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

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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. MATa 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 transmembrane 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 chemotactants. Cells find one another in space by polarizing their growth toward each other along gradients of pheromone (Jackson and Hartwell, 1990a,b), a behavior that involves polarized changes in the actin cytoskeleton and in secretion (for a review see Chen, 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).

Jackson and Hartwell (1990b) 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 c~ cells and pheromoneless a cells, a cells discriminate between these two partners and mate with a pheromoneless cell partner only once in 10⁵ 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; Haseloff 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, 1990b; 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 (1990b) proposed that when cells cannot sense pheromone gradients, they...
execute a default pathway in which they select a mating partner at random.

The existence of a default pathway may explain why wild-type α cells form a shmoo in the presence of an isotropic concentration of α-pheromone (Jackson and Hartwell, 1990b). 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; Fujita 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 α 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 MATα cary1 ade2-1 his4-580 lys2-0 trpl1 cary1 SLP4.9 (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. Dohman 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 STE6α allele (Whiteway et al., 1994) in the vector pRS313 (Sikorski and Hieter, 1989). 7611-AMafala2 was created by sequential gene replacement using S:SM8 ( safα::LEU2) and S:SM35 ( safα::URA3) (Michaelis and Herskowitz, 1988) and confirmed by Southern hybridization. FUSI-lacZ fusions were integrated at the FUSI locus by transformation with plasmid pSB286 or pFL-lys that had been linearized with SphI; pFL-lys is a derivative of the integrating FUSI-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 ste6-4thm 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, 1990a).

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 × OD900 of reaction)/(OD900 of responding cell culture × volume of responding cell culture × minutes of assay). The OD900 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 was 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-L4 was grown to a density of 5 × 10⁷ cells/ml in YEPD containing 4 μM α-factor; the cells were sonicated, and then 10⁵ 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 pathway assay, a wild-type α parent, a transduction assay, and, a-pheromone, we used an assay as described in Hartwell (1980), except that 3 × 10⁶ 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 concentrations 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 α cells on the filter secrete the Barf 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 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, 1993) at a density of 10⁷ 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*  | a hom3 ilv1 can1 | Hartwell Laboratory |
| 3284-12* | a ade3 lys2* tyr1* trpl1 can1 cyh2 SUP4-3* | Hartwell Laboratory |
| 4213-67* | a ade3 lys2* tyr1* trpl1 can1 cyh2 ss2-1 SUP4-3* | Hartwell Laboratory |
| 3284-12FL* | a ade3 lys2* tyr1* trpl1 can1 cyh2 Fus1::Fusi-lacZ(LYS2) SUP4-3* | This study |
| 4213-67FL* | a ade3 lys2* tyr1* trpl1 can1 cyh2 ss2-1 Fus1::Fusi-lacZ(LYS2) SUP4-3* | This study |
| 7609-1-4* | a cry1 ade2-1* lys2* tyr1* cyh2 SUP4-3* | Hartwell Laboratory |
| 7609-6-4* | a cry1 lys2* tyr1* ura2 SUP4-3* | Hartwell Laboratory |
| 7611-2* | a cry1 his4-580* lys2* trpl1 ura3-52 leu2-3,112 cyh2 SUP4-3* | Hartwell Laboratory |
| 7611-4* | a cry1 his4-580* lys2* tyr1* ura3-52 leu2-3,112 cyh2 SUP4-3* | Hartwell Laboratory |
| 7611-4FL* | isogenic with 7611-4, except Fus1::Fusi-lacZ(Ura3) | This study |
| 7623-16-3FL* | isogenic with 7623-16-3, except Fus1::Fusi-lacZ(Lys2) | This study |
| 7623-16-3FL* | isogenic with 7623-16-3, except Fus1::Fusi-lacZ(Lys2) | This study |
| 7647-20-1* | a cry1 lys2* trpl1 ura2-3,112 ura3-52 SUP4-3* | Hartwell Laboratory |
| 7680-8-1FL* | a cry1 his4-580* lys2* trpl1 tyr1* ura3-52 ss2-1 cyh2 SUP4-3*Fusi::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* lys2* trpl1 tyr1* ura3-52 cyh2 farl1::Ura3 SUP4-3* | Kathrin Schrick |
| 8940-6-3* | a cry1 ade6 his4-580* lys2* trpl1 tyr1* ura3-52 cyh2 SUP4-3* | Kathrin Schrick |
| 8941-1-4* | a cry1 ade6 his4-580* trpl1 tyr1* ura3-52 leu2-3,112 mfa1::Ura3 D mfa2::LEU2C | Kathrin Schrick |
| 8941-12-2* | a cry1 ade6 his4-580* trpl1 ura3-52 leu2-3,112 mfa1::Ura3 D mfa2::LEU2C con1 cyh3 SUP4-3* | Kathrin Schrick |
| 8948-4-2* | a cry1 his3 leu2-3,52 cyh2 SUP4-3* | Kathrin Schrick |
| 8948-3-3* | a cry1 ade6 his3 lys2* tyr1* ura3-52 leu2-3,112 SUP4-3* | Kathrin Schrick |
| 10703* | a/a ade2/ade2 cryl/cryl his4-580/a his4-580/a lys2*/lys2* trpl1/trpl1 ura3-52/ura3-52 leu2-3,112/leu2-3,112 cyh2/cyh2 SUP4-3*/SUP4-3* | This study |
| 10815-14-4* | a cry1 lys2* trpl1 tyr1* ura3-52 leu2-3,112 cyh2 SUP4-3* Farl1-16D | This study |
| 10843-9-2* | a cry1 his3 ura5-52 trpl1 leu2-3,112 cyh2 mfa1::Ura3 D mfa2::LEU2C SUP4-3* | This study |
| Y6011 | a spa2-Δ3::URA3 ura3-52 lys2-801 ade2-101 trpl1-901 his1-Δ200 | Gehring and Snyder, 1990 |
| Y6033 | a SPA2 ura2-52 lys2-801 ade2-101 trpl1-901 his1-Δ200 | Gehring and Snyder, 1990 |
| Y6044 | a SPA2 ura2-52 lys2-801 ade2-101 trpl1-901 his3-Δ200 | Gehring and Snyder, 1990 |
| Y6092 | a spa2::TRP1 ura2-52 lys2-801 ade2-101 trpl1-901 his3-Δ200 | Gehring and Snyder, 1990 |
| Y604far1Δ1 | isogenic with Y604, except farl1::URA3 | This study |
| Y609far1Δ1 | isogenic with Y609, except farl1::URA3 | This study |
| Y604ste2Δ1 | isogenic with Y604, except ste2a::URA3 | This study |
| Y609ste2Δ1 | isogenic with Y609, except ste2a::URA3 | This study |
| W303-1BΔ | a ade2-1 trpl1-1 ura2-3,112 his3-11,15 can1 | Kurjan, 1985 |
| mfal::LEU2CΔ | a ade2-1 trpl1-1 ura2-3,112 his3-11,15 can1 mfal::LEU2C Mfa2 | Kurjan, 1985 |
| mfa1::mfa2Δ | a ade2-1 trpl1-1 ura2-3,112 his3-11,15 can1 mfal::LEU2C mfa2::ura3D | Kurjan, 1985 |

*31810 strain background.
3288C strain background.
W303 strain background.

Results

**MATa Cells Execute a Default Matting Pathway in the Presence of High, Isotopic Pheromone Concentrations**

Jackson and Hartwell (1990b) proposed that there is a default pathway that MATa 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 *α* cells. Cells that are deleted for both of the α-pheromone structural genes, *Mfa1* and *Mfa2*, produce no pheromone and are sterile (Kurjan, 1985; Table II; Fig. 1). Therefore, if a wild-type *α* 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 *α* cells over a large range of exogenous pheromone concentrations, and since the experimental conditions cause the actual concentration of pheromone experienced by the *α* cells to be less than the concentration in the media (see Materials and Methods),

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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 α-factor could partially suppress the sterility of α cells deleted for both α-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 α 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 α-factor inhibited the mating efficiency of wild-type α and α cells. To determine whether these matings occurred at a reduced efficiency because α cells were unable to use the chemotropic pathway and instead mated by the default pathway, we measured the behavior of α cells in a discrimination assay (Jackson and Hartwell, 1990b) to which we added increasing amounts of α-factor. In this assay, a wild-type α 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 α cell mates almost exclusively with the wild-type α cell partner, the randomness index is low. When the α 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 α cell mated with an α-pheromoneless cell as the sole mating partner (9.0%) and an α 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 α cell in the above experiments. When an α 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 α 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 α 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.

| α Cell partner(s) | α-factor | Mating efficiency | Randomness index | Fold inhibition by α-factor |
|------------------|----------|------------------|------------------|---------------------------|
| MFa1/MFa2        | -        | 150 ± 16 (3)     | NA               | NA                        |
| MFa1/MFa2        | +        | 7.8 ± 0.50 (3)   | NA               | 19                        |
| mfα1mfα2         | -        | <3.8 × 10⁻² (3)  | NA               | NA                        |
| mfα1mfα2         | +        | 9.0 ± 1.8 (3)    | NA               | NA                        |
| MFa1/MFa2, mfα1mfα2 | -    | 160 ± 16 (3)     | 9.0 × 10⁻⁴ ± 3.5 × 10⁻⁴ (3) | NA                        |
| MFa1/MFa2, 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 α strain (7611-4). Equal numbers of cell partners were used in all matings.

1Matings 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.

2Percent of input haploid cells that formed diploids.

3Calculated as the fraction of diploids formed by the α cells and the α-pheromoneless cells, and divided by the fraction of α cells that were α-pheromoneless in the mating reaction.

4The total mating efficiency of the α strain in the absence of α-factor, divided by the mating efficiency of the α strain in the presence of α-factor.
The mating efficiency with a-phero-

nless 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 maxi-

mum 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 a cell partners were present. The percent-

age 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 experi-

ments in A, B, and C were all performed in parallel; these data

represent the average of duplicate experiments. The a strain

used was 7623-16-3IL, and the α strains used were 8941-1-4 and

8998-4-3.

Figure 1. The behaviors of wild-type cells executing the default

pathway. (A and B) Wild-type a cells were mated either with

pheromoneless a 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 of 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 7623-16-3IL, and the α strains used were 8941-1-4 and 8998-4-3.

Shmoo Formation Correlates with the Execution of the

Default Pathway

When a cells are exposed to high concentrations of exoge-

nous pheromone, they form a shmoos (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 incorpo-

rates into the cell wall, and observed that bud scars were

predominantly in the third of the cell where the shmoos

were located. We have made a similar observation in the

381G strain background. We found that when a cells were

grown to mid-logarithmic phase, and then spread on a

YEPD plate containing a high concentration of α-pheromone

(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 re-

sponse 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
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 Matting 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 shmoo 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 MATa 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 a cells (50% and 112%, respectively) or with spa2Δ a 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 pathway in a cells, since SPA2 is not required for chemo-tropic 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 nM α-factor), a 1,100-fold reduction. In contrast, the presence of 100 nM 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 nM, 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 2. MATa cells produce shmoos adjacent to their last budding site in the presence of a high concentration of α-factor. Cells were grown to mid-logarithmic 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 adjacent to the other cell in the pair. In class II one shmoo tip is in the half of the cell that is opposite the other cell in the pair, in class III 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).](image-url)
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, 1990b; Schrick, 1994); (b) sst2-1 mutants mate with wild-type cells ~10-fold less efficiently than SST2 cells (Jackson and Hartwell, 1990b; 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 MATα mutants that mate poorly with an sst2-1 α strain (Dorer, R., 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 α-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 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 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 (open symbols) and with the pheromoneless a cell partner (closed symbols) and with the pheromoneless a cell partner (open symbols) we calculated for each mating assay (see Materials and Methods).

Table IV. sst2-1 Mutants in the Default Assay

| α Strain | α-factor | α Strain | Mating efficiency |
|----------|----------|----------|-------------------|
| SST2     |          |          |                   |
| sst2-1   |          |          |                   |
| sst2-1   | +        | MFA1MFA2 | 190 ± 32 (3)      |
| sst2-1   | +        | mFA1mfa2 | 3.5 ± 0.59 (3)    |
| sst2-1   | +        | mFA1mfa2 | 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 a strains used were 7611-2 (lines 1 and 2) and 8941-12-2 (lines 3 and 4).
§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 a 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 a 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 chemotropip 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;}

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

| α-Strain | Pheromone-producing α strain | Total mating efficiency4 | Randomness index1 |
|---------|-----------------------------|--------------------------|-------------------|
| SST2    | STE6                        | 180 ± 20 (3)             | 5.9 x 10^-6 ± 3.4 x 10^-5 (3) |
| SST2    | ste6-81HM                   | 0.85 ± 0.69 (3)          | <9.1 x 10^-4 ± 6.9 x 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) |

1. The α strains used were 3284-12 (SST2) and 4213-67 (sst2-1).
2. The pheromone producing α strains used in the discrimination assay are isogenic with 7623-16-3. All assays used 7611-4mfal MFa2 as the pheromoneless α strain.
3. The percentage of α 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.
4. See footnote 1, Table II.

### 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) ||
|-----------------------|----------------|-----------|-------------------------------------------------------------|
| a                     | mfa1 mfaf2     | −         | 0.66 ± 0.25 (8)                                             |
| a                     | Mfa1 Mfa2      | −         | 8.5 ± 1.4 (8)                                               |
| a                     | STE6-81HM      | +         | 60 ± 8.1 (8)                                               |
| a                     | mfa1 mfaf2     | −         | 0.59 ± 0.18 (4)                                             |
| a                     | ste6-81HM      | −         | 2.8 ± 0.33 (4)                                              |
| a                     | Mfa1 Mfa2      | −         | 38 ± 0.87 (4)                                              |

1. The strain containing the FUS1-lacZ construct. The strains used were 7611-4FL (α SST2), 7680-8-1FL (α sst2-1), 3484-2FL (α SST2), and 4213-6FL (α sst2-1).
2. The mating partner of the responding cell. The strains used, in order from top to bottom, were mfa1 mfaf2, mfa1/MU2/C, W303-1B, 7647-20-1, 7611-4mfal mfaf2, 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.
3. Cells were mated in either the presence (+) or absence (−) of 20 μM α-factor.
4. The percentage of the maximum level of FUS1-lacZ induction observed. The maximum level of β-galactosidase activity was 60.5 units for α 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

| α Strain | Total mating efficiency | Randomness index |
|----------|-------------------------|------------------|
| SST2 SPA2 | 120 ± 13 (4)            | <5.2 × 10^{-6} ± 1.4 × 10^{-6} (4) |
| sst2Δ SPA2 | 32 ± 3.0 (4)           | 0.51 ± 0.083 (4) |
| SST2 spa2Δ | 120 ± 17 (4)          | <8.1 × 10^{-6} ± 1.4 × 10^{-6} (4) |
| sst2Δ spa2Δ | 0.74 ± 0.14 (4)      | 0.0088 ± 0.0021 (4) |

*The α strains used in this study were, in order from top to bottom, Y604, Y604sst2Δ, Y609, and Y609sst2Δ. The α strains used were 8998-4-2 and 8941-12-2.
1See footnote 1, Table V.

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, STE4Hpl (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 STE4Hpl 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 STE4Hpl 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, STE4Hpl 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 10⁵ cells of the indicated α 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) Y604ss2Δ and Y609ss2Δ α strains were tested with 10 μM, 1 μM, and 100 nM α-factor (counterclockwise from upper right).
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Table VIII. STE4Δa 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-STE4Δa | 0.37 ± 0.016 (3) |
| spa2-Δ2:TRP1 STE2 + pRS313 | <0.00038 (3) |
| spa2-Δ2:TRP1 STE2 + pGAL-STE4Δa | 0.012 ± 0.0012 (3) |
| SPA2 ste2Δ + pGAL-STE4Δa | 1.2 ± 0.10 (3) |
| spa2-Δ2:TRP1 ste2Δ + pGAL-STE4Δa | 0.078 ± 0.013 (3) |

*The a strains used were Y604 (lines 1 and 2), Y609 (lines 3 and 4), Y604ste2Δ, and Y609ste2Δ: these strains contained either pRS313 or p211(pGAL-STE4Δa), as indicated. All matings were with 8941-12-2 as the α cell partner.

†The percentage of α 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. 1A; 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 as 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 α 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 × 10⁻⁶ ± 4.6 × 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, MATA 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 α strain by about eightfold (160 ± 25% to 20 ± 2.5% [Table IX]), far1Δ α 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 α cells was not inhibited by the loss of pheromone gradients, we conclude that far1Δ α 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 × 10⁻⁴ ± 3.4 × 10⁻⁴ [Table IX]; Chenever, 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 × 10⁻⁶ ± 4.6 × 10⁻⁶ (6) | 6.2 |
| SST2      | +         | 26 ± 6.1 (6)             | 0.58 ± 0.20 (6)    | 0.58  |
| sst2-1    | –         | 11 ± 1.4 (3)             | 0.63 ± 0.098 (3)   | 1.7  |
| sst2-1    | +         | 6.3 ± 0.78 (3)           | 0.89 ± 0.087 (3)   | 0.89  |
| FAR1      | –         | 160 ± 25 (3)             | 8.6 × 10⁻⁶ ± 8.6 × 10⁻⁶ (3) | 8.0  |
| FAR1      | +         | 20 ± 2.5 (3)             | 0.73 ± 0.021 (3)   | 1.0  |
| far1Δ     | –         | 3.3 ± 0.55 (3)           | 5.6 × 10⁻⁴ ± 3.4 × 10⁻⁴ (3) | 5.6  |
| far1Δ     | +         | 3.2 ± 0.21 (3)           | 0.67 ± 0.16 (3)    | 0.67  |

*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 3, Table V.

See footnote 4, 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.

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farl Mutants Display a Fixed Polarization

We wished to test the hypothesis (Chang, 1991) that farl 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 chemotropically induced 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 farl mutants have their conjugation site fixed at the incipient bud site, then the Far1 protein may be the molecular eraser of the axial 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, farl-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 farl-16D mutation is hypomorphic; this allele still retains partial mating function of the FAR1 gene (data not shown). In addition, a farlΔ strain budded at axial sites in the presence of 0 M, 6 nM, and 4 μM α-factor (Table X).

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

| Strains* | Hours after plating1 | α-factor4 | Bud pattern class5 | Predominant pattern |
|----------|----------------------|-----------|-------------------|---------------------|
| a FAR1   | 2.2–2.4              | –         | 0.5              | <0.25              | 11 88 axial         |
| a FAR1   | 4.6–5.0              | +         | 59.7             | 5.7                | 17 18 bipolar       |
| a-farl-16D | 2.3–2.7              | –         | 0.2              | 0.75               | 12 87 axial         |
| a-farl-16D | 4.6–4.9              | +         | 6.8              | 4.5                | 16 73 axial         |
| a-farl-16D | 2.3–2.5              | –         | 0.3              | 0.3                | 14 85 axial         |
| a farlΔ  | 2.2–2.7              | +         | <0.3             | 1.7                | 73 91 axial         |
| a farlΔ  | 3.2–3.7              | 4 μM      | 1.0              | 2.7                | 11 85 axial         |
| a/a FAR1 | 2.2–2.4              | –         | 48.9             | 90.0               | 16 28 bipolar       |

1*The α strains used were 7611-4 (FAR1), 10815-14-4 (farl-16D), 8940-4-3 (farlA), and 10763 (a/aFAR1).
2Four cell microcolonies were scored, the indicated number of hours after cells were plated on YEPD plates. All plates were incubated at 30°C.
3Cells were incubated on YEPD plates either in the presence (+) or absence (−) of 6 nM α-factor.
4The 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 500 and 400 microcolonies were scored for each line above.

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 farl-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).
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 *far1Δ spa2Δ* 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, 1990a, 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 α 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 α 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 α cells in saturating pheromone is morphologically distinct from the response of α 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 (1990b) demonstrated that when wild-type α cells are surrounded by an excess of wild-type α cells, the fraction of α 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, 1990b).

**Table XI. Deletions of FAR1 and SPA2 Display Synthetic Sterility**

| α Strain* | Mating efficiency1 |
|-----------|---------------------|
| FAR1 SPA2 | 225                 |
|           | 246                 |
| *FAR1 spa2Δ | 93                  |
|           | 80                  |
| *far1Δ SPA2 | 2.0                |
|           | 1.8                 |
| *far1Δ spa2Δ | 0.0018             |
|           | 0.0014              |

*The α strains used were, in order from top to bottom, Y604, Y609, Y604far1Δ, and Y609far1Δ. The wild-type α strain used was 7611-2.

1The percentage of 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* α strain mated very poorly in the default mating assay, where α cells are mated poorly in the default mating assay, where α cells are mated poorly in the presence of high, isotropic pheromone concentrations (Table III). Second, the mating ability of a *spa2Δ* α 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* α 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; Gehring and Snyder, 1990).

In contrast to *SPA2*, the *SST2* gene is not required for the default pathway, since an *sst2-1* α strain mated as well as a wild-type α 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 α cells from pheromoneless cells and mate at a reduced efficiency (Jackson and Hartwell, 1990b; Scharf, 1994). Second, we demonstrate that both of these mating defects are suppressible in *sst2-1* α cells by mating to an α 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 SP A2 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 (afriΔ [Konopka et al., 1995], bem1Δ [Chenevert et al., 1992, 1994], ste2-T326 [Konopka et al., 1988], and myf [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,
**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 Farlp promotes this gradient-dependent change in cell polarity. Specifically, we show that unlike wild-type cells, the mating efficiency of farlΔ cells is not inhibited by the induction of the default mating pathway (Table IX), suggesting that farlΔ cells do not use pheromone gradients as a directional cue to find a mate. Farlp inhibits the function of the bud site selection genes at the incipient bud site, acting as a molecular eraser of this predetermined site, or Farlp may directly stimulate chemotropic morphogenesis by interacting with the pheromone receptor or heterotrimeric G-protein. Although these models predict very different molecular roles for Farlp, they both predict that farlΔ 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 farlΔ 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 *farl* mutants having a fixed polarity. First, we found that in response to low uniform levels of pheromone, unlike wild-type cells, farlΔ 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 Farlp 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, farlΔ mutants mate by a mechanism that requires SPA2. This observation is consistent both with farl 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 farl 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, farl mutants cannot orient mating projections along gradients of pheromone created by micropipets (Valtz et al., 1995). Moreover, farl 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 farlΔ cells discriminate wild-type α cells from pheromoneless α cells (Table IX; Cheney, 1994; Schrick, 1994). We suggest that the key factor that prevents farlΔ mutants from mating with pheromoneless cells in a discrimination assay is that farlΔ 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 farlΔ 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, farlΔ cells do not complete mating events with pheromoneless cells because their signal transduction pathway is not saturated. As a result, farlΔ 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, chemotactants 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.

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