone response pathway is saturated, wild-type **a** cells execute a default pathway in order to select a mate (Fig. 1, *A* and *B*). Since the shmoo tip formed in high, isotropic α-factor concentrations is positioned near the incipient bud site (Madden and Snyder, 1992; Fig. 2), and since shmoo formation correlates with the onset of the default mating pathway (Fig. 1 *C*), we suggest that when **a** cells execute the default pathway they choose a site near the incipient bud site as the mating site by default. In addition, these observations may explain why the shmoo response of **a** cells in saturating pheromone is morphologically distinct from the response of **a** cells in pheromone gradients; in saturating pheromone cells, form multiple projections that are short, pointed, and produced successively (Lipke et al., 1976; Tkacz and MacKay, 1979; Moore, 1983; Baba et al., 1989; Segall, 1993), but in nonsaturating pheromone and in gradients, cells form single projections that are much longer and wider (Levi, 1953; Segall, 1993; Yorihuzi and Ohsumi, 1994). These two responses may reflect the induction of the default and chemotropic pathways, respectively.

What is the role of the default pathway in the normal yeast life cycle? Jackson and Hartwell (1990*b*) demonstrated that when wild-type **a** cells are surrounded by an excess of wild-type α cells, the fraction of **a** cells that mate by the default pathway is high. Therefore, we speculate that the default pathway is activated when cells are surrounded by an overwhelming excess of opposite mates. This situation could occur when a rare cell in a growing colony of a natural heterothallic yeast strain spontaneously switches mating type. Alternatively, the default pathway could be the major pathway for mating when the pH of the media is low, a condition that inactivates the Bar1 protein, the protease that degrades α-factor (Hartwell, 1980; MacKay et al., 1988); this hypothesis is consistent with the fact that *bar1-1* mutants mate frequently with pheromoneless cells in a discrimination assay (Jackson and Hartwell, 1990*b*).

Table XI. Deletions of FAR1 and SPA2 Display Synthetic Sterility

a Strain*	Mating efficiency‡
	%
FAR1 SPA2	225
	246
FAR1 spa2Δ	93
	80
far1Δ SPA2	2.0
	1.8
far1Δ spa2Δ	0.0018
	0.0014

*The **a** strains used were, in order from top to bottom, Y604, Y609, Y604*far1Δ*, and Y609*far1Δ*. The wild-type α strain used was 7611-2.
‡The percentage of input haploid cells that mated. Matings were allowed to occur for 3 h at 30°C in the absence of added α-factor.

Two Classes of Genes Indicate Two Pathways for Mating Partner Selection

The key observation that distinguishes the default mating pathway from the chemotropic mating pathway is that these pathways require different genes; *SPA2* is required for default, and *SST2*, *STE2*, and *FAR1* are required for chemotropism. The following observations lead us to conclude that *SPA2* is required for default matings and not for chemotropic matings. First, a *spa2Δ* **a** strain mated very poorly in the default mating assay, where **a** cells are mated with pheromoneless α cells in the presence of high, isotropic pheromone concentrations (Table III). Second, the mating ability of a *spa2Δ* **a** strain was inhibited 1,100-fold by the addition of exogenous pheromone; therefore a *SPA2* -dependent mating pathway is induced in wild-type cells by high pheromone concentrations (Fig. 3). Third, *SPA2* is not required for chemotropic matings: a *spa2Δ* **a** strain mated very efficiently with both wild-type α cells and *spa2Δ* α cells on media lacking exogenous pheromone when cell densities were high (Table III; Fig. 3; Gehrung and Snyder, 1990).

In contrast to *SPA2*, the *SST2* gene is not required for the default pathway, since an *sst2-1* **a** strain mated as well as a wild-type **a** strain in the default mating assay (Table IV). Instead, three observations suggest that *SST2* is required for chemotropism because it mediates the sensitivity of cells to pheromone gradients and ensures that cells can orient over a large range of pheromone concentrations. First, previous experiments showed that *sst2-1* cells fail to discriminate wild-type from pheromoneless mating partners and mate at a reduced efficiency (Jackson and Hartwell, 1990*b*; Schrick, 1994). Second, we demonstrate that both of these mating defects are suppressible in *sst2-1* α cells by mating to an **a** strain that produces less pheromone because it contains a hypomorphic mutation in

STE6, the gene encoding the **a**-factor transporter (Table V). Third, *sst2-1* mutants can orient projections along an artificial gradient of α-pheromone if the pheromone concentration in the gradient is very low (Segall, 1993).

In addition, we observed that *sst2-1* cells are saturated for pheromone-induced transcription of *FUS1-lacZ* in a mating assay with wild-type partners (Table VI). Taken together with the above data, these data suggest that *SST2* prevents cells from executing the default pathway when wild-type mating partners are present (Fig. 6). This hypothesis makes the explicit predictions that the deletion of *SPA2* in an *sst2Δ* strain should suppress the discrimination defect caused by the *sst2Δ* mutation and that deletions of *SPA2* and *SST2* should display a synthetic decrease in mating efficiency; both of these predictions have been confirmed (Table VII).

The Role of SPA2 in the Default Mating Pathway

Early in the G1 phase of the cell cycle and before bud emergence, a cell is poised to polarize its growth at the incipient bud site. Several components that are important for cell polarity are positioned at the bud site before bud emergence, including Cdc42p and Spa2p, among others (for reviews see Chant, 1994; Chenevert, 1994). *CDC42*, and two other genes, *BEM1* and *CDC24*, are members of a group of polarity establishment genes that organize the actin cytoskeleton toward the bud site (Drubin, 1991). In addition, *BEM1* and *CDC24* are important for efficient mating (Chenevert et al., 1992; Chenevert, 1994; Reid and Hartwell, 1977), and therefore this group of genes appears to be generally required for the emergence of polarized structures in yeast. During default matings, cells use the incipient bud site to construct a mating projection by a process that requires *SPA2*; thus the Spa2 protein may interact with the polarity establishment proteins or cytoskeletal proteins in order to modify the incipient bud site to produce a mating projection. Alternatively, Spa2p may not interact with the polarity establishment proteins, but may restrict components that are required for cell wall or membrane fusion to the incipient bud site. The Spa2 protein localizes as a sharp patch to the tip of the growing shmoo (Snyder, 1989; Snyder et al., 1991), and as a result it is an excellent candidate for a protein that polarizes the cytoskeleton, secretion, or cell fusion machinery to the shmoo tip. Since preliminary observations indicate that other shmoo-defective mutants (*afr1Δ* [Konopka et al., 1995], *bem1Δ* [Chenevert et al., 1992, 1994], *ste2-T326* [Konopka et al., 1988], and *tny1* [Chenevert et al., 1994]) do not show specific defects in the default pathway (Dorer, R., unpublished observations), we suggest that the default mating defect of *spa2Δ* cells may not be caused by the shmoo defect, but may be a result of some other requirement for *SPA2* during default matings. Unfortunately, the sequence of *SPA2* reveals little about its function; it encodes a 180-kD protein that displays some low level sequence similarities to proteins containing coiled-coil structures (Gehrung and Snyder, 1990).

While *SPA2* certainly performs a function that is required for default matings and for shmoo formation, its role in vegetatively growing cells is unclear. Even though the *SPA2* gene is not required for bud emergence, bud growth, or cytokinesis, the protein localizes to the tip of the growing bud and to the cytokinesis ring (Snyder, 1989; Snyder et al., 1991); and *spa2* mutants display a mildly randomized budding pattern (Snyder, 1989), a rounder cell shape than wild-type cells, synthetic lethality with a *cdc10* mutation, which causes a cytokinesis defect (Flescher et al., 1993), and synthetic lethality with a deletion of the *SLK1/BCK1* gene, a *STE11* homologue that is required for cell wall integrity (Costigan et al., 1992; Lee and Levin, 1992).

The Role of the Signal Transduction Pathway in the Default Mating Pathway

Our results demonstrate that *SPA2* is required for matings induced by the overexpression of an activated *STE4* allele,

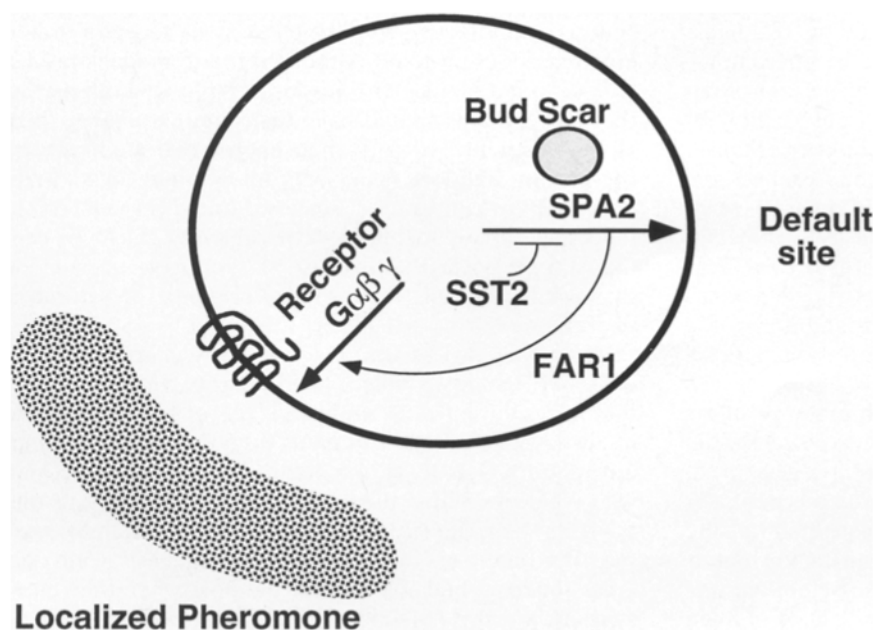


Figure 6. A genetic model for mating partner selection. The pheromone receptor and heterotrimeric G-protein are required for chemotropic morphogenesis (Jackson et al., 1991; Schrick, 1994), and *SPA2* is required for default matings. The *SST2* gene is required for inhibiting the execution of the default pathway because it mediates the sensitivity of cells to pheromone, and *FAR1* is required for chemotropic morphogenesis because it prevents cells from using the default site to mate.

$STE4^{Hpl}$, in both wild-type **a** cells and in *ste2Δ* **a** cells (Table VIII). Therefore, *STE4* or components of the signal transduction pathway downstream of *STE4* activate the default pathway by a process that requires *SPA2*. In addition, we conclude that beyond their role in activating the signal transduction pathway, pheromone receptors are not required for default matings, a conclusion that is consistent with the observation that receptorless cells are capable of shmoo formation if the signal transduction pathway is activated downstream (Jahng et al., 1988; Clark and Sprague, 1989). Therefore, while pheromone receptors are required for selecting a conjugation site when gradients of pheromone are present, they are not required for selecting the default mating site. It is interesting to note that in addition to *STE4*, activated *STE5* and *STE11* alleles are capable of causing smaller, but significant, numbers of mating events in receptorless cells (Hasson et al., 1994; Stevenson et al., 1992), suggesting that the default pathway may be at least partially activated by the MAP kinase cascade. It will be important to determine whether individual components of the signal transduction pathway activate the default pathway by a mechanism that is distinct from their known role in activating the transcription of mating-specific genes; some components may perform two functions, one in activating the next component of the cascade and another in activating proteins that are required for chemotropic or default matings.

How does the signal transduction pathway activate two behaviorally distinct mating pathways, chemotropic and default, in response to two different levels of pheromone? There must be a molecular explanation for the dramatic increase in default mating efficiency compared to the more gradual increase in *FUS1-lacZ* expression in **a** cells in response to increasing concentrations of exogenous α-factor (Fig. 1, *A* and *B*). Two general models could explain these behaviors. In the first model the default pathway differs from the chemotropic pathway in a quantitative sense, in that default matings may require an increase in the level of activity or expression of pheromone-inducible gene products over the level in chemotropic matings. At least two molecular explanations are consistent with this model. First, there may be cooperative effects or oligomers formed among proteins that are activated by pheromone, and subtle changes in the relative levels of these proteins may determine which mating pathway is used. For instance, Ste20p may interact with Cdc42p in yeast (Manser et al., 1994); perhaps saturating pheromone changes the levels or spatial arrangements of activated polarity establishment proteins on the cell surface, and by this means, causes cells to use the incipient bud site for mating. Second, the dramatic increase in default mating efficiency may occur when the concentration of an inhibitor of the default pathway is exceeded by the concentration of an activator.

In the second general model, the default pathway differs from the chemotropic pathway in a qualitative sense: default matings may require a different selection of gene products to be activated or expressed. There are precedents for this type of mechanism. In the amphibian embryo, the concentration of activin, a peptide growth factor, determines the selection of genes that are expressed in blastula cells (Gurdon et al., 1994; Green and Smith, 1990; Green et al., 1992); and in the *Drosophila* embryo a gradient of the *bicoid* protein determines the anterior-posterior axis by controlling the transcription of *hunchback* (Driever and Nüsslein-Volhard, 1988; Driever et al., 1989; Struhl et al., 1989). In yeast, the overexpression of Ste12p, a transcription factor, can partially suppress the sterility of receptorless cells (Dolan and Fields, 1990), suggesting that the default pathway can be activated by affecting the transcription of certain genes. In addition, proteins that are not required for chemotropic matings, such as Spa2p, may be activated or modified only in response to saturating pheromone. It is interesting to note that Mpk1p, a MAP kinase homolog that is required for cell wall integrity, is activated when cells are exposed to α-factor, coincident with shmoo formation (Levin and Errede, 1995); perhaps Mpk1p activates the default pathway. Alternatively, a combination of mechanisms may be important. For example, *FAR1* is required for cell cycle arrest and is regulated in both RNA and protein level (Chang and Herskowitz, 1990; McKinney et al., 1993), and in response to pheromone the Far1 protein is phosphorylated by Fus3p (Chang and Herskowitz, 1992; Elion et al., 1993; Peter et al., 1993).

The Role of FAR1 in Mating Partner Selection

When a cell is exposed to a gradient of pheromone, it probably reorients the polarity establishment proteins (Cdc42p, etc.) from the incipient bud site to the site of highest pheromone concentration on the cell surface. This reorientation requires pheromone receptors and their associated heterotrimeric G-proteins (Jackson et al., 1991; Schrick, 1994), and in this paper we propose that Far1p also promotes this gradient-dependent change in cell polarity. Specifically, we show that unlike wild-type **a** cells, the mating efficiency of *far1Δ* **a** cells is not inhibited by the induction of the default mating pathway (Table IX), suggesting that *far1Δ* cells do not use pheromone gradients as a directional cue to find a mate. Far1p may inhibit the function of the bud site selection genes at the incipient bud site, acting as a molecular eraser of this predetermined site, or Far1p may directly stimulate chemotropic morphogenesis by interacting with the pheromone receptor or heterotrimeric G-protein. Although these models predict very different molecular roles for Far1p, they both predict that *far1* mutants should have their mating polarity fixed at the incipient bud site when pheromone gradients are present. In addition, *FAR1* may be required for an additional step in conjugation, since we found that *far1Δ* mutants mate about sixfold more poorly with wild-type cells (3.3 ± 0.55%) in the absence of saturating pheromone than wild-type cells mate in the presence of saturating pheromone (20 ± 2.5% [Table IX]).

We report two pieces of evidence that are consistent with *far1* mutants having a fixed polarity. First, we found that in response to low uniform levels of pheromone, unlike wild-type cells, *far1* mutants do not change their budding pattern from axial to bipolar (Table X). The fact that wild-type cells bud at the poles as they adapt suggests that Far1p may specifically erase the axial bud site and not affect cues that mark the pole for morphogenesis. Cells also erase the axial bud site but not the pole when they enter stationary phase (Madden and Snyder, 1992; Chant and

Pringle, 1995); perhaps there is a protein equivalent to Far1p that specifically erases the axial bud site when cells enter stationary phase. It is interesting to note that the Bud3 protein, which is required for the axial signal, displays a transient localization in exponentially growing cells (Chant et al., 1995). Since a signal must be present continuously in the G1 phase that marks the axial bud site, the Far1p protein may directly affect the function of Bud3p or a protein that Bud3p localizes during the G1 phase. Alternatively, cells may not need to directly erase the axial bud site in stationary phase if the axial signal created by Bud3p has a short half-life and disassembles spontaneously over time. Second, we found that mutations in *FAR1* and *SPA2* are synthetic sterile (Table XI), demonstrating that in contrast to wild-type cells, *far1Δ* mutants mate by a mechanism that requires *SPA2*. This observation is consistent both with *far1* mutants having their polarity fixed at the incipient bud site and with *SPA2* being required to construct a mating projection at the incipient bud site (Fig. 6). It will be interesting to determine whether *far1* mutants are permanently fixed at the incipient bud site for shmoo formation and are unable to form multiple mating projections when incubated in the presence of saturating pheromone for several hours. This process of reorienting the polarity axis in saturating pheromone is not understood, but probably involves many of the genes discussed in this paper, since cells must establish and develop a new site of polarity on their surface.

In addition to showing a defect in the pheromone confusion assay, *far1* mutants cannot orient mating projections along gradients of pheromone created by micropipets (Valtz et al., 1995). Moreover, *far1* mutants fuse with their mating partners at a site that is adjacent to their last bud site (Valtz et al., 1995). These observations, coupled with ours, cause us to wonder how *far1Δ* **a** cells discriminate wild-type α cells from pheromoneless α cells (Table IX; Chenevert, 1994; Schrick, 1994). We suggest that the key factor that prevents *far1Δ* mutants from mating with pheromoneless cells in a discrimination assay is that *far1Δ* mutants display wild-type sensitivity to pheromone (Chang and Herskowitz, 1990). In this paper, we show that mating events with pheromoneless cells require the execution of the default pathway, and *FUS1-lacZ* data suggest that this default pathway is only activated when the pheromone response pathway is saturated (Fig. 1 *B*). We do not understand why cells require saturating levels of signal to mate with pheromoneless cells. Perhaps a high, local pheromone signal is required for the completion of all mating events, a requirement that is normally met when partners are closely aligned just before fusion. Nevertheless, in a discrimination assay to which no pheromone is added, all cells with wild-type sensitivity to pheromone, including *far1Δ* mutants, should not mate with pheromoneless cells because their pheromone response pathway is not saturated (Fig. 1 *B*). Thus, even though they have their polarity fixed at the incipient bud site, and even though they may produce a projection that orients toward a pheromoneless cell, *far1Δ* cells do not complete mating events with pheromoneless cells because their signal transduction pathway is not saturated. As a result, *far1Δ* mutants still require their partner to produce pheromone in order to complete conjugation. This model makes the explicit prediction that cells that display wild-type sensitivity to pheromone will not mate with pheromoneless cells in the discrimination assay, even if they are chemotropism-defective.

Analogies between Yeast and Other Systems

Cell exhibits two types of cell polarity mechanisms, one in which their polarity is determined by an internal program, and another in which their polarity is determined by external cues. For example, the axes of cell division during the early development of *C. elegans* (Priess, 1994) and during many developmental stages of plants (Meyerowitz, 1994) are genetically predetermined. On the other hand, chemoattractants guide many changes in cell polarity, such as the migration of leukocytes through endothelial cells (Springer, 1994), the chemotaxis of dictyostelium amoebae toward one another to form multicellular structures (Devreotes and Zigmond, 1988; Gross, 1994), and the chemotropism of nerve cell axons toward their target tissues during development (Goodman, 1994; Goodman and Shatz, 1993). These two mechanisms are conceptually similar to the budding and chemotropic mating polarity pathways in yeast, respectively. The existence of a default pathway that uses components of both pathways provides a relationship between these two types of cell polarity mechanisms.

We thank Kathrin Schrick and Michael Snyder for yeast strains, Ira Herskowitz, Jamie Konopka, Henrik Dohlman, Jeremy Thorner, Malcolm Whiteway, Susan Michaelis, and Aaron Neiman for plasmids, Josh Kim for technical assistance, Michael Snyder, an anonymous reviewer, members of the Hartwell lab, and Breck Byers for critical review of the manuscript, and Nicole Valtz, Matthias Peter, and Ira Herskowitz for communicating results before publication and suggesting the term, "pheromone confusion assay".

This work was supported by a grant from the National Institutes of Health, General Medical Sciences grant GM61-4590. R. Dorer was supported by the National Institutes of General Medical Sciences, Medical Scientist Training Program grant GM07266, and training grant T32GM-07735. P. M. Pryciak was supported by the Damon Runyon-Walter Winchell Cancer Research Fund (DRG1158).

Received for publication 6 June 1995 and in revised form 24 August 1995.

References

Baba, M., N. Baba, Y. Ohsumi, K. Kanaya, and M. Osumi. 1989. Three-dimensional analysis of morphogenesis induced by mating pheromone α-factor in *Saccharomyces cerevisiae*. *J. Cell Sci.* 94:207–216.

Bardwell, L., J. G. Cook, C. J. Inouye, and J. Thorner. 1994. Signal propagation and regulation in the mating pheromone response pathway of the yeast *Saccharomyces cerevisiae*. *Dev. Biol.* 166:363–379.

Bender, A., and J. R. Pringle. 1989. Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the ras-related gene RSR1. *Proc. Natl. Acad. Sci. USA.* 86:9916–9980.

Byers, B. 1981. Cytology of the yeast life cycle. *In* The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance. J. N. Strathern, E. W. Jones, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 59–96.

Byers, B., and L. Goetsch. 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. *J. Bacteriol.* 124:511–523.

Chang, F. 1991. Regulation of the cell cycle by a negative growth factor in yeast. Ph.D. Thesis University of California, San Francisco, CA. pp. 97–118.

Chang, F., and I. Herskowitz. 1990. Identification of a gene necessary for cell cycle arrest by a negative growth factor of yeast: *FAR1* is an inhibitor of a G1 cyclin, CLN2. *Cell.* 63:999–1011.

Chang, F., and I. Herskowitz. 1992. Phosphorylation of FAR1 in response to α-factor: a possible requirement for cell-cycle arrest. *Mol. Biol. Cell.* 3:445–450.

Chant, J. 1994. Cell polarity in yeast. *Trends. Genet.* 10:328–333.

Chant, J., and I. Herskowitz. 1991. Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. *Cell.* 65: 1203–1212.

Chant, J., and J. R. Pringle. 1995. Patterns of bud site selection in the yeast *Sac-*

charomyces cerevisiae. J. Cell. Biol. 129:751–765.

Chant, J., K. Corrado, J. R. Pringle, and I. Herskowitz. 1991. Yeast *BUD5*, encoding a putative GDP-GTP exchange factor, is necessary for bud site selection and interacts with bud formation gene *BEM1. Cell.* 65:1213–1224.

Chant, J., M. Mischke, E. Mitchell, I. Herskowitz, and J. R. Pringle. 1995. Role of Bud3p in producing the axial budding pattern of yeast. *J. Cell Biol.* 129: 767–778.

Chan, R. K., and C. A. Otte. 1982. Physiological characterization of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a**-factor and α-factor pheromones. *Mol. Cell. Biol.* 2:21–29.

Chenevert, J., K. Corrado, A. Bender, J. Pringle, and I. Herskowitz. 1992. A yeast gene (*BEM1*) necessary for cell polarization whose product contains two SH3 domains. *Nature (Lond.).* 356:77–79.

Chenevert, J., N. Valtz, and I. Herskowitz. 1994. Identification of genes required for normal pheromone-induced cell polarization in *Saccharomyces cerevisiae. Genetics.* 136:1287–1296.

Chenevert, J. 1994. Cell polarization directed by extracellular cues in yeast. *Mol. Biol. Cell.* 5:1169–1175.

Clark, K. L., and G. F. Sprague. 1989. Yeast pheromone response pathway: characterization of a suppressor that restores mating to receptorless mutants. *Mol. Cell. Biol.* 9:2682–2694.

Costigan, C., S. Gehrung, and M. Snyder. 1992. A synthetic lethal screen identifies *SLK1*, a novel protein kinase homolog implicated in yeast cell morphogenesis and cell growth. *Mol. Cell. Biol.* 12:1162–1178.

Devreotes, P. N., and S. H. Zigmond. 1988. Chemotaxis in eukaryotic cells: a focus on leukocytes and *Dictyostelium. Annu. Rev. Cell Biol.* 4:649–686.

Dietzl, C., and J. Kurjan. 1987. Pheromonal regulation and sequence of the *Saccharomyces cerevisiae SST2* gene: a model for desensitization to pheromone. *Mol. Cell. Biol.* 7:4169–4177.

Dolan, J. W., and S. Fields. 1990. Overproduction of the yeast Ste12 protein leads to constitutive transcriptional induction. *Genes & Dev.* 4:492–502.

Driever, W., and C. Nüsslein-Volhard. 1988. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell.* 54:95–104.

Driever, W., G. Thoma, and C. Nüsslein-Volhard. 1989. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature (Lond.).* 340:363–367.

Drubin, D. G. 1991. Development of cell polarity in budding yeast. *Cell.* 65: 1093–1096.

Elion, E. A., B. Satterberg, and J. E. Kranz. 1993. FUS3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAR1. *Mol. Biol. Cell.* 4:495–510.

Flescher, E. G., K. Madden, and M. Snyder. 1993. Components required for cytokinesis are important for bud site selection in yeast. *J. Cell Biol.* 122:373–386.

Fugita, A., C. Oka, Y. Arikawa, T. Katagai, A. Tonouchi, S. Kuhara, and Y. Misumi. 1994. A yeast gene necessary for bud-site selection encodes a protein similar to insulin-degrading enzymes. *Nature (Lond.).* 372:567–570.

Gehrung, S., and M. Snyder. 1990. The *SPA2* gene of *Saccharomyces cerevisiae* is important for pheromone-induced morphogenesis and efficient mating. *J. Cell Biol.* 111:1451–1464.

Gietz, R. D., and R. H. Schiestl. 1991. Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. *Yeast.* 7:253–263.

Goodman, C. S. 1994. The likeness of being: phylogenetically conserved molecular mechanisms of growth cone guidance. *Cell.* 78:353–356.

Goodman, C. S., and C. J. Shatz. 1993. Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell.* 72(suppl):77–98.

Green, J. B. A., and J. C. Smith. 1990. Graded changes in dose of a *Xenopus* activin A homologue elicit stepwise transitions in embryonic cell fate. *Nature (Lond.).* 347:391–394.

Green, J. B. A., H. New, and J. C. Smith. 1992. Responses of embryonic *Xenopus* cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell.* 71:731–739.

Gross, J. D. 1994. Developmental decisions in *Dictyostelium discoideum. Microbiol. Rev.* 58:300–351.

Gurdon, J. B., P. Harger, A. Mitchell, and P. Lemaire. 1994. Activin signalling and response to a morphogen gradient. *Nature (Lond.).* 371:487–492.

Hartwell, L. H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* 93:1662–1670.

Hartwell, L. H. 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. *J. Cell Biol.* 85:811–822.

Hasson, M. S., D. Blinder, J. Thorner, and D. Jenness. 1994. Mutational activation of the *STE5* gene product bypasses the requirement for G protein β and γ subunits in the yeast pheromone response pathway. *Mol. Cell. Biol.* 14: 1054–1065.

Herskowitz, I. 1995. MAP kinase pathways in yeast: formating and more. *Cell.* 80:187–197.

Jackson, C. L., and L. H. Hartwell. 1990*a*. Courtship in *Saccharomyces cerevisiae:* an early cell-cell interaction during mating. *Mol. Cell. Biol.* 10:2202–2213.

Jackson, C. L., and L. H. Hartwell. 1990*b*. Courtship in *S. cerevisiae:* both cell types choose mating partners by responding to the strongest pheromone signal. *Cell.* 63:1039–1051.

Jackson, C. L., J. B. Konopka, and L. H. Hartwell. 1991. *S. cerevisiae* α pheromone receptors activate a novel signal transduction pathway for mating partner discrimination. *Cell.* 67:389–402.

Jahng, K., J. Ferguson, and S. I. Reed. 1988. Mutations in a gene encoding the α subunit of a *Saccharomyces cerevisiae* G protein indicate a role in mating pheromone signalling. *Mol. Cell. Biol.* 8:2484–2493.

Jenness, D. D., A. C. Burkholder, and L. H. Hartwell. 1983. Binding of α-factor pheromone to yeast cells: chemical and genetic evidence for an α-factor receptor. *Cell.* 35:521–529.

Jenness, D. D., A. C. Burkholder, and L. H. Hartwell. 1986. Binding of α-factor pheromone to *Saccharomyces cerevisiae* **a** cells: dissociation constant and number of binding sites. *Mol. Cell. Biol.* 6:318–320.

Konopka, J. B., D. D. Jenness, and L. H. Hartwell. 1988. The C-terminus of the *S. cerevisiae* α-pheromone receptor mediates an adaptive response to pheromone. *Cell*. 54:609–620.

Konopka, J. B., C. DeMattei, and C. Davis. 1995. *AFR1* promotes polarized apical morphogenesis in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 15:723–730.

Kurjan, J. 1985. α-Factor structural gene mutations in *Saccharomyces cerevisiae:* effects on α-factor production and mating. *Mol. Cell. Biol.* 5:787–796.

Kurjan, J. 1992. Pheromone response in yeast. *Annu. Rev. Biochem.* 61:1097–1129.

Lee, K. S., and D. E. Levin. 1992. Dominant mutations in a gene encoding a putative protein kinase (*BCK1*) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. *Mol. Cell. Biol.* 12:172–182.

Levi, J. D. 1956. Mating reaction in yeast. *Nature (Lond.).* 177:153–154.

Levin, D. E. and B. Errede. 1995. The proliferation of MAP kinase signaling pathways in yeast. *Curr. Opin. Cell Biol.* 7:197–202.

Lipke, P. N., A. Taylor, and C. E. Ballou. 1976. Morphogenic effects of α-factor on *Saccharomyces cerevisiae* cells. *J. Bacteriol.* 127:610–618.

MacKay, V. L., S. K. Welch, M. Y. Insley, T. R. Manney, J. Holly, G. C. Saari, and M. L. Parker. 1988. The *Saccharomyces cerevisiae BAR1* gene encodes an exported protein with homology to pepsin. *Proc. Natl. Acad. Sci. USA.* 85:55–59.

Madden, K., and M. Snyder. 1992. Specification of sites for polarized growth in *Saccharomyces cerevisiae* and the influence of external factors on site selection. *Mol. Biol. Cell.* 3:1025–1035.

Madden, K., C. Costigan, and M. Snyder. 1992. Cell polarity and morphogenesis in *Saccharomyces cerevisiae. Trends Cell Biol.* 2:22–29.

Manser, E., T. Leung, H. Salihuddin, Z. S. Zhao, and L. Lim. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature (Lond.).* 367:40–46.

Marcus, S., G. A. Caldwell, D. Miller, C. B. Xue, F. Naider, and J. M. Becker. 1991. Significance of C-terminal cysteine modifications to the biological activity of the *Saccharomyces cerevisiae* **a**-factor mating pheromone. *Mol. Cell. Biol.* 11:3603–3612.

Marsh, L., A. M. Neiman, and I. Herskowitz. 1991. Signal transduction during pheromone response in yeast. *Annu. Rev. Cell Biol.* 7:699–728.

McCaffrey, G., F. J. Clay, K. Kelsey, and G. F. Sprague. 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae. Mol. Cell. Biol.* 7:2680–2690.

McKinney, J. D., F. Chang, N. Heintz, and F. R. Cross. 1993. Negative regulation of *FAR1* at the start of the yeast cell cycle. *Genes & Dev.* 7:833–843.

Meyerowitz, E. 1994. Pattern formation in plant development: four vignettes. *Curr. Opin. Gen. Dev.* 4:602–608.

Michaelis, S. 1993. *STE6*, the yeast **a**-factor transporter. *Semin. Cell. Biol.* 4:17–27.

Michaelis, S., and I. Herskowitz. 1988. The **a**-factor pheromone of *Saccharomyces cerevisiae* is essential for mating. *Mol. Cell. Biol.* 8:1309–1318.

Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Moore, S. A. 1983. Comparison of dose response curves for α-factor induced cell division arrest, agglutination, and projection formation of yeast cells. *J. Biol. Chem.* 258:13849-13856.

Peter, M., A. Gartner, J. Horeka, G. Ammerer, and I. Herskowitz. 1993. FAR1 links the signal transduction pathway to the cell cycle machinery in yeast. *Cell.* 73:747–760.

Priess, J. R. 1994. Establishment of initial asymmetry in early *Caenorhabditis elegans* embryos. *Curr. Opin. Gen. Dev.* 4:563–568.

Reid, B. J., and L. H. Hartwell 1977. Regulation of mating in the cell cycle of *Saccharomyces cerevisiae. J. Cell Biol.* 75:355–365.

Schrick, K. 1994. Mating Partner Discrimination in Yeast: The Role of the Pheromone Response Signal Transduction Pathway. Ph. D. Thesis. University of Washington, Seattle, WA. pp. 1–80.

Segall, J. E. 1993. Polarization of yeast cells in spatial gradients of α mating factor. *Proc. Natl. Acad. Sci. USA.* 90:8332–8336.

Sikorski, R. S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae. Genetics.* 122:19–27.

Snyder, M. 1989. The *SPA2* protein of yeast localizes to sites of cell growth. *J. Cell Biol.* 108:1419–1429.

Snyder, M., S. Gehrung, and B. D. Page. 1991. Studies concerning the temporal and genetic control of cell polarity in *Saccharomyces cerevisiae. J. Cell Biol.* 114:515–532.

Sprague, G. F., Jr., and J. Thorner. 1992. Pheromone response and signal trans-

duction during the mating process of *Saccharomyces cerevisiae. In* The Molecular and Cellular Biology of the Yeast *Saccharomyces:* Gene Expression. E. W. Jones, J. R. Pringle, and J. R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 657–744.

Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 76:301–314.

Stevenson, B. J., N. Rhodes, B. Errede, and G. F. Sprague, Jr. 1992. Constitutive mutants of the protein kinase *STE11* activate the yeast pheromone response pathway in the absence of the G protein. *Genes & Dev.* 6:1293–1304.

Struhl, G., K. Struhl, and P. M. Macdonald. 1989. The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell.* 57:1259–1273.

Tkacz, J. S., and V. L. MacKay. 1979. Sexual conjugation in yeast. *J. Cell Biol.* 80:326–333.

Trueheart, J., J. D. Boeke, and G. R. Fink. 1987. Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. *Mol. Cell. Biol.* 7:2316–2328.

Valtz, N., M. Peter, and I. Herskowitz. 1995. *FAR1* is required for oriented polarization of yeast cells in response to mating phenomones. *J. Cell Biol.* 131: 863–873.

Whiteway, M., L. Hougan, and D. Y. Thomas. 1990. Overexpression of the *STE4* gene leads to mating response in haploid *Saccharomyces cerevisiae. Mol. Cell. Biol.* 10:217–222.

Whiteway, M., K. L. Clark, E. Leberer, D. Dignard, and D. Y. Thomas. 1994. Genetic identification of residues involved in association of α and β G-protein subunits. *Mol. Cell. Biol.* 14:3223–3229.

Yorihuzi, T., and Y. Ohsumi. 1994. *Saccharomyces cerevisiae MAT**a*** mutant cells defective in pointed projection formation in response to α-factor at high concentrations. *Yeast.* 10:579–594.