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Genetic Analysis of Default Mating Behavior in *Saccharomyces cerevisiae*

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ABSTRACT

Haploid *Saccharomyces cerevisiae* cells find each other during conjugation by orienting their growth toward each other along pheromone gradients (chemotropism). However, when their receptors are saturated for pheromone binding, yeast cells must select a mate by executing a default pathway in which they choose a mating partner at random. We previously demonstrated that this default pathway requires the *SPA2* gene. In this report we show that the default mating pathway also requires the *AXL1, FUS1, FUS2, FUS3, PEA2, RVS161,* and *BNI1* genes. These genes, including *SPA2,* are also important for efficient cell fusion during chemotropic mating. Cells containing null mutations in these genes display defects in cell fusion that subtly affect mating efficiency. In addition, we found that the defect in default mating caused by mutations in *SPA2* is partially suppressed by multiple copies of two genes, *FUS2* and *MFA2.* These findings uncover a molecular relationship between default mating and cell fusion. Moreover, because *axl1* mutants secrete reduced levels of **a**-factor and are defective at both cell fusion and default mating, these results reveal an important role for **a**-factor in cell fusion and default mating. We suggest that default mating places a more stringent requirement on some aspects of cell fusion than does chemotropic mating.

DURING conjugation, haploid yeast cells communicate with each other by secreting small peptide pheromones. *MAT***a** cells secrete **a**-factor and *MAT*α cells secrete α-factor. Each pheromone binds to a seven-pass transmembrane receptor that activates a heterotrimeric G-protein, which in turn activates a signal transduction (MAP kinase) cascade (for reviews see Kurjan 1992; Sprague and Thorner 1992; Bardwell 1994; Herskowitz 1995). As a result, cells arrest in the G1 phase of the cell cycle and induce the expression of mating-specific genes. Pheromones also act as chemoattractants. To find the location of their mate, each mating partner senses a gradient of pheromone and polarizes its growth along the gradient (Jackson and Hartwell 1990a,b; Segall 1993). Mating partners touch at the tips of their projections, fuse cell walls, then membranes, and finally nuclei (Byers and Goetsch 1975; Byers 1981). Pheromone receptors, the heterotrimeric G protein, and the Far1 protein are all required for this chemotropic growth (Jackson *et al.* 1991; Schrick 1994; Dorer *et al.* 1995; Valtz *et al.* 1995). These gene products may polarize growth by recruiting or organizing a set of proteins, including those encoded by the *CDC24, CDC42,* and *BEM1* genes, which then establish polarity at the site of highest pheromone concentration on the cell surface (for reviews see Chenevert 1994; Kron and Gow 1995).

Yeast cells exhibit a second mating behavior that uses internal cues to direct cell polarity: when their receptors are saturated with pheromone, wild-type *MAT***a** cells execute a default pathway in which they select a mate at random by initially choosing a site near their incipient bud site to form a mating projection (Madden and Snyder 1992; Dorer *et al.* 1995). Similarly, haploid *MAT***a** and *MAT*α cells position their buds directly adjacent to their last bud site (Chant *et al.* 1995). This budding pattern is determined by the products of the *RSR1, BUD2, BUD3, BUD4, BUD5, AXL1, AXL2,* neck filaments, and other genes (Bender and Pringle 1989; Chant and Herskowitz 1991; Chant *et al.* 1991, 1995; Flescher *et al.* 1993; Fujita *et al.* 1994; Chant and Pringle 1995; Chant 1996; Halme *et al.* 1996; Roemer *et al.* 1996). In saturating α-factor the initial position of the mating projection is dependent on at least four of these budding pattern genes [*RSR1, BUD2, BUD3,* and *BUD4* (Madden and Snyder 1992)]. Therefore, when cells use a site near the incipient bud site to form a mating projection in saturating pheromone, they recognize these intrinsic cues for polarity, and instead of forming a bud, form a mating projection.

We are interested in understanding how the signal transduction pathway activates two behaviorally distinct mating pathways in response to two levels of pheromone, a chemotropic pathway in response to pheromone gradients, and a default pathway in response to saturating pheromone. We previously demonstrated that these two mating pathways are genetically distinct. The Spa2 protein is required for default mating but not for chemotropism (Dorer *et al.* 1995): *spa2*Δ mutants

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cannot mate if pheromone concentrations are high and gradients are absent, but can mate if gradients are present. In contrast, pheromone receptors, the Sst2 protein, and the Far1 protein are required for chemotropism but not for default mating. These findings suggest that pheromone receptors, Sst2p, and Far1p promote cell growth along pheromone gradients and Spa2p promotes growth during default mating.

In this paper we used two approaches to further analyze the default mating pathway. First, we identified seven other genes that are more important for default mating than for chemotropic mating: the *AXL1, FUS1, FUS2, FUS3, BNI1, RVS161* and *PEA2* genes. We found that all of these genes, including *SPA2,* are also mildly important for efficient cell fusion during chemotropic matings. In fact, for cells carrying mutations in these genes, the degree of defect in cell fusion correlates with the degree of defect in default mating. These results suggest that default matings place a stringent requirement on the fidelity of cell fusion. Moreover, because *axl1* mutants secrete reduced levels of **a**-factor (Adames *et al.* 1995) and are defective at both cell fusion and default mating, these results reveal an important role for **a**-factor in cell fusion and default mating. Second, we identified *MFA2* and *FUS2* as multicopy suppressors of the defect in default mating caused by mutations in *SPA2.* These results support a relationship between *SPA2,* cell fusion, and **a**-factor. Finally, because many of the genes that are important for default mating are involved in other aspects of yeast cell polarity, we tested other cell polarity mutants for their ability to mate by default. We found that many mutants defective in bud site selection, Ash1 protein localization, and mating projection formation are not defective in either chemotropism or default mating. These findings demonstrate a specific connection between default mating and some aspects of cell fusion.

MATERIALS AND METHODS

Strains, plasmids, and media: The strains used in this study are listed in Table 1. Strains containing the *axl1::URA3* mutation were constructed by transformation [using lithium acetate method (Gietz and Schiestl 1991)] with the plasmid p98 (Adames *et al.* 1995) that was digested with *Eco*RI and *Bam*HI. Strains containing the *bni1*Δ*::URA3* mutation were constructed by transformation with *Hin*dIII- and *Xho*I-digested p321 (C. Boone, unpublished data), a plasmid containing a substitution of *BNI1* sequences with the *URA3* gene. Strains containing the *chs5*Δ*::URA3* mutation were constructed by transformation with *Xho*I- and *Sst*I-digested p220 (C. Boone, unpublished data), a plasmid containing a substitution of *CHS5* sequences with the *URA3* gene. Strains containing a deletion of *FUS2* were constructed by transforming with p81 cut with *Hpa*I (C. Boone, unpublished data), which contains the *FUS2* gene deleted for a *Hin*dIII-*Hin*dIII fragment in the vector pRS306; colonies were selected on synthetic media lacking uracil, and prototrophs were replica plated to synthetic media containing 5-FOA to obtain popouts; these colonies were then tested for the presence or absence of a deletion of *FUS2.* Strains containing a deletion of *PEA2* were constructed by transformation with a 2.0-kb *Bam*HI-*Kpn*I fragment of pNV44 (a gift from Nicole Valtz), containing a substitution of *PEA2* sequences with *URA3,* and confirmed by PCR using oligonucleotides oNV40 and oNV41 (gifts from Nicole Valtz). Strains containing the *spa2*Δ*::URA3* mutation were constructed by transforming with a *Hin*dIII-*Sal*I fragment of p210 (Gehrung and Snyder 1990), which contains a substitution of *SPA2* sequences with *URA3.* Deletions of *AXL1, BNI1, CHS5, FUS2,* and *SPA2* were confirmed by PCR analysis of genomic DNA using oligonucleotides that flank the deleted regions of the wild-type genes. Plasmid pMA106 contains a fusion of *RAS2* and *GFP* in the centromere-containing, *TRP1* vector, YCplac22 (a gift of Jennifer Whistler, Department of Molecular and Cellular Biology, University of California, Berkeley). pMFA2 contains *MFA2* in YEp352 (Jackson and Hartwell 1990a). p203 contains *FUS2* in YEp24, described in C. Boone (unpublished data). Complete descriptions of plasmids p321, p220, p81, and p203 are available upon request (contact C. Boone).

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 (Dorer *et al.* 1995), *MAT***a**- and *MAT*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.), 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 *MAT***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. The mating efficiency was calculated as follows: 100% × (the number of diploids formed/the smallest number of input haploids of one mating type); efficiencies >100% occur if haploids divide before mating or if diploids divide after mating.

In the cell fusion assay (Figure 2) 3×10^6 *MAT***a** cells containing pMA106 were mated quantitatively with 3×10^6 wild-type *MAT*α cells. Matings were resuspended in -N media and viewed at 400–600× magnification under a fluorescent microscope (Nikon). GFP-staining zygotes were scored as fused if both mating partners contained GFP (Figure 1, A and B); unfused prezygotes were those zygote pairs in which only one partner contained visible GFP (Figure 1, C and D). At least 200 cell pairs were scored for each independent experiment.

Screen for multicopy suppressors of *spa2*: *MAT***a** *spa2-1* and *spa2-2* strains (JC-D6 and JC-J9) were transformed [using lithium acetate method (Gietz and Schiestl 1991)] with a yeast genomic library contained in the 2-μm vector YEp24 (Carlson and Botstein 1982). Forty thousand individual transformants were pooled and mated for 3 hr at 30° on Noble agar plates in a quantitative discrimination as described above. The *MAT*α cell mating partners were 7609-7-3 and 8907-4-1. Diploids produced by matings with the pheromoneless cell partner (4360 colonies with JC-J9, 1360 colonies with JC-D6) were collected on selective media and pooled. Plasmid DNA was isolated (Hoffman and Winston 1987), amplified in *Escherichia coli,* and transformed back into the respective yeast strains. Individual transformants were screened by replica plating to identify plasmids that increased the mating efficiency of the *spa2* mutant with the pheromoneless partner in the discrimination assay. Six different plasmids were isolated and shown to suppress the default mating defect caused by the *spa2* mutations, as determined

by restriction analysis and a sequence analysis of the genomic DNA insert. Two of these suppressors (*MFA2* and *FUS2*) are described in this report. The other four suppressors were not extensively analyzed.

Screen for mutants defective at default mating: A 381G *MAT***a** *bar1* strain was mutagenized to 10% viability with EMS. About 10,000–15,000 individual colonies were screened by a replica plating protocol for colonies that could mate with a wild-type *MATα* strain (7611-2) in the absence of pheromone (chemotropism), but failed to mate with a pheromoneless *MAT***a** strain (8941-12-2) in the presence of 1 μM pheromone (default). Candidates were purified and retested. Five mutants were isolated, all of which were complemented by a plasmid containing the *AXL1* gene.

RESULTS

AXL1, BNI1, FUS1, FUS2, FUS3, PEA2, RVS161* and *SPA2* are important for default mating:** To further analyze the default mating pathway, we wished to identify genes in addition to *SPA2* that are important for default mating. We assayed the default mating behavior of a given mutant *MATa** strain by quantitating the mating efficiency of the *MAT***a** strain with a pheromoneless *MATα* strain in the presence of a high concentration of synthetic α-factor (see MATERIALS AND METHODS; DORER *et al.* 1995). All matings that occur with the pheromoneless *MATα* strain must occur by the default pathway because this *MATα* strain cannot create a pheromone gradient. When presented with a choice between a pheromone-producing and a pheromoneless *MATα* mating partner, a wild-type *MAT***a** strain senses a pheromone gradient and mates by chemotropism with the pheromone-producing partner 10^5 times more frequently than with the pheromoneless partner [randomness index of $<10^{-5}$ (JACKSON and HARTWELL 1990b)]. In contrast, when saturating α-factor is added exogenously to the mating mixture, a wild-type *MAT***a** strain chooses a partner by default and mates as efficiently with the pheromoneless partner as it mates with the pheromone-producing partner (randomness index of 1.0). These default matings are ~10-fold less efficient than chemotropic matings, revealing how important it is that both partners are able to undergo a chemotropic response during mating. Using this assay, we previously showed that *SPA2* is specifically required for default matings: *spa2Δ* mutants mate efficiently by chemotropism in the absence of exogenous pheromone, but fail to mate by default with pheromoneless cells in the presence of saturating pheromone (DORER *et al.* 1995).

We tested the *axl1::URA3*, *bni1Δ*, *fus1Δ*, *fus2Δ*, *fus3Δ*, *pea2Δ*, and *spa2Δ* mutants in this default mating assay, and all were defective at default mating (Table 2). These mutants were identified in three ways. First, we found *axl1* mutants as a result of a screen for mutants defective at default mating (see MATERIALS AND METHODS). Second, we tested a *pea2Δ* mutant in the default assay because *pea2* mutants share many phenotypes in common with *spa2* mutants (CHENEVERT *et al.* 1994; VALTZ and HERSKOWITZ 1996). Third, we tested strains containing mutations in *FUS1, FUS2, FUS3, RVS161,* and *BNI1* because C. BOONE (unpublished data) found that mutations in these genes are each synthetic sterile with a deletion of *SST2*, similar to the synthetic sterility we previously observed with *spa2Δ* and *sst2Δ* (DORER *et al.* 1995).

We found that the *axl1::URA3* mutant mated extremely poorly with pheromoneless cells in the presence of saturating pheromone [0.0074% (Table 2)]. Moreover, the *axl1::URA3* mutant displayed a 1500-fold inhibition in mating efficiency by the addition of exogenous pheromone, a much greater inhibition than we observed for wild type [8.1-fold (Table 2)]. Therefore, *AXL1* is required for default matings. *AXL1* is mildly important for matings that occur in the absence of exogenous pheromone (ADAMES *et al.* 1995); in our strain background (381G) the *axl1::URA3* mutant mated 4.3-fold less efficiently than wild-type cells (Table 2).

We found that *pea2Δ* mutants are about as defective as *spa2Δ* mutants at default mating. In the presence of saturating pheromone, both mutants mated with pheromoneless cells very poorly [0.067% and 0.021% for *pea2Δ* and *spa2Δ*, respectively, compared to 6.9% for wild type (Table 2)]. In addition, the total mating efficiency of the *pea2Δ* and *spa2Δ* mutants was inhibited dramatically by the addition of saturating pheromone (Table 2). *PEA2* and *SPA2* are not very important for chemotropic matings, since *pea2Δ* and *spa2Δ* mutants mated nearly as efficiently as wild-type *MAT***a** cells mated with wild-type *MATα* cells in the absence of exogenous pheromone (Table 2).

We deleted *BNI1, FUS1, FUS2, FUS3,* and *RVS161* and found that all of these genes are important for default mating (Table 2). The *bni1Δ*, *fus1Δ*, *fus2Δ*, *fus3Δ*, and *rvs161Δ* mutants mated with pheromoneless cells very poorly in the presence of saturating pheromone (Table 2). Furthermore, their mating efficiencies were inhibited by the addition of exogenous pheromone to a greater degree than the wild-type control strain. Despite their significant defects in default mating, we found that the *bni1Δ*, *fus1Δ*, *fus2Δ*, and *rvs161Δ* mutants mated as efficiently as wild-type cells mated when mating by chemotropism with a wild-type *MATα* cell in the absence of exogenous α-factor (Table 2). On the other hand, consistent with the results of ELION *et al.* (1990), *fus3Δ* mutants mated inefficiently with the wild-type *MATα* strain, even in the absence of saturating pheromone [Table 2 (6.0% and 130% for *fus3Δ* and wild type, respectively)].

To support the conclusion that these genes are important for default mating, we performed a second assay for default mating. Cells containing the *sst2-1* mutation, a null mutation in the *SST2* gene, are supersensitive to pheromone because Sst2p negatively regulates the activity of the heterotrimeric G-protein (CHAN and OTTE 1982; DIETZL and KURJAN 1987; DOHLMAN *et al.*

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source
7609-7-3[a]	*MATα cry1 ade2-1° his4-580^a lys2^o ura2 SUP4-3^{ts}*	Hartwell Laboratory
7611-2[a]	*MATα cry1 his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 cyh2 SUP4-3^{ts}*	Hartwell Laboratory
7623-16-3[a]	*MAT***a** *cry1 ade2-1° his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 cyh2 SUP4-3^{ts}*	Hartwell Laboratory
7623-16-3*ax11::URA3*[a]	Isogenic with 7623-16-3, except *ax11::URA3*	This study
7623-16-3*bnil*Δ[a]	Isogenic with 7623-16-3, except *bni1Δ::URA3*	This study
7623-16-3*chs5*Δ[a]	Isogenic with 7623-16-3, except *chs5Δ::URA3*	This study
7623-16-3*fus2*Δ[a]	Isogenic with 7623-16-3, except *fus2Δ*	This study
7623-16-3*pea2*Δ[a]	Isogenic with 7623-16-3, except *pea2Δ::URA3*	This study
7623-16-3*spa2*Δ[a]	Isogenic with 7623-16-3, except *spa2-Δ3::URA3*	This study
8882-7-2[a]	*MAT***a** *cry1 ade6 his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 sst2-1 SUP4-3^{ts}*	Kathrin Schrick
8882-7-2*ax11::URA3*[a]	Isogenic with 8882-7-2, except *ax11::URA3*	This study
8882-7-2*bnil*Δ[a]	Isogenic with 8882-7-2, except *bni1Δ::URA3*	This study
8882-7-2*fus2*Δ[a]	Isogenic with 8882-7-2, except *fus2Δ*	This study
8882-7-2*chs5*Δ[a]	Isogenic with 8882-7-2, except *chs5Δ::URA3*	This study
8882-7-2*pea2*Δ[a]	Isogenic with 8882-7-2, except *pea2Δ::URA3*	This study
8882-7-2*spa2*Δ[a]	Isogenic with 8882-7-2, except *spa2-Δ3::URA3*	This study
8907-4-1*ura3*^{FOAR}[a]	*MATα cry1 ade6 his4-580^a tyr1^o ura3-52 leu2-3,112 ura3-52 leu2-3, 112 can1 cyh2 mfα1::ura3^{FOAr} mfα2::LEU2C*	Kathrin Schrick
8941-12-2[a]	*MATα cry1 ade6 his4-580^a trp1^a ura3-52 leu2-3,112 mfα1::URA3D mfα2::LEU2C can1 cyh2 SUP4-3^{ts}*	Kathrin Schrick
10838-12-1[a]	*MAT***a** *cry1 his4-580^a trp1^a lys2^o ura3-52 leu2-3,112 cyh2 fus3Δ::LEU2 SUP4-3^{ts}*	This study
10848A-7-1[a]	*MAT***a** *cry1 his4-580^a trp1^a lys2^o ura2 ura3-52 leu2-3,112 sst2-1 fus3Δ::LEU2 SUP4-3^{ts}*	This study
11236-12-2[a]	*MATα cry1 ade6 his4-580^a trp1^a lys2^o ura3-52 leu2-3,112 SUP4-3^{ts}*	Kathrin Schrick
DJ211-1-2[a]	*MAT***a** *cry1 ade2-1° his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 bar1-1 SUP4-3^{ts}*	James Konopka
JK26[a]	*MAT***a** *cry1 ade2-1° his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 bar1-1 afr1Δ::URA3 SUP4-3^{ts}*	James Konopka
DJ147-1-2[a]	*MAT***a** *cry1 ade2-1° his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 SUP4-3^{ts}*	James Konopka
JKY7441-4[a]	*MAT***a** *cry1 ade2-1° his4-580^a lys2^o trp1^a ura3-52 leu2-3,112 ste2-T326 SUP4-3^{ts}*	James Konopka
Y96[b]	*MAT***a** *sst2Δ::LEU2 lys2Δ::GAL-SST2 his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
Y106[b]	*MAT***a** *fus1Δ sst2Δ::LEU2 lys2Δ::GAL-SST2 his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
Y331[b]	*MAT***a** *rvs161Δ sst2Δ::LEU2 lys2Δ::GAL-SST2 his3::FUS1-HIS3 mfa2Δ::FUS1-lac ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
Y414[b]	*MAT***a** *exs1/chs5Δ::URA3 his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
Y432[b]	*MAT***a** *rvs161Δ::LEU2 his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
Y448[b]	*MAT***a** *fus1Δ::LEU2 his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
SY2585[b]	*MAT***a** *his3::FUS1-HIS3 mfa2Δ::FUS1-lacZ ura3-1 leu2-3,112 trp1-1 ade2-1 can1-100*	Charles Boone
JC2-1b	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1*	Chenevert *et al.* (1994)
JC-D6	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 spa2-1*	Chenevert *et al.* (1994)
JC-J9	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 spa2-2*	Chenevert *et al.* (1994)
JC2-G11	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 bem1-s1*	Chenevert *et al.* (1994)
JC2-F5	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 bem1-s2*	Chenevert *et al.* (1994)
JC2-G16	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 tny1-1*	Chenevert *et al.* (1994)
JC2-H9	*MAT***a** *HML***a** *HMR***a** *ura3-52 ade2-1 met1 bar1-1 tnyl-2*	Chenevert *et al.* (1994)
NVY139	*MAT***a** *HML***a** *HMR***a** *spa2-Δ3::URA3 ura3-52 ade2-1 met1 bar1-1*	Nicole Valtz
IH2393	*MAT***a** *HMRα HMLα his4 trp1 ura3 can1*	Chant and Herskowitz (1991)
IH2407	*MAT***a** *HMRα HMLα his4 trp1 ura3 can1 bud1-*	Chant and Herskowitz (1991)
IH2408	*MAT***a** *HMRα HMLα his4 trp1 ura3 can1 bud2-*	Chant and Herskowitz (1991)

TABLE 1

Continued

Strain	Genotype	Source
IH2409	*MAT***a** *HMRα HMLα his4 trp1 ura3 can1 bud3-*	CHANT and HERSKOWITZ (1991)
IH2410	*MAT***a** *HMRα HMLα his4 trp1 ura3 can1 bud4-*	CHANT and HERSKOWITZ (1991)
YJZ354	*MAT***a** *bud6Δ::TRP1 trp1 leu2 lys2 his3 ura3*	JOHN R. PRINGLE
YJZ355	*MAT***a** *BUD6 trp1 leu2 lys2 his3 ura3*	JOHN R. PRINGLE
YJZ355 *spa2Δ::URA3*	*MAT***a** *BUD6 trp1 leu2 lys2 his3 ura3 spa2Δ::URA3*	This study
YHH113	*MAT***a** *ura3 his4 trp1 bud7-1*	ZAHNER *et al.* (1996)
YHH114	*MAT***a** *ura3 his4 trp1 bud9-1*	ZAHNER *et al.* (1996)
YHH394	*MAT***a** *bud8Δ::TRP1 trp1 leu2 lys2 his3 ura3*	JOHN R. PRINGLE
Y604[c]	*MAT***a** *SPA2 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200*	GEHRUNG and SNYDER (1990)
Y609[c]	*MAT***a** *spa2-Δ2::TRP1 ura3-52 lys2-801 ade2-101 trp1-901 his3-Δ200*	GEHRUNG and SNYDER (1990)
Y604*sst2Δ*[c]	isogenic with Y604, except *sst2Δ*	DORER *et al.* (1995)
Y609*sst2Δ*[c]	isogenic with Y609, except *sstΔ*	DORER *et al.* (1995)
Y1153	*MAT***a** *ura3 lys2 ade2 trp1 his3 axl2Δ::URA3*	ROEMER *et al.* (1996)
Y1155	*MAT***a** *ura3 lys2 ade2 trp1 his3 AXL2*	ROEMER *et al.* (1996)
5104-1-3[b]	*MAT***a** *his3-11 leu2 ade2-1 trp1-1 ura3 can1-100 SHE*	JANSEN *et al.* (1996)
5104-2-2[b]	*MAT***a** *his3-11 leu2 ade2-1 trp1-1 ura3 can1-100 she1::URA3(myo4::URA3)*	JANSEN *et al.* (1996)
10900-7-2[b]	*MAT***a** *HO-ADE2 HO-CAN1 his3::FUS1-HIS3 leu2 ade2-1 trp1-1 lys2 ura3 can1-100 she2::URA3*	This study
5213-1-1[b]	*MAT***a** *his3-11 leu2 ade2-1 trp1-1 ura3 can1-100 she3::URA3*	JANSEN *et al.* (1996)
5102-2-2[b]	*MAT***a** *his3-11 leu2 ade2-1 trp1-1 ura3 can1-100 she5::URA3(bni1::URA3)*	JANSEN *et al.* (1996)

[a] 381G strain background (HARTWELL 1980).
[b] W303 strain background.
[c] S288C strain background.

1995). As a result, *sst2-1* mutants behave as if their pheromone-response pathway is saturated when wild-type mating partners are present. Therefore, even in the absence of exogenously added pheromone, these mutants mate by default with the pheromoneless partner in a discrimination assay at a high frequency (JACKSON and HARTWELL 1990b; DORER *et al.* 1995). The deletion of *SPA2* in an *sst2-1* mutant prevents these default matings with the pheromoneless partner and causes a synthetic drop in mating efficiency (DORER *et al.* 1995; Table 3). Similarly, we found that the *axl1::URA3* and *pea2Δ* mutants are synthetic sterile with the *sst2-1* mutation (Table 3), and we confirmed that the *bni1Δ*, *fus2Δ*, *fus3Δ*, *rvs161Δ*, and *fus1Δ* mutations are all synthetic sterile with a null mutation in *SST2* (Table 3; C. BOONE, unpublished data). For example, the *sst2-1 axl1::URA3* *double mutant mated with an efficiency of 0.031% (Table 3), 610-fold less efficient than the sst2-1 single mutant [19% (Table 3)] and 970-fold less efficient than the axl1::URA3* single mutant [30% (Table 2)]. Moreover, we found that each double mutant had a lower randomness index than the *sst2* single mutant control. For example, the *sst2-1 axl1::URA3* double mutant displayed a randomness index of 0.14 (Table 3), significantly lower than the randomness index of the *sst2-1* mutant [0.57 (Table 3)]. Therefore, the *axl1::URA3*, *bni1Δ*, *pea2Δ*, *fus2Δ*, *fus3Δ*, *rvs161Δ*, and *fus1Δ* mutations prevent the *sst2-1* mutant from mating by default with the pheromoneless *MATα* strain. These data support the conclusion that *AXL1*, *BNI1*, *PEA2*, *FUS2*, *FUS3*, *RVS161*, and *FUS1* are important for default mating behavior.

The degree of defect in cell fusion is directly proportional to the degree of defect in default mating: Since *FUS1*, *FUS2* and *FUS3* are important for cell fusion during chemotropic mating (MCCAFFREY *et al.* 1987; TRUEHEART *et al.* 1987; ELION *et al.* 1990), we wished to determine whether the other default mating genes are important for cell fusion. Therefore, we quantitated the percentage of cells that formed prezygotes during chemotropic matings because they failed to complete zygote formation. We were able to distinguish prezygotes from zygotes by transforming the *MAT***a** cell mating partner with a plasmid containing a RAS2-GFP fusion gene (kindly provided by JENNIFER WHISTLER, University of California, Berkeley). The fusion protein localizes to the cell membrane of the *MAT***a** cell. When the *MAT***a** cell fuses its cell membrane with *MATα* cell mating partner, the protein diffuses into the *MATα* cell. A fused zygote contains GFP staining in both partners (Figure 1, A and B), and a prezygote, in which the cell walls fuse but the membranes fail to fuse, contains the GFP staining in only one partner (Figure 1, C and D). We found that in addition to the *fus2Δ* mutant, the *axl1Δ*, *spa2Δ*, and *pea2Δ* mutants all displayed defects in cell fusion when mated with a wild-type *MATα* cell

TABLE 2

The *AXL1*, *BNI1*, *FUS1*, *FUS2*, *FUS3*, *PEA2*, *SPA2*, and *RVS161* genes are important for the ability of cells to mate by default in the presence of saturating pheromone

*MAT***a** strain[a]	α-factor[b]	Total mating efficiency (%)[c]	Mating efficiency with pheromoneless (%)[d]	Randomness index[e]	Fold inhibition[f]
			381G strain background		
Wild-type	−	130 ± 13 (4)	<8.6 × 10^{-4} ± 4.0 × 10^{-4} (4)	<1.1 × 10^{-5} ± 5.6 × 10^{-6} (4)	
	+	16 ± 1.2 (4)	6.9 ± 0.38 (4)	0.79 ± 0.14 (4)	8.1
axl1::URA3	−	30 ± 4.1 (4)	2.0 × 10^{-3} ± 7.0 × 10^{-4} (4)	4.8 × 10^{-5} ± 1.5 × 10^{-5} (4)	
	+	0.020 ± 0.0028 (4)	0.0074 ± 0.0023 (4)	0.69 ± 0.18 (4)	1500
bni1Δ	−	100 ± 10 (4)	8.5 × 10^{-4} ± 2.6 × 10^{-4} (4)	1.6 × 10^{-5} ± 4.4 × 10^{-6} (4)	
	+	0.71 ± 0.23 (4)	0.21 ± 0.74 (4)	0.56 ± 0.051 (4)	140
chs5Δ	−	160 ± 10 (4)	≤5.4 × 10^{-4} ± 3.0 × 10^{-5} (4)	≤5.5 × 10^{-6} ± 3.5 × 10^{-7} (4)	
	+	6.4 ± 0.56 (4)	2.3 ± 0.80 (4)	0.62 ± 0.19 (4)	25
fus2Δ	−	90 ± 7.8 (4)	≤4.1 × 10^{-4} ± 1.3 × 10^{-4} (4)	≤8.2 × 10^{-6} ± 2.4 × 10^{-6} (4)	
	+	2.2 ± 0.18 (4)	0.83 ± 0.21 (4)	0.66 ± 0.16 (4)	41
pea2Δ	−	84 ± 1.9 (4)	≤6.4 × 10^{-4} ± 1.5 × 10^{-4} (4)	≤1.2 × 10^{-5} ± 3.4 × 10^{-6} (4)	
	+	0.59 ± 0.25 (4)	0.067 ± 0.030 (4)	0.20 ± 0.074 (4)	140
spa2Δ	−	70 ± 2.2 (4)	7.0 × 10^{-4} ± 1.1 × 10^{-4} (4)	1.6 × 10^{-5} ± 2.9 × 10^{-6} (4)	
	+	0.16 ± 0.11 (4)	0.021 ± 0.0084 (4)	0.28 ± 0.80 (4)	440
fus3Δ	−	6.0 ± 0.30 (3)	0.015 ± 0.0023 (3)	0.0049 ± 0.00078 (3)	
	+	0.068 ± 0.011 (3)	0.013 ± 0.0056 (3)	0.39 ± 0.19 (3)	88
			W303 strain background		
Wild-type	−	200 ± 12 (5)	≤4.7 × 10^{-4} ± 2.2 × 10^{-5} (5)	≤4.2 × 10^{-6} ± 2.6 × 10^{-7}	
	+	9.8 ± 2.0 (5)	4.7 ± 0.91 (5)	0.87 ± 0.050 (5)	20
fus1Δ	−	140 ± 7.1 (3)	≤7.7 × 10^{-4} ± 3.9 × 10^{-4} (3)	≤9.7 × 10^{-6} ± 4.5 × 10^{-6} (3)	
	+	1.1 ± 0.086 (3)	0.53 ± 0.078 (3)	0.91 ± 0.12 (3)	130
rvs161Δ	−	150 ± 8.7 (3)	≤5.0 × 10^{-4} ± 0 (3)	≤6.0 × 10^{-6} ± 3.8 × 10^{-7} (3)	
	+	0.30 ± 0.071 (3)	0.14 ± 0.0050 (3)	0.83 ± 0.012 (3)	500
chs5Δ	−	170 ± 22 (5)	≤6.2 × 10^{-4} ± 4.7 × 10^{-5} (5)	≤5.6 × 10^{-6} ± 1.1 × 10^{-6} (5)	
	+	5.2 ± 2.1 (5)	2.1 ± 0.89 (5)	0.72 ± 0.087 (5)	33

[a] *MAT***a** strains used, in order from top to bottom, were as follows: 7623-16-3, 7623-16-3 *axl1::URA3*, *7623-16-3 bni1Δ::URA3*, 7623-16-3 *chs5Δ::URA3*, 7623-16-3 *fus2Δ*; 7623-16-3 *pea2Δ::URA3*, 7623-16-3 *spa2Δ::URA3*, 10838-12-1, SY2585, Y448, Y432, and Y414. The *MATα* strains used as mating partners with the 381G *MAT***a** strains were 7609-7-3 and 8907-4-1. The *MATα* strains used with the W303 *MAT***a** strains were 7609-7-3 and 8941-12-2.

[b] Equal numbers of *MAT***a** cells, wild-type *MATα* cells, and pheromoneless *MATα* cells were mixed, filtered onto 25-mm Millipore filters, and allowed to mate for 3 hr at 30° on Noble Agar plates in the absence (−) or in the presence (+) of 40 μM α-factor.

[c] The percentage of *MAT***a** cells that formed diploids with either the *MATα* wild-type or the α-pheromoneless strain partners. The mean and standard error of the number of independent observations shown in parentheses is indicated, where appropriate.

[d] The percentage of *MAT***a** cells that formed diploids with the α-pheromoneless strain.

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

[f] Calculated as the mean total mating efficiency of the *MAT***a** strain in the absence of pheromone, divided by the mean total mating efficiency of the *MAT***a** strain in the presence of pheromone.

in the absence of exogenous α-factor, and we confirmed the observation (C. Boone, unpublished data) that *bni1Δ* mutants are defective at cell fusion (Figure 2). In addition, C. Boone (unpublished data) found that *rvs161Δ* mutants are defective at cell fusion. The most defective strain was the *axl1::URA3* mutant, for which 23 ± 3.5% of the zygotes were prezygotes, compared to ≤0.5 ± 0% for the wild-type control strain (Figure 2). The other mutants were less defective for cell fusion, but still were between 8.4- and 24-fold more defective than the wild-type control strain.

We found that the strains most defective in default mating, *axl1Δ* and *spa2Δ*, also displayed the greatest defect in cell fusion. *fus2Δ* mutants were less defective for default mating (Table 2) and less defective for cell fusion; only 4.2 ± 2.3% of the zygotes were prezygotes (Figure 2). Therefore, it appears that the degree of defect in cell fusion is directly proportional to the degree of defect in default mating.

Cell fusion and default mating: Are all aspects of cell fusion required for default mating? Perhaps some functions that are important for cell fusion are no more important during default mating than during chemotropic mating. The *CHS5* gene is required for efficient cell fusion during conjugation (Cid *et al.* 1995; C. Boone, unpublished data), and a deletion of *CHS5/*

TABLE 3

Synthetic sterility and discrimination of *axl1*::*URA3*, *bni1Δ*, *chs5Δ*, *pea2Δ*, *spa2Δ*, *rvs161Δ*, *fus1Δ*, *fus2Δ*, and *fus3Δ* mutations with mutations in *SST2*

*MAT***a** strain[a]	Total mating efficiency (%)[b]	Mating efficiency with pheromoneless (%)[c]	Randomness index[d]
	381G strain background		
sst2-1	19 ± 2.1 (7)	5.2 ± 0.42 (7)	0.57 ± 0.043 (7)
sst2-1 axl1::*URA3*	0.031 ± 0.0071 (3)	0.0021 ± 0 (3)	0.14 ± 0.030 (3)
sst2-1 bni1Δ::*URA3*	4.4 ± 0.70 (3)	0.40 ± 0.045 (3)	0.18 ± 0.015 (3)
sst2-1 chs5Δ::*URA3*	2.8 ± 2.0 (4)	0.43 ± 0.19 (4)	0.36 ± 0.13 (4)
sst2-1 fus2Δ	7.6 ± 0.85 (3)	0.20 ± 0.026 (3)	0.051 ± 0.011 (3)
sst2-1 pea2Δ::*URA3*	1.2 ± 0.68 (4)	0.042 ± 0.0090 (4)	0.084 ± 0.065 (4)
sst2-1 spa2Δ::*URA3*	1.1 ± 0.52 (4)	0.084 ± 0.074 (4)	0.12 ± 0.076 (4)
sst2-1 fus3Δ::*LEU2*	0.022 ± 0.010 (3)	0.0035 ± 0.0018 (3)	0.30 ± 0.11 (3)
	W303 strain background		
sst2Δ	29 ± 5.5 (4)	5.7 ± 1.0 (4)	0.35 ± 0.039 (4)
sst2Δ fus1Δ	0.065 ± 0.015 (4)	0.0017 ± 0.00052 (4)	0.046 ± 0.011 (4)
sst2Δ rvs161Δ	0.044 ± 0.018 (4)	≤0.00076 ± 0.00043 (4)	≤0.038 ± 0.020 (4)

[a] The 381G *MAT***a** strains used in this study were as follows, in order from top to bottom: 8882-7-2, 8882-7-2 *axl1*::*URA3*, 8882-7-2 *bni1Δ*::*URA3*, 8882-7-2 *chs5Δ*::*URA3*, 8882-7-2 *fus2Δ*, 8882-7-2 *pea2Δ*::*URA3*, 8882-7-2 *spa2Δ*::*URA3*, 10838-12-1, and 10848A-7-1. The W303 *MAT***a** strains used were Y96, Y106, and Y331. For the 381G *MAT***a** strains, the *MATα* mating partners used were 7609-7-3 and 8907-4-lura3^{FOAr}. For the W303 *MAT***a** strains, the *MATα* mating partners were 7609-7-3 and 8941-12-2.
[b] See footnote *c*, Table 2.
[c] See footnote *d*, Table 2.
[d] See footnote *e*, Table 2.

EXS1 is synthetic sterile with the *sst2Δ* mutation (C. Boone, unpublished data). We confirmed that *chs5Δ* mutants are defective at cell fusion during chemotropic matings with a wild-type *MATα* cell partner in the absence of exogenous pheromone, forming prezygotes ~30 times more frequently than a wild-type control strain [≤0.5 ± 0% and 15 ± 5.3% prezygotes, respectively (Figure 2)]. However, despite this defect in cell fusion, *chs5Δ* mutants are not very defective at default mating (Table 2). In the 381G background the *chs5Δ* strain mated about as efficiently as wild-type cells mated with pheromoneless cells in saturating pheromone [2.3% and 6.9%, respectively (Table 2)]. Similar results were obtained for the *chs5Δ* mutation in the W303 background (Table 2). Therefore, not all aspects of cell fusion are more important during default mating than during chemotropic mating.

Multicopy suppression of mutations in *SPA2*: To investigate the defect in default mating caused by mutations in *SPA2*, we isolated high copy suppressors of the *spa2-1* and *spa2-2* mutations (see Materials and Methods). Because these *spa2* mutations were isolated in a *bar1* mutant background (Chenevert *et al.* 1994), we analyzed the default mating ability of these strains using a discrimination assay. Bar1p is a protease that degrades α-factor (Mackay *et al.* 1988). Thus *MAT***a** *bar1* strains fail to degrade the α-factor secreted by their mating partner and are supersensitive to α-factor. This failure leads to a high concentration of α-factor in the media, a situation analogous to adding exogenous α-factor to the mating mixture. Therefore, even without adding exogenous α-factor to the media, *bar1* mutants mate by default with the pheromoneless partner in a discrimination assay at a high frequency (Jackson and Hartwell 1990b).

The *spa2-1* and *spa2-2* mutations prevent default matings, lowering the high randomness index caused by the *bar1* mutation in a *MAT***a** strain (Table 4). We found that when transformed with a high copy 2μ plasmid containing either *MFA2* or *FUS2*, the mating efficiencies of the *spa2-1* and *spa2-2* strains with the pheromoneless strain in the discrimination assay were increased by at least 7.9- to 26-fold (Table 4).

In addition, since the molecular nature of these *spa2* mutations is unknown, we asked if muliple copies of *MFA2* or *FUS2* could suppress a deletion of *SPA2*. We found that multiple copies of both *MFA2* and *FUS2* partially suppressed the low mating efficiency of an *sst2Δspa2Δ* double mutant with a pheromoneless *MATα* cell partner. The mating efficiency with the pheromoneless strain increased from 0.00049 to 0.060% for the strains containing the vector control and pMFA2, respectively (Table 5), and the mating efficiency increased from 0.0015 to 0.014% for the vector control and pFUS2, respectively. Therefore, multiple copies of *MFA2* and *FUS2* partially suppress the requirement for *SPA2* during default mating.

The *MFA2* gene is one of the two **a**-factor genes

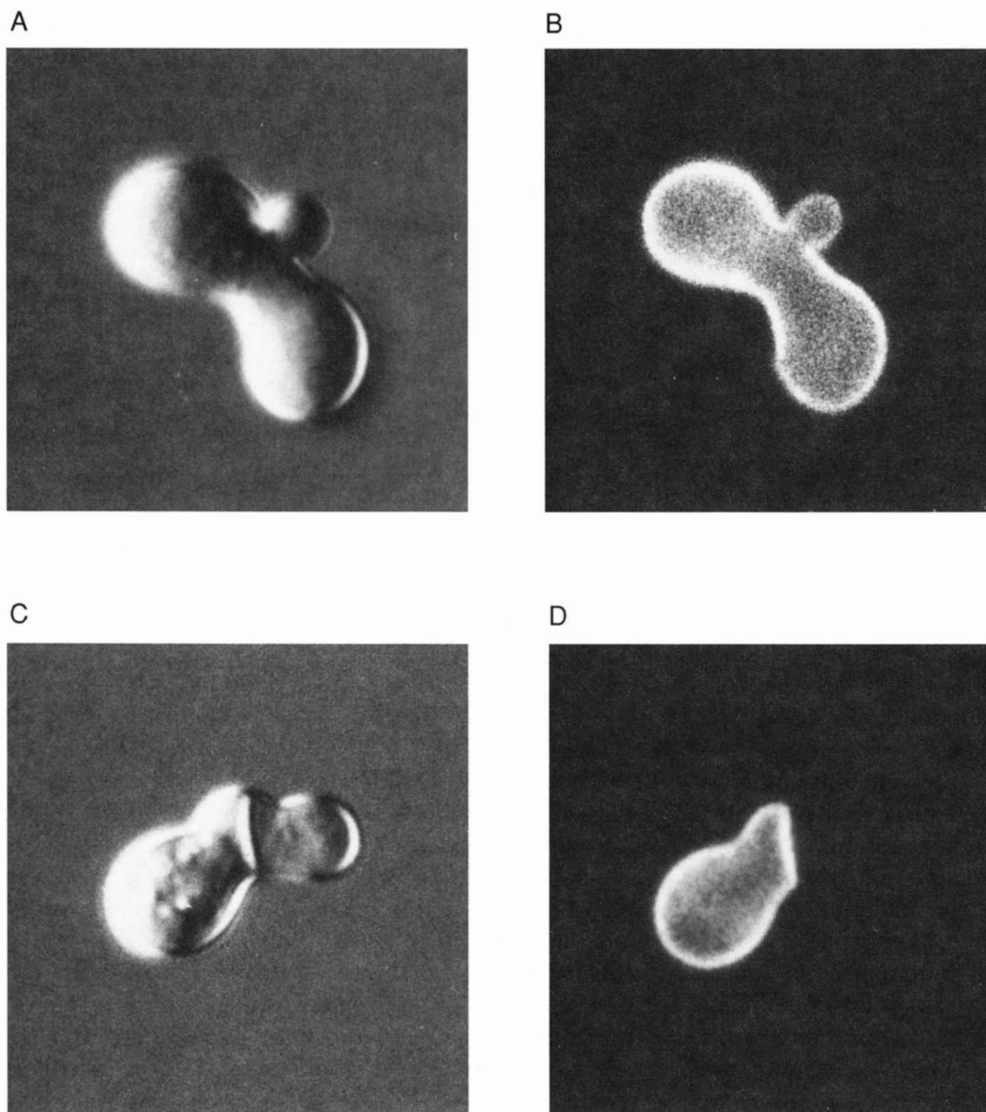

FIGURE 1.—An assay for cell fusion. *MAT***a** strains were transformed with a plasmid containing a fusion of *RAS2* with the gene encoding the Green Fluorescent Protein [(pMA106) kindly provided by JENNIFER WHISTLER, University of California, Berkeley]. The fusion protein localizes to the cell membrane, and when partners fuse, the protein diffuses from the *MAT***a** cell into the membrane of the *MATα* cell partner. Therefore, fused cells [A (Nomarski image) and B (fluorescent image)] contain GFP staining throughout the zygote, and unfused mating pairs (prezygotes) contain GFP staining in only the *MAT***a** mating partner [C (Nomarski) and D (fluorescent image)]. The *MAT***a** strains used were 7623-16-3 *AXL1* (A and B) and 7623-16-3 *axl1::URA3* (C and D); the *MATα* strain used was 7609-7-3. Matings were for 3 hr at 30° on Noble Agar plates.

(MICHAELIS and HERSKOWITZ 1988). A *MAT***a** strain containing the high copy plasmid pMFA2 secretes more **a**-factor than a strain containing a control vector (JACKSON and HARTWELL 1990a). Therefore, these results indicate that the overexpression of **a**-factor can partially bypass the need for *SPA2* during default mating. This conclusion is related to the observation that *axl1::URA3* mutants, which secrete less **a**-factor than wild type (ADAMES *et al.* 1995), are defective at default mating and cell fusion (for possible explanations of these data, see DISCUSSION).

Default mating and bud site selection: It is noteworthy that several of the mutants described above that are defective in default mating are also defective in either axial (*axl1* mutants) or bipolar (*spa2Δ, pea2Δ, bni1Δ,* and *rvs161Δ* mutants) bud site selection (FUJITA *et al.* 1994; ADAMES *et al.* 1995; SIVADON *et al.* 1995; ZAHNER *et al.* 1996; VALTZ and HERSKOWITZ 1996). Is it possible that these mutants fail to mate in saturating pheromone because they have not established a bud site at the correct location on the cell surface? To answer this question we tested several bud site selection mutants in the default mating assay. The *BUD3, BUD4,* and *AXL2* genes are required for axial bud site selection, the *BUD6, BUD7, BUD8,* and *BUD9* genes are required for bipolar bud site selection, and the *RSR1* and *BUD2* genes are required for both axial and bipolar bud site selection (BENDER and PRINGLE 1989; CHANT and HERSKOWITZ 1991; CHANT *et al.* 1995; ROEMER *et al.* 1996; ZAHNER *et al.* 1996). We found that the *rsr1-, bud2-, bud3-, bud4-, bud6Δ, bud7-1, bud8Δ, bud9-1,* and *axl2Δ* mutants all mated in saturating pheromone as well as the wild-type control strains (Table 6). Therefore, improper bud site selection does not necessarily cause a defect in default mating, and *AXL1, BNI1, SPA2, PEA2,* and *RVS161* can function during default mating independent of a defective bud site selection machinery. Furthermore, because the bud site selection mutants all mated as efficiently as the wild-type control strains and because they all were inhibited to the same degree as the wild-type strains by saturating pheromone (Table 6), these data suggest that none of these genes are required for chemotropic

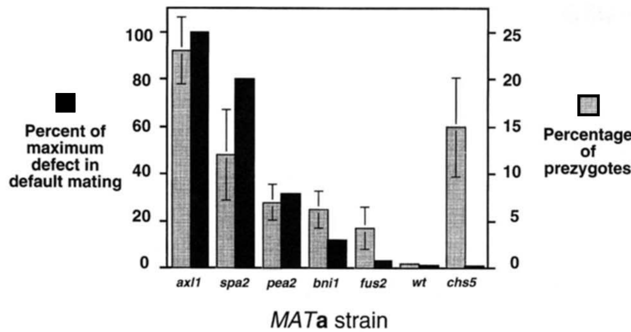

FIGURE 2.—The degree of defect in cell fusion correlates with the degree of defect in default mating. The level of defect in cell fusion was calculated by determining the percentage of all zygotes that were prezygotes in a quatitative mating (see MATERIALS AND METHODS). Matings were for 3 hr at 30° on Noble Agar plates. A total of 200 zygotes were scored for each mating, and the mean and standard error of at least three independent experiments are shown. The *MAT***a** strains used contained pMA106 and were isogenic with 7623-16-3, except for the following indicated mutations: *axl1::URA3, bni1Δ::URA3, chs5Δ::URA3, fus2Δ::URA3, pea2Δ::URA3,* and *spa2Δ::URA3.* The *MATα* strain used was 7609-7-3. The degree of default mating defect was calculated (using data in Table 2) as the ratio of the mean of the mating efficiency of the indicated *MAT***a** strain in the absence of pheromone (chemotropism) divided by the mean of mating efficiency of the *MAT***a** strain with pheromoneless cells in the presence of saturating pheromone (default); the ratios were 4100, 3300, 1300, 480, 110, 19, 25 for *axl1::URA3, spa2Δ, pea2Δ, bni1Δ, fus2Δ,* wild type, and *chs5Δ,* respectively. The ratios were normalized to 100% for the *axl1::URA3* strain.

matings. Therefore, regulated bud site selection is not required for chemotropism.

Default mating and Ash1p localization: *BNI1* was independently identified by its role in mating type switching (BOBOLA *et al.* 1996; JANSEN *et al.* 1996). When haploid yeast cells divide, a mother and daughter cell are generated. The mother cell is capable of switching mating type and the daughter is not. Switching is stimulated by the HO endonuclease, which is only expressed in the mother cell. The *ASH1* gene prevents expression of HO in daughter cells (BOBOLA *et al.* 1996; SIL and HERSKOWITZ 1996), and the Ash1 protein is preferentially localized in daughter cell nuclei at the end of anaphase by a mechanism that requires the products of the *SHE1/MYO4, SHE2, SHE3, SHE4,* and *SHE5/BNI1* genes (BOBOLA *et al.* 1996; JANSEN *et al.* 1996). The fact that *SHE5/BNI1* is required for default mating suggests that default mating and Ash1p localization could be dependent on the same genes. Therefore, we tested the *she1Δ/myo4Δ, she2Δ, she3Δ,* and *she5Δ/bni1Δ* mutants in the default mating assay (Table 7). Consistent with our earlier observations (Table 2), we found that the mating efficiency of *she5Δ/bni1Δ* mutant was reduced in the presence of saturating pheromone (from 81 to 0.0077%). In contrast, the mating effiencies of the *she1Δ/myo4Δ, she2Δ,* and *she3Δ* strains were reduced to the same degree as the *SHE* control strain [between 16- and 28-fold (Table 7)], and the *she1/myo4, she2,* and *she3* mutants mated as well as the *SHE* strain mated with pheromoneless cells in saturating pheromone. Therefore, default mating and Ash1p localization do not require all of the same genes. Consistent with this observation, *SPA2* is not required for Ash1p localization (JANSEN *et al.* 1996). Furthermore, these data strongly suggest that the *SHE1/MYO4, SHE2,* and *SHE3* genes are not required for chemotropic morphogenesis, because the mutants mated as efficiently as the *SHE* strain mated in the absence of pheromone and were inhibited

TABLE 4

The defect in default mating caused by mutations in *SPA2* is partially suppressed by multiple copies of *FUS2* and *MFA2*

*MAT***a** strain[a]	Total mating efficiency (%)[b]	Mating efficiency with pheromoneless (%)[c]	Randomness index[d]
bar1 SPA2 (pMFA2)	34 ± 2.5 (3)	9.1 ± 0.23 (3)	0.56 ± 0.042 (3)
bar1 SPA2 (YEp352)	39 ± 3.5 (3)	10 ± 0.95 (3)	0.54 ± 0.055 (3)
bar1 SPA2 (pFUS2)	41 ± 3.6 (3)	6.3 ± 0.64 (3)	0.33 ± 0.026 (3)
bar1 SPA2 (pRS426)	40 ± 4.6 (3)	7.7 ± 0.81 (3)	0.41 ± 0.059 (3)
bar1 spa2-1 (pMFA2)	4.0 ± 0.67 (3)	0.11 ± 0.023 (3)	0.060 ± 0.0038 (3)
bar1 spa2-1 (YEp352)	3.3 ± 1.0 (3)	0.014 ± 0.0035 (3)	0.0096 ± 0.0044 (3)
bar1 spa2-1 (pFUS2)	4.9 ± 1.1 (3)	0.21 ± 0.057 (3)	0.093 ± 0.015 (3)
bar1 spa2-1 (pRS426)	2.5 ± 0.47 (3)	0.0081 ± 0.0012 (3)	0.0073 ± 0.0022 (3)
bar1 spa2-2 (pMFA2)	9.0 ± 0.89 (3)	0.49 ± 0.14 (3)	0.12 ± 0.039 (3)
bar1 spa2-2 (YEp352)	4.9 ± 0.98 (3)	0.056 ± 0.011 (3)	0.025 ± 0.0078 (3)
bar1 spa2-2 (pFUS2)	12 ± 3.4 (5)	0.50 ± 0.049 (5)	0.097 ± 0.028 (5)
bar1 spa2-2 (pRS426)	21, 30	0.039, 0.020	0.0033, 0.0012

[a] The *MAT***a** strains used were JC2-1B (*bar1*), JC2-D6 (*spa2-1*), and JC2-J9 (*spa2-2*), transformed with the indicated plasmids. The *MATα* strains used were 7609-7-3 and 8907-4-1.

[b] See footnote *c,* Table 2.

[c] See footnote *d,* Table 2.

[d] See footnote *e,* Table 2.

TABLE 5

Multicopy *FUS2* and *MFA2* partially suppress the defect in default mating caused by *spa2Δ* and the synthetic sterility of *spa2Δ* and *sst2Δ*

*MAT***a** strain[a]	Total mating efficiency (%)[b]	Mating efficiency with pheromoneless (%)[c]	Randomness index[d]
SPA2 (pMFA2)	96 ± 14 (2)	$\leq 6.9 \times 10^{-4} \pm 4.1 \times 10^{-4}$ (2)	$\leq 1.4 \times 10^{-5} \pm 7.0 \times 10^{-6}$ (2)
SPA2 (YEp352)	120 ± 0 (2)	$<6.2 \times 10^{-4} \pm 0$ (2)	$<1.1 \times 10^{-5} \pm 0$ (2)
SPA2 (pFUS2)	120 ± 0 (2)	$<3.4 \times 10^{-4} \pm 0$ (2)	$<6.0 \times 10^{-6} \pm 0$ (2)
SPA2 (pRS426)	120 ± 5 (2)	$<3.4 \times 10^{-4} \pm 0$ (2)	$<5.8 \times 10^{-6} \pm <2.0 \times 10^{-7}$ (2)
spa2Δ (pMFA2)	61 ± 3 (2)	$\leq 4.8 \times 10^{-4} \pm 1.6 \times 10^{-4}$ (2)	$\leq 1.7 \times 10^{-5} \pm 6.0 \times 10^{-6}$ (2)
spa2Δ (YEp352)	58 ± 10 (2)	$<2.9 \times 10^{-4} \pm 0$ (2)	$<1.1 \times 10^{-5} \pm 2.0 \times 10^{-6}$ (2)
spa2Δ (pFUS2)	120 ± 0 (2)	$<8.3 \times 10^{-4} \pm 0$ (2)	$<1.5 \times 10^{-5} \pm 0$ (2)
spa2Δ (pRS426)	55 ± 8 (2)	$<3.3 \times 10^{-4} \pm 0$ (2)	$<1.1 \times 10^{-5} \pm 0$ (2)
sst2Δ (pMFA2)	18 ± 3.5 (3)	6.1 ± 1.7 (3)	0.70 ± 0.060 (3)
sst2Δ (YEp352)	26 ± 2.5 (3)	8.0 ± 0.41 (3)	0.67 ± 0.046 (3)
sst2Δ (pFUS2)	15 ± 1.2 (3)	3.9 ± 0.61 (3)	0.54 ± 0.10 (3)
sst2Δ (pRS426)	17 ± 1 (2)	6.2 ± 0.40 (2)	0.78 ± 0.01 (2)
sst2Δspa2Δ (pMFA2)	1.9 ± 0.61 (3)	0.060 ± 0.029 (3)	0.063 ± 0.015 (3)
sst2Δspa2Δ (YEp352)	0.80 ± 0.27 (3)	0.00049 ± 0.00021 (3)	0.0017 ± 0.00087 (3)
sst2Δspa2Δ (pFUS2)	0.89 ± 0.16 (3)	0.014 ± 0.0025 (3)	0.033 ± 0.0016 (3)
sst2Δspa2Δ (pRS426)	0.26 ± 0 (2)	0.0015 ± 0.00060 (2)	0.012 ± 0.0050 (2)

[a] The *MAT***a** strain used, in order from top to bottom, were as follows: Y604, Y609, Y604*sst2Δ*, and Y609*sstΔ*, transformed with the indicated plasmid. The *MATα* strains used were 8907-4-1 and 7609-7-3.

[b] The percentage of *MAT***a** cells that formed diploids with either the *MATα* wild-type or the α-pheromoneless strain partners. The mean and standard error of the number of independent observations shown in parentheses is indicated, where appropriate. When there were two independent experimental observations, the mean and the range are shown.

[c] See footnote *d*, Table 2.

[d] See footnote *e*, Table 2.

by pheromone to the same degree as the *SHE* strain (Table 7).

Default mating and projection formation: Mutants defective in the *SPA2, PEA2, BNI1,* and *FUS3* genes are defective at mating projection formation. Instead of forming pointed projections, these cells form broader, peanut-shaped projections (Elion *et al.* 1990; Chenevert *et al.* 1994; Yorihuzi and Ohsumi 1994; C. Boone, unpublished data). Do defects in projection formation cause defects in default mating? Perhaps cells require a pointed projection to localize components that are required for cell fusion and default mating to the tip of the projection. To answer this question, we tested the *bem1-s1* and *bem1-s2* mutants, which form spherical cells in the presence of pheromone, for their ability to mate by default. In addition, we tested the *tny1-1* and *tny1-2* mutants, which form tiny mating projections (Chenevert *et al.* 1992, 1994), and we tested the *afr1Δ* mutation, which causes cells to form broader projections than wild type (Konopka 1993; Konopka *et al.* 1995). Because these mutations were isolated in a *bar1* strain (Chenevert *et al.* 1994), we tested their effect on the default mating ability of a *bar1* mutant. In contrast to a *spa2Δ* control strain, we found that the *bem1-s1, bem1-s2, tny1-1, tny1-2,* and *afr1Δ* mutations did not affect the ability of the *MAT***a** *bar1* cell to mate by default with the pheromoneless partner in a discrimination assay in the absence of exogenous pheromone (Table 8). Therefore, defects in projection formation do not necessarily cause defects in default mating. As further support of this conclusion, we found that the *ste2-T326* mutation, which also causes cells to form broader shmoo tips than wild type (Konopka *et al.* 1988), does not cause a defect in default mating, as measured by the ability of the *ste2-T326* mutant to mate with pheromoneless cells in the presence of saturating pheromone (Table 8). These results suggest that the defects in default mating that are caused by *spa2Δ, pea2Δ, bni1Δ,* and *fus3Δ* are a result of a defect in a process that can function indepenently of some aspects of projection formation, since the Spa2, Pea2, Fus3, and Bni1 proteins are still able to perform their default mating functions, despite the above defects in projection formation. Perhaps the Pea2, Spa2, and Bni1 proteins are still localized and activated correctly in the *bem1, tny1, afr1Δ,* and *ste2-T326* mutants.

DISCUSSION

Yeast cells are capable of choosing a mating partner by two different mechanisms, by chemotropism or by default. We are interested in understanding how the default mating pathway is activated in response to saturating pheromone. In this report we used two approaches to begin to address this question. First, we identified seven genes, in addition to *SPA2*, that are more important for default mating than for chemotropic mating: the *FUS1, FUS2, FUS3, AXL1, BNI1,*

TABLE 6

***rsr1-*, *bud2-*, *bud3-*, *bud4-*, *bud6Δ*, *bud7-1*, *bud8Δ*, *bud9-1*, and *axl2Δ* mutants are not defective in their ability to mate by default in saturating α-factor**

*MAT***a** strain[a]	α-factor[b]	Total mating efficiency (%)[c]	Mating efficiency with pheromoneless (%)[d]	Randomness index[e]	Fold inhibition[f]
BUD	−	150 ± 16 (3)	0.0081 ± 0.00064 (3)	$9.7 \times 10^{-5} \pm 3.3 \times 10^{-5}$ (3)	
	+	11 ± 1.1 (3)	5.7 ± 0.43 (3)	0.99 ± 0.11 (3)	14
rsr1-	−	140 ± 7.1 (3)	0.0085 ± 0.0015 (3)	$1.2 \times 10^{-4} \pm 2.0 \times 10^{-5}$ (3)	
	+	8.1 ± 1.3 (3)	4.2 ± 1.0 (3)	0.96 ± 0.029 (3)	17
bud2-	−	130 ± 15 (3)	0.0069 ± 0.00074 (3)	$1.0 \times 10^{-4} \pm 1.4 \times 10^{-5}$ (3)	
	+	12 ± 0.27 (3)	6.0 ± 0.30 (3)	0.92 ± 0.037 (3)	11
bud3-	−	170 ± 15 (3)	0.0051 ± 0.0038 (3)	$5.6 \times 10^{-5} \pm 2.7 \times 10^{-5}$ (3)	
	+	15 ± 3.4 (3)	6.8 ± 1.6 (3)	0.87 ± 0.090 (3)	11
bud4-	−	150 ± 12 (3)	0.0078 ± 0.0015 (3)	$9.9 \times 10^{-5} \pm 4.6 \times 10^{-6}$ (3)	
	+	18 ± 4.3 (3)	5.1 ± 0.51 (3)	0.58 ± 0.16 (3)	8.3
BUD	−	120 ± 7.1 (3)	0.00056 ± 0.00037 (3)	$8.2 \times 10^{-6} \pm 3.8 \times 10^{-6}$ (3)	
	+	7.9 ± 2.7 (3)	2.7 ± 1.3 (3)	0.60 ± 0.039 (3)	15
spa2Δ::URA3	−	83 ± 5.8 (3)	≤0.00056 ± 0.00019 (3)	$\leq 1.7 \times 10^{-5} \pm 6.6 \times 10^{-6}$ (3)	
	+	0.33 ± 0.12 (3)	0.051 ± 0.0081 (3)	0.42 ± 0.14 (3)	250
bud6Δ::TRP1	−	88 ± 2.5 (3)	0.0014 ± 0.0012 (3)	$2.9 \times 10^{-5} \pm 1.7 \times 10^{-5}$ (3)	
	+	7.0 ± 0.36 (3)	3.2 ± 0.17 (3)	0.79 ± 0.069 (3)	13
bud7-1	−	110 ± 5.0 (3)	0.0019 ± 0.0011 (3)	$3.4 \times 10^{-5} \pm 1.3 \times 10^{-5}$ (3)	
	+	9.9 ± 1.2 (3)	3.7 ± 0.46 (3)	0.73 ± 0.14 (3)	11
bud8Δ::TRP1	−	170 ± 15 (3)	0.0019 ± 0.00023 (3)	$2.0 \times 10^{-5} \pm 1.9 \times 10^{-6}$ (3)	
	+	13 ± 1.3 (3)	6.4 ± 0.80 (3)	0.82 ± 0.0071 (3)	13
bud9-1	−	130 ± 8.7 (3)	0.0025 ± 0.0014 (3)	$3.6 \times 10^{-5} \pm 1.7 \times 10^{-5}$ (3)	
	+	8.3 ± 0.74 (3)	3.1 ± 0.23 (3)	0.72 ± 0.051 (3)	16
AXL2	−	92 ± 8 (2)	<0.00036 ± 0 (2)	$<7.2 \times 10^{-6} \pm 6.0 \times 10^{-7}$ (2)	
	+	10 ± 0 (2)	5.4 ± 0.3 (2)	0.97 ± 0.40 (2)	9.2
axl2Δ::URA3	−	94 ± 6 (2)	<0.00036 ± 0 (2)	$<7.6 \times 10^{-6} \pm 0$ (2)	
	+	10 ± 2 (2)	4.2 ± 1.2 (2)	0.78 ± 0.60 (2)	9.4

[a] The *MAT***a** strains used, in order from top to bottom, were as follows: IH2393, IH2407, IH2408, IH2409, IH2410, YJZ355, YJZ355*spa2Δ::URA3*, YJZ354, HH113, HH394, HH114, Y1155, and Y1153. The *MATα* cells used were 11236-12-2 and 8941-12-2 (for IH2393, IH2407, IH2408, IH2409, IH2410, HH113, and HH114), 7609-7-3 and 8907-4-1 (for YJZ355, YJZ355*spa2Δ::URA3*, YJZ354, and HH394), or 7609-7-3 and 8941-12-2 (for Y1153 and Y1155).

[b] See footnote *b*, Table 2.

[c] See footnote *c*, Table 5.

[d] See footnote *d*, Table 2.

[e] See footnote *e*, Table 2.

[f] See footnote *f*, Table 2.

RVS161, and *PEA2* genes. All of these genes are also important for cell fusion during chemotropic mating, and we found that the degree of defect in cell fusion is proportional to the degree of defect in default mating. Second, we identified *MFA2* and *FUS2* as multicopy suppressors of the defect in default mating caused by mutations in *SPA2*. These observations support the existence of a molecular relationship between cell fusion, **a**-factor secretion, and default mating. Our findings raise the following three questions: (1) what are the molecular roles of these eight genes in cell membrane fusion? (2) what is the role of **a**-factor in cell fusion? and (3) why are *SPA2* and these other genes more important for default mating than for chemotropic mating?

The roles of *FUS1*, *FUS2*, *FUS3*, *BNI1*, *RVS161*, *SPA2*, *PEA2*, and *AXL1* in cell fusion: The phenotypes of the *fus1Δ*, *fus2Δ*, *fus3Δ*, *bni1Δ*, *rvs161Δ*, *pea2Δ*, *spa2Δ*, and *axl1::URA3* mutants argue strongly that the proteins promote cell fusion. These mutations block cell fusion and generate aberrant zygotes [called prezygotes (Figure 1)], in which the cell walls are fused but the membranes remain unfused and separated by cell wall material (Trueheart *et al.* 1987). To accomplish cell fusion, cells must contact each other at the tips of their mating projections, fuse their intervening cell walls, and then locally fuse their plasma membranes. Several molecular events are coordinated to insure the fidelity of these events. First, cells choose a site for conjugation. This site is used as a cue toward which cells reorient their actin cytoskeleton and secretory apparatus. Second, a set of polarity establishment proteins promote the formation of a mating projection. These proteins include Cdc24p, Cdc42p and Bem1p, which are generally required for the emergence of polarized structures in yeast (for a review, see Drubin and Nelson 1996). Third, cells must localize proteins that promote cell wall and membrane fusion to the mating projection tip. Bem1p and Cdc42p are localized to the tip of the mat-

TABLE 7

she1/myo4Δ, she2Δ, and she3Δ mutants are not defective in their ability to mate by default in saturating pheromone

*MAT***a** strain[a]	α-factor[b]	Total mating efficiency (%)[c]	Mating efficiency with pheromoneless (%)[d]	Randomness index[e]	Fold inhibition[f]
SHE	–	220 ± 31 (4)	≤0.00048 ± 0.000029 (4)	≤$3.7 \times 10^{-6} \pm 3.8 \times 10^{-7}$ (4)	
	+	15 ± 2.1 (4)	5.6 ± 1.0 (4)	0.65 ± 0.16 (4)	15
she1Δ/myo4Δ	–	260 ± 30 (2)	<0.00056 ± 0 (2)	<$4.0 \times 10^{-4} \pm 5.0 \times 10^{-5}$ (2)	
	+	14 ± 1 (2)	4.4 ± 0.4 (2)	0.56 ± 0.010 (2)	18
she2Δ	–	220 ± 10 (2)	0.00050 ± 0 (2)	$3.8 \times 10^{-6} \pm 2.0 \times 10^{-7}$ (2)	
	+	7.8 ± 0.8 (2)	3.2 ± 0.5 (2)	0.70 ± 0.30 (2)	28
she3Δ	–	260 ± 0 (2)	0.0092 ± 0.0018 (2)	$7.0 \times 10^{-5} \pm 6.0 \times 10^{-6}$ (2)	
	+	14 ± 4.0 (2)	4.8 ± 0.70 (2)	0.66 ± 0.10 (2)	19
she5Δ/bni1Δ	–	81 ± 4.0 (2)	<0.0019 ± 0 (2)	<$4.2 \times 10^{-5} \pm 2.0 \times 10^{-6}$ (2)	
	+	0.0077 ± 0.0019 (2)	0.0038 ± 0 (2)	0.96 ± 0.25 (2)	10,000

[a] The *MAT***a** strains used, in order from top to bottom, were as follows: 5104-1-3, 5104-2-2, 10900-7-2, 5213-1-1, and 5102-2-2. The *MAT*α strains used were 7609-7-3 and 8907-4-1.
[b] See footnote *b*, Table 2.
[c] See footnote *c*, Table 5.
[d] See footnote *d*, Table 2.
[e] See footnote *e*, Table 2.
[f] See footnote *f*, Table 2.

ing projection and interact with the yeast mating signal transduction pathway (Ziman *et al.* 1993; Cvrckova *et al.* 1995; Simon *et al.* 1995; Zhao *et al.* 1995; Kao *et al.* 1996). Therefore, these polarity establishment proteins could promote cell fusion by localizing or activating proteins that are important for cell fusion. Finally, cells may communicate with each other just before initiating the fusion reaction to insure that both mating partners are prepared to fuse.

Given the diversity of their mutant phenotypes and protein sequences, it is likely that the Fus1, Fus2, Fus3, Bni1, Spa2, Pea2, Rvs161, and Axl1 proteins are each required to perform somewhat different functions during the fusion process. These proteins may interact with the polarity establishment proteins to organize the cytoskeleton and secretory apparatus at the conjugation site, directly catalyze the cell fusion reaction at the mating projection tip, localize or activate proteins that directly catalyze cell fusion, or participate in intercellular communication just before cell fusion.

Fus1p and Fus2p could be involved in any of these processes. While neither the Fus1 nor the Fus2 protein

TABLE 8

bem1-s, tny1, ste2-T326, and afr1Δ mutants are not defective in default mating

*MAT***a** strain[a]	α-factor[b]	Total mating efficiency (%)[c]	Mating efficiency with pheromoneless (%)[d]	Randomness index[e]	Fold inhibition[f]
bar1	–	34 ± 6.6 (4)	9.1 ± 2.7 (4)	0.53 ± 0.15 (4)	
bar1 spa2Δ	–	6.2 ± 1.1 (4)	0.013 ± 0.0067 (4)	0.0048 ± 0.0023 (4)	
bar1 bem1-s1	–	14 ± 7.8 (4)	5.7 ± 4.2 (4)	0.70 ± 0.18 (4)	
bar1 bem1-s2	–	14 ± 6.9 (4)	6.0 ± 3.5 (4)	0.77 ± 0.086 (4)	
bar1-1 tny1-1	–	32 ± 5.8 (4)	4.2 ± 0.85 (4)	0.27 ± 0.088 (4)	
bar1-1 tny1-2	–	6.3 ± 0.41 (4)	1.8 ± 0.12 (4)	0.56 ± 0.087 (4)	
bar1-1 AFR1	–	34 ± 2.6 (3)	12 ± 1.7 (3)	0.61 ± 0.042 (3)	
bar1-1 afr1Δ	–	26 ± 3.8 (3)	5.6 ± 0.55 (3)	0.38 ± 0.041 (3)	
STE2	–	140 ± 36 (4)	0.0011 ± 0.0010 (4)	$1.4 \times 10^{-5} \pm 6.9 \times 10^{-6}$ (4)	
	+	17 ± 6.9 (5)	7.5 ± 2.9 (5)	0.89 ± 0.072 (5)	8.2
ste2-T326	–	66 ± 13 (5)	0.0018 ± 0.0014 (4)	$5.8 \times 10^{-5} \pm 4.4 \times 10^{-5}$ (5)	
	+	11 ± 1.4 (5)	5.3 ± 0.69 (5)	0.95 ± 0.069 (5)	6.0

[a] The *MAT***a** strains used, in order from top to bottom, were as follows: JC2-1B, NVY139, JC2-G11, JC2-F5, JC2-G16, JC2-H9, DJ211-1-2, JK26, DJ147-1-2, and JKY7441-4a. The *MAT*α strains used were 7611-2 and 8941-12-2.
[b] See footnote *b*, Table 2.
[c] See footnote *c*, Table 2.
[d] See footnote *d*, Table 2.
[e] See footnote *e*, Table 2.
[f] See footnote *f*, Table 2.

is required for projection formation or for signal transduction in the pheromone-response pathway, both proteins are important for cell fusion (McCaffrey *et al.* 1987; Trueheart *et al.* 1987). *FUS1* encodes an O-linked glycoprotein that spans the plasma membrane of the projection tip (Trueheart and Fink 1989). *FUS2* is predicted to encode a coiled-coil protein, and Fus2p localizes to discrete structures at the projection tip and at the junction of fused, mating cells (Elion *et al.* 1995). Therefore, both Fus1p and Fus2p could either be part of the fusion machinery or act as cytoskeletal components that aid in organizing the fusion machinery. *FUS3* is also important for cell fusion (Elion *et al.* 1990). However, it is likely that Fus3p does not directly catalyze the fusion reaction, but instead acts more indirectly by phosphorylating proteins that directly promote cell fusion (Elion *et al.* 1993). *FUS3* encodes a MAP kinase that is an integral part of the pheromone-reponse pathway and functionally overlaps with the *KSS1* gene, a MAP kinase that is required for signal transduction but not for cell fusion. *FUS3* is required for multiple functions during mating [G1 phase arrest, projection formation, and cell fusion (Elion *et al.* 1990, 1991, 1993)]. Defects in any one of these processes could contribute to the low mating efficiency of *fus3*Δ mutants.

The *BNI1, SPA2,* and *PEA2* genes are all important for projection formation. *bni1*Δ, *spa2*Δ, and *pea2*Δ mutants form abnormal projections in the presence of saturating pheromone that are oval, broader, or more peanut-shaped than wild type, depending on the strain background and allele (Gehrung and Snyder 1990; Chenevert *et al.* 1994; Yorihuzi and Ohsumi 1994; Valtz and Herskowitz 1996; C. Boone, unpublished data). During mating both Pea2p and Spa2p localize as a sharp patch to the projection tip, and during vegetative growth both localize to the presumptive bud site in unbudded cells, to the tip of the growing bud, and to the bud neck in cells undergoing cytokinesis (Snyder 1989; Gehrung and Snyder 1990; Valtz and Herskowitz 1996). The localization of Bni1p is similar to Spa2p (Longtine *et al.* 1996). Moreover, *BNI1, SPA2,* and *PEA2* are similar in their roles in other aspects of cell polarity: (1) all three genes are required for bipolar bud site selection (Zahner *et al.* 1996); (2) *spa2*Δ is synthetic lethal with a mutation in the septin gene *CDC10* (Flescher *et al.* 1993); (3) *bni1*Δ is synthetic lethal with a mutation in the septin gene *CDC12* (Longtine *et al.* 1996); and (4) the localizations of Pea2p and Spa2p are interdependent (Valtz and Herskowitz 1996). Despite these similarities, the proteins are not homologous. *BNI1* encodes a 220-kDa protein that contains coiled-coil domains flanking a proline-rich region and is homologous to several proteins involved in polarity establishment and cytokinesis, including the Drosophila proteins *diaphanous* and *cappuccino* and the product of the mouse *limb deformity* gene (Castrillion and Wasserman 1994; Emmons *et al.* 1995; Longtine *et al.* 1995). *SPA2* encodes a 180-kDa protein that displays some low level sequence similarities to proteins containing coiled-coil structures (Gehrung and Snyder 1990). *PEA2* is not homologous to *SPA2* or to any other proteins (Valtz and Herskowitz 1996). Bni1p, but not Spa2p, is required for the localization of the Ash1 protein (Jansen *et al.* 1996) (the role of Pea2p in this process has not been reported), and the localization of Spa2p and Pea2p do not require *BNI1* (Valtz and Herskowitz 1996). However, the mating projection tip localization of the Spa2 and Pea2 proteins (and perhaps Bni1p), coupled with their common mutant phenotypes, suggests that these three proteins may act as a functional group. Perhaps one or all three of these proteins localizes cell fusion components to the projection tip or interacts with the polarity establishment proteins to organize the secretory apparatus and cytoskeleton at the projection tip.

RVS161 encodes a 30-kDa protein that is similar to the Rvs167 protein, an actin-binding protein (Bauer *et al.* 1993; Amberg *et al.* 1995). Both Rvs161p and Rvs167p are similar to amphiphysin, a neuronal protein that was first identified in chicken synaptic vesicles and is the autoantigen of Stiff-Man Syndrome associated with breast cancer (Crouzet *et al.* 1991; Bauer *et al.* 1993; David *et al.* 1994; Sivadon *et al.* 1995). *RVS161* is also required for viability in stationary phase (Crouzet *et al.* 1991), for endocytosis (Munn *et al.* 1995), and for bipolar, but not axial, bud site selection (Durrens *et al.* 1995; Sivadon *et al.* 1995). The role of Rvs161p in cell fusion is unknown, but since amphiphysin is a synaptic vesicle associated protein, it is possible that Rvs161p is required for a specialized fusion of vesicles during cell membrane fusion. Perhaps there is a regulated vesicle fusion process that delivers enzymes that degrade cell wall material or promote membrane fusion during the mating process. Alternatively, Rvs161p could physically interact with the actin cytoskeleton to directly organize the cell fusion machinery at the mating projection tip.

The role of a-factor in cell fusion: It is intriguing that the *AXL1* gene is required for both cell fusion and for default mating. Axl1p shares homology with the insulin-degrading enzyme family of endoproteases and is involved in **a**-factor propheromone processing. As a result, *axl1* mutants secrete reduced levels of **a**-factor (Adames *et al.* 1995). However, **a**-factor secretion is not abolished in *axl1* mutants, because *AXL1* is redundant with a homologous gene, *STE23,* for propheromone processing. *AXL1* is also required for axial bud site selection in yeast (Fujita *et al.* 1994). But this function appears to be distinct from the function of *AXL1* in propheromone processing, since mutations that abolish the propheromone processing function of *AXL1* do not affect bud site selection (Adames *et al.* 1995). Why are *axl1* mutants defective at cell fusion? We suggest that cells may require a high level of pheromone secretion

just before cell fusion, a level that goes beyond their requirement for agglutination, cell cycle arrest, and partner selection. This possibility is consistent with the observation that cells display graded levels of response to pheromone (JENNESS *et al.* 1983, 1986; MOORE 1983; DORER *et al.* 1995). Cells require more pheromone to form a mating projection than they require to arrest in the G1 phase of the cell cycle (MOORE 1983). It is possible that cell fusion requires the highest level of pheromone expression from a mating partner. This requirement might insure that mating partners are in very close proximity before they initiate the membrane fusion reaction. This level may occur in a chemotropic mating only at the site of fusion, where pheromone receptors may be saturated. In the absence of *AXL1,* the levels of pheromone may be too low.

The relationship between cell fusion and default mating: We describe eight genes that are more important for default matings than chemotropic matings. Cells containing mutations in any one of these eight genes (except *fus3*Δ mutants) mate efficiently when they mate by chemotropism, but mate between 8.3-fold (*fus2*Δ) and 930-fold (*axl1::URA3*) more poorly by default with pheromoneless cells in saturating pheromone than wild type (Table 2). Why are these genes more important for default mating than for chemotropic mating? We suggest two possibilities that are not mutually exclusive.

First, chemotropism may compensate for the defect in cell fusion by more closely aligning partners before fusion. Cells that are defective at fusion may present a less active fusion apparatus on their mating projection. For example, Spa2p may help organize or restrict the fusion machinery to a point on the projection tip; in the absence of *SPA2,* cells form a peanut-shaped projection (GEHRUNG and SNYDER 1990; CHENEVERT *et al.* 1994; YORIHUZI and OHSUMI 1994) that may not allow a tightly localized fusion mechanism. During chemotropism, mating partners may be able to compensate for this defect by communicating with each other and mutually aligning their fusion machinery. However, when a *MAT***a** cell chooses its mate by default, only the *MAT*α cell partner is capable of aligning its projection tip and fusion machinery toward the *MAT***a** cell. This lack of coordination may exacerbate defects in the fusion step.

Second, chemotropism may compensate for a defect in cell fusion by allowing more time for the fusion step. During default mating cells initially produce a projection near their incipient bud site. However, cells are not fixed in their polarity at this default site. If they do not find a mate near their incipient bud site, they will adapt and choose another site, producing a second projection, and then subsequent projections, one after another in succession until a mate is found (LIPKE *et al.* 1976; TKACZ and MACKAY 1979; MOORE 1983; BABA *et al.* 1989; SEGALL 1993). In contrast, during chemotropic mating, cells produce a single projection. Even in the absence of mating, these projections continue to grow for hours in the same direction, up the gradient (LEVI 1953; SEGALL 1993). Mating partners remain committed to each other, as long as the gradient remains, until cell fusion occurs. Therefore, if a cell is partially defective at inducing cell fusion, a longer chemotropic mating may compensate for the defect. For example, the Ste6 protein, which is required for **a**-factor secretion, is localized to the tip of the mating projection, where it facilitates the secretion of pheromone toward a mate (KUCHLER *et al.* 1992). A longer period of mating might allow the accumulation of more **a**-factor at the junction where cell walls are fused, over time inducing fusion. However, if one cell chooses another by default, then the cell mating by default may abort the mating early and attempt to mate in another direction by choosing another default site. This lack of attention during courtship may also exacerbate defects in the fusion step.

These models may explain why multiple copies of *FUS2* and *MFA2* suppress the default mating defect caused by *spa2* mutations (Tables 4 and 5). Increasing the copy number of *FUS2* may help localize the fusion machinery at the projection tip in *spa2* mutants, and thus reduce the stringent need for alignment during mating. Multiple copies of *MFA2* may locally increase the secretion of **a**-factor at the junction between mating cells and increase the efficiency of cell fusion.

The finding that *CHS5* is required for cell fusion (Figure 2; C. BOONE, unpublished data; CID *et al.* 1995) but not very important for default mating (Table 2) indicates that not all aspects of cell fusion are more important for default mating than chemotropic mating. This finding suggests that *CHS5* performs a function that is quite distinct from the functions of other genes that are involved in cell fusion. *CHS5* is required for chitin synthase III activity, and *chs5*Δ mutants have reduced amounts of chitin in their cell walls (reviewed in CID *et al.* 1995). This suggests that chitin synthase III activity may be required for cell fusion. The Chs5 protein displays some homology to protein H of neurofilaments (CID *et al.* 1995; C. BOONE, unpublished data), but the molecular role of Chs5p in chitin synthase III activity has not been reported. Perhaps a reduction in cell wall chitin inhibits cell fusion uniformly on the cell surface so that fusion is no more efficient during chemotropism than during default.

The relationship between default mating and budding polarity: Given that each of the default mutants we described above is required for other aspects of yeast cell polarity, including bud site selection, Ash1p localization, and mating projection formation, it was important to determine whether their role in default mating is a consequence of their role in these other process. We considered and rejected the possibility that proper bud site selection is necessary for default mating (Table 6). Therefore, despite the fact that cells initially appear to use a site near their last bud site for mating in saturat-

ing pheromone, cells are not restricted to that site for default mating. Moreover, our data also strongly argue that the bud site selection genes are not necessary for chemotropism (Table 6). This observation suggests that during chemotropism, the bud site selection machinery is not used to mark the site of polarity on the cell surface that is organized by pheromone receptors. In addition, we found that despite the requirement for *BNI1* in Ash1 protein localization, not all genes required for localizing Ash1p are required for default mating (Table 7). The opposite is also true. Not all genes required for default mating are required for Ash1p localization, since *SPA2* is not required for localizing Ash1p (JANSEN *et al.* 1996). These data indicate that the processes of Ash1p localization and default mating are genetically distinct.

The mating and budding processes are conceptually very similar. During both processes cells recognize a cue on their cell surface and organize their cytoskeleton and secretory apparartus toward that cue. In fact, many of the same molecular components are recruited and organized during both mating and budding (for a review see DRUBIN and NELSON 1996). However, during bud formation cells recognize an intrinsic cue, determined by the bud site selection machinery. During chemotropic mating these intrinsic cues are overridden by an extrinsic cue, the pheromone gradient. How cells make use of distinct cues to create different polarized structures is a central question in cell polarity. For example, neurons form axons, dendrites and synapses, all within a single cell, and all are highly polar structures that form in response to different cues and require an organized actin cytoskeleton and secretory apparatus (GOODMAN and SHATZ 1993; GOODMAN 1994). Leukocytes not only respond to chemoattractants and migrate through endothelial cells (SPRINGER 1994), but also undergo cytokinesis, a process that involves the retraction of cytoplasmic extensions and the reorientation of the cytoskeleton inward toward the cleavage furrow. The findings reported here of a unique set of proteins involved in default mating, cell fusion, and certain aspects of bud polarity may contribute to our understanding of these issues.

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