MOLECULAR AND CELLULAR BIOLOGY, July 1987, p. 2316–2328
0270-7306/87/072316-13$02.00/0

Vol. 7, No. 7

Two Genes Required for Cell Fusion during Yeast Conjugation: Evidence for a Pheromone-Induced Surface Protein

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Received 7 January 1987/Accepted 30 March 1987

We characterized two genes, *FUS1* and *FUS2*, which are required for fusion of *Saccharomyces cerevisiae* cells during conjugation. Mutations in these genes lead to an interruption of the mating process at a point just before cytoplasmic fusion; the partition dividing the mating pair remains undissolved several hours after the cells have initially formed a stable "prezygote." Fusion is only moderately impaired when the two parents together harbor one or two mutant *fus* genes, and it is severely compromised only when three or all four *fus* genes are inactivated. Cloning of *FUS1* and *FUS2* revealed that they share some functional homology; *FUS1* on a high-copy number plasmid can partially suppress a *fus2* mutant, and vice versa. *FUS1* remains essentially unexpressed in vegetative cells, but is strongly induced by incubation of haploid cells with the appropriate mating pheromone. Immunofluorescence microscopy of α factor-induced a cells harboring a *fus1-LACZ* fusion showed the fusion protein to be localized at the cell surface, concentrated at one end of the cell (the shmoo tip). *FUS1* maps near *HIS4*, and the intervening region (including *BIK1*, a gene required for nuclear fusion) was sequenced along with *FUS1*. The sequence of *FUS1* revealed the presence of three copies of a hexamer (TGAAAC) conserved in the 5′ noncoding regions of other pheromone-inducible genes. The deduced FUS1 protein sequence exhibits a striking concentration of serines and threonines at the amino terminus (46%; 33 of 71), followed by a 25-amino acid hydrophobic stretch and a predominantly hydrophilic carboxy terminus, which contains several potential N-glycosylation sites (Asn-X-Ser/Thr). This sequence suggests that *FUS1* encodes a membrane-anchored glycoprotein with both N- and O-linked sugars.

Conjugation in the yeast *Saccharomyces cerevisiae* involves the fusion of haploid cells of opposite mating type followed by the fusion of nuclei to form a diploid. The zygote formed by this process buds off diploid cells capable of vegetative growth. Formation of the zygote requires the coordination of two processes—cell fusion and nuclear fusion. Both processes are initiated by mating pheromones: a cells produce a factor, to which α cells respond, and α cells produce α factor, which specifically acts on a cells (13, 44). Cells stimulated by the appropriate mating pheromone produce surface agglutinins (resulting in extensive clumping of conjugating cultures) (33, 40), arrest their cell cycle at the G1 stage, and elongate to form a discernible tip (a process dubbed shmooing) (12, 44; for a review, see reference 41). When the appropriate partners have achieved contact, presumably at the shmoo tip, the cells rapidly fuse, a process that requires the degradation or reorganization (or both) of the cell wall and the fusion of the two plasma membranes (see Fig. 8). The nuclei subsequently fuse within this dumbbell-shaped zygote, and the resultant diploid nucleus begins a series of division cycles, each of which yields a new diploid nucleus that enters an emerging bud. Recent experiments (7, 31) have shown that nuclear fusion is not a passive process; like cell fusion, it requires potentiation by the mating factors.

In this report we describe two genes involved in the initial stage of zygote formation, that of cell surface reorganization leading to cytoplasmic fusion. Mutations in these genes block the conjugation process at a point after cell contact, preventing cytoplasmic fusion and, as a result, nuclear fusion. We designated such mutants *fus*, for cell fusion defective. Mutations similar to *fus* have been described previously in *Schizosaccharomyces pombe*; such mutants similarly fail to dissolve the partition between the mating pair (2).

The expression of one of the *FUS* genes, *FUS1*, is activated by mating pheromones, an induction that leads to the appearance of the gene product at the tip of the shmoo. This localization suggests that the defect observed in matings between mutants is a direct result of the absence of the *FUS1* gene product, rather than a symptom of some general metabolic defect. Furthermore, the gene is not expressed in vegetative haploid a and α cells and diploid a/α cells and achieves high levels only in the presence of mating pheromones, suggesting that it functions exclusively during the actual conjugation process. The genetic and molecular behavior of *FUS1* and *FUS2* leads to the conclusion that their gene products act at the point of contact between mating pairs to facilitate cell wall degradation and reorganization and plasma membrane fusion.

MATERIALS AND METHODS

Media and strains. YPD (rich medium) consisted of 1% yeast extract (Difco Laboratories, Detroit, Mich.), 2% Bacto-Peptone (Difco), and 2% glucose. YPG was the same except for the substitution of 3% glycerol for glucose; this medium was supplemented with 3 μg of cycloheximide (Sigma Chemical Co., St. Louis, Mo.) per ml when appropriate. YNB (minimal medium) consisted of 0.15% Difco yeast nitrogen base (without amino acids and ammonium sulfate), 0.5% ammonium sulfate, and 2% glucose. SC-ura was the same except for the addition of a dry powder (2 g/liter) containing equal amounts (except twofold leucine) of all amino acids plus adenine (0.25-fold). All media were

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TABLE 1. Strains used in this work

Strain	Genotype	Source
XT300-3A	α *ade2-1*	R. K. Mortimer
C52a	**a** *exb1-1*	C. Nombela
L483	α *his4 ade2 kar1-1* Can^r [K$^+$]	Laboratory collection
L1052	**a** *ura3-52 ade2 ino1 ino4 his4Δ453*	30
L1543	α *lys9*	This work
L1546	α *lys9 his4Δ453 ino*	This work
L2498	**a**/α +/*cry1 his4Δ5/his4Δ29 arg11/arg11*	Laboratory collection
L2499	L2498 α/α *cry1/cry1*	Laboratory collection
L2500	**a**/α *cry1/+ his4Δ5/his4Δ29 arg11/arg11*	Laboratory collection
L2501	L2500 **a**/**a** *cry1/cry1*	Laboratory collection
8964-9C	**a** *his4 leu2 lys2 kar1-1*	Laboratory collection
EEX8	**a**/α *ura3/ura3 leu2/+ his4/+ ade2/+ trp1/+ can1/+ cry1/+ cyh2/+*	E. Elion
JY132	**a** *ura3-52 lys2-801 trp1Δ1 his4-34*	This work
JY133	α *ura3-52 leu2-3,112 his4-34*	This work
JY138	**a** *ura3-52 lys2-801 trp1Δ1 fus1-483* [K$^-$]	This work
JY146	α *ura3-52 leu2-3,112 fus1-483*	This work
JY186	α *ura3-52 leu2-3,112 fus1-483 cyh2* [*rho*^{0}]	This work
JY215	**a** *ura3-52 lys2-801 trp1Δ1 fus1-483 fus2-1 exb1-1*	This work
JY217	α *leu2-3,112 fus1-483 fus2-1*	This work
JY240	**a** *ura3-52 lys2-801 fus1-483 fus2-1*	This work
JY245	α *ade2-1 fus1-483* [K$^+$]	This work
JY248	α *ade2-1 fus2-1* [K$^+$]	This work
JY283	α *ade2-1 fus1-483 fus2-1* [K$^+$]	This work
JY284	**a** *ade2-1 fus1-483 fus2-1*	This work
JY306	**a** *ura3-52 trp1Δ1 fus2-1* [K$^+$]	This work
JY317	JY306 [K$^-$]	This work
JY336	α *ura3-52 ade2 cyh2 fus2-1* [*rho*^{0}]	This work

solidified with 2% Difco agar in petri dishes. Table 1 lists the strains used in this work. [*rho*^{0}] strains were derived by inoculation of respiration-proficient strains at a low density (<10^5 cells per ml) in YPD containing 10 μg of ethidium bromide per ml (16), incubation of the culture for 36 h at 30°C, and then isolation of single colonies that were unable to grow on YPG (S. Dutcher, Ph.D. thesis, University of Washington, Seattle, 1982).

Mating assays. Three different methods were used to test the ability of a strain to conjugate.

(i) Replica plating. Lawns of strains containing markers complementary to the strains under analysis were made by inoculating approximately 10^7 cells onto YPD plates, or by replica plating from previously grown lawns onto fresh YPD plates. At the same time, strains to be tested were patched onto YPD plates (or the appropriate minimal plate to select for plasmids). After overnight growth at 30°C, patches and lawns were replica plated together onto fresh YPD plates and incubated for 4 h at 30°C. At that time the mating cells were replica plated onto minimal medium which selected for diploids. Mating responses were scored by the intensity of prototrophic growth observed the next day.

(ii) Quantitative mass mating. Quantitative mass mating was performed by the method of Dutcher (Ph.D. thesis). Exponentially growing cells were mixed together and concentrated onto a 0.45-μm-pore-size nitrocellulose filter (Millipore Corp., Bedford, Mass.) at a density of 3×10^6 cells per parent. The filter was then transferred aseptically to a YPD plate and incubated for 4 h (or 5.5 h for the selection of cytoductants [see below]) at 30°C. At that time the cells were washed from the filter into sterile water, and various dilutions were plated on YPD plates to determine the titer of total cells, and on YNB plates to determine the titer of prototrophic diploids. Mating frequencies are expressed as a ratio of these two numbers. YPG plus cycloheximide was used to determine the titer of cytoductants; the frequency of cytoduction is expressed as the ratio of cytoductants to total cells (able to form a colony on YPD). For microscopic examination of zygotes (all steps at room temperature), cells were suspended in methanol-glacial acetic acid (3:1, vol/vol), fixed for at least 45 min, washed with 0.85% saline, incubated for 30 min in 1 μg of 4′,6-diamidino-2-phenylindole (DAPI) per ml, and washed extensively with saline (Dutcher, Ph.D. thesis). Cells were sonicated to remove clumps before viewing.

(iii) Micromanipulation of zygotes (or "prezygotes"). Exponentially growing cells were mixed together in liquid YPD at a density of 3×10^6 to 5×10^6 cells per ml per parent. After 2 h of growth at 30°C, when extensive clumping was apparent, cells were agitated vigorously in a mixer and spread at the edge of a YPD plate. Unbudded zygotes were extracted from microclumps and manipulated to regular positions on the surface of the agar. Forty zygotes were manipulated in each experiment, which typically took 45 min. The zygotes were allowed to form colonies at 30°C, and the zygotic clones were subsequently replica plated to media that established their nuclear genotype (**a** or α parent, diploid, or sectored) as well as their killer phenotype (15). The frequency of successful diploid formation is expressed as the ratio of unsectored diploid colonies to total colonies. Viability is expressed as the number of total colonies divided by the number of zygotes manipulated.

To test the resistance of paired cells, or prezygotes (see Results), to Zymolyase, 10 ml of 2-h-old mating mixtures were washed and resuspended in 1 ml of 1.2 M sorbitol–100 mM potassium phosphate (pH 6.5). A 5-μl aliquot of β-mercaptoethanol and 30 μl of a solution of Zymolyase 100,000 (10 mg/ml in sorbitol-potassium phosphate; Kirin Breweries, Osaka, Japan) were added, and incubation proceeded for 40 min at 30°C. Zygotes or prezygotes were counted before and after such treatment. Whereas *FUS*$^+$ × *FUS*$^+$ zygote frequencies were unaffected by Zymolyase

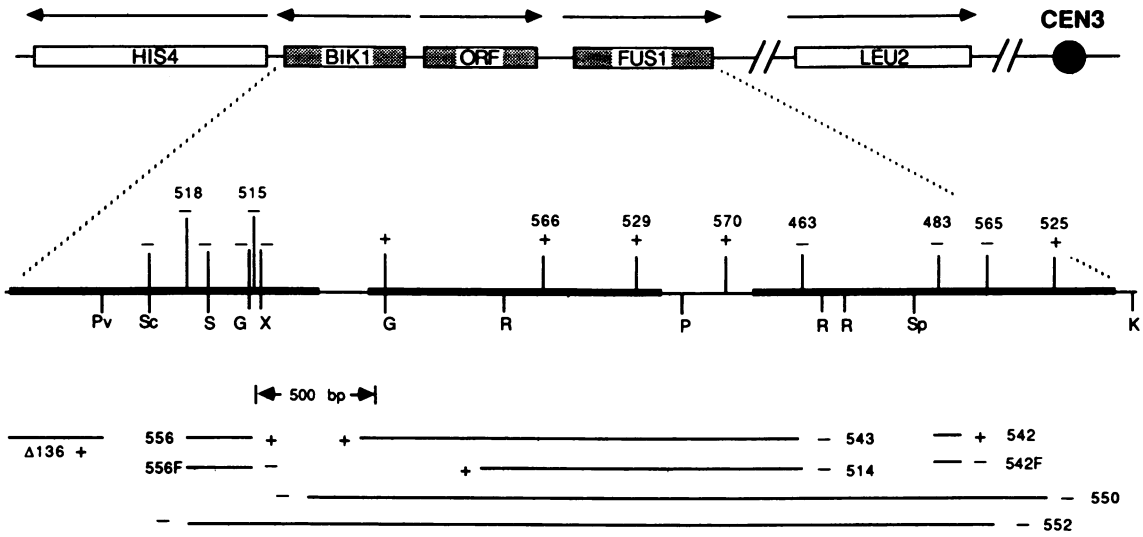


FIG. 1. *BIK1-FUS1* region adjacent to *HIS4* on chromosome III. The hatched boxes at the top show the order of genes on the chromosome. The 6-kb *Hin*dIII fragment extends from ~600 bp within the 5′ end of the *HIS4* coding region to ~900 bp beyond the 3′ end of *FUS1*. The arrow above each box indicates the direction of transcription of that gene. The insert below the top line shows the region that has been sequenced (see Fig. 7). The thick black lines represent each of the genes, and numbers above these lines refer to linker insertions (often associated with small deletions of less than 50 nucleotides). Letters refer to restriction sites (Pv, *Pvu*II; Sc, *Sac*I; S, *Sal*I; G, *Bgl*II; X, *Xho*I; R, *Eco*RI; P, *Pst*I; Sp, *Sph*I; K, *Kpn*I). Below the insert are lines representing large, linker-associated deletions. The phenotypes of mutations caused by linker insertions (those altered by filling in with Klenow fragment [see text]) are indicated with an F, and mutated restriction sites (also altered by filling in) are shown as + (functional) or − (nonfunctional). For large deletions these phenotypes are indicated at their left and right ends for their ability to complement *bik1* and *fus1*, respectively. These phenotypes were determined by comparing the mating response of *MAT***a** Δ453 (Δ*bik1* Δ*fus1*) (L1052) transformants with *MAT*α *BIK*$^+$ *FUS*$^+$ (L1543) and *MAT*α Δ453 (L1546) tester lawns.

digestion, *fus1 fus2* × *fus1 fus2* prezygotes were efficiently disrupted (their frequency decreasing from 19% to less than 0.4% of the total cells).

DNA manipulations. The 6-kilobase (kb) *Hin*dIII fragment (14) containing *FUS1* and *BIK1* was inserted into the unique *Hin*dIII site of YCp50, in the orientation *bla-BIK1-FUS1-tet*; the unique *Bam*HI site of this plasmid was subsequently destroyed by filling in the ends with Klenow fragment and religating the blunt ends, generating pJEF423. All complementation analyses were performed with derivatives of this plasmid. Linker insertion mutagenesis was done by DNase I nicking, *Micrococcus luteus* DNA polymerase exonucleolytic digestion followed by single-strand digestion with S1 nuclease, and ligation of *Bam*HI linkers, essentially as described previously (37). Certain *Bgl*I, *Bgl*II, *Sal*I, *Sac*I, and *Xho*I sites, as well as certain *Bam*HI linker-derived sites (556, 542), were destroyed by opening the plasmid with the appropriate enzyme (which required partial digestion in some cases), filling in the ends with Klenow fragment, and religating (24). The sequence of the *BIK1-FUS1* region was obtained by inserting various restriction fragments, including those obtained from the *Bam*HI insertions, into the M13 bacteriophage derivatives mp18 and mp19 and sequencing by the dideoxy chain termination method as described previously (1, 34).

Genetic analysis. A strain (L1052) containing a large deletion (Δ453) extending from *HIS4* to *LEU2* (see Results) was transformed with various derivatives of pJEF423 harboring linker insertions within the 6-kb *Hin*dIII fragment. These transformants were assayed by the replica plating test for their ability to mate successfully with a Δ453 tester lawn, L1546. Large linker-associated deletions (e.g., 550, 552; Fig. 1) in the *Hin*dIII fragment completely abolished the ability of the plasmid to complement the Δ453 defect; however, less extensive linker-associated deletions (e.g., 514, 543) and simple linker insertions (<50 base pairs deleted; 518, 483) only partially compromised the complementing activity of the *Hin*dIII insert. From this mutational analysis, a portion of which is presented in Fig. 1, a map was constructed which contains two distinct regions of complementing activity; either region alone is able to restore only partial mating competence to the Δ453 recipient.

With the exception of 565, 566, 514, and Δ136, all the mutations shown in Fig. 1 were integrated into the chromosome of wild-type cells (JY132 and JY133). The linker insertions were moved to *URA3*-integrating vectors, and the resultant plasmids were directed to integrate (27) at chromosome III by cutting at the unique *Kpn*I site before transformation. Since the recipient strains harbored the *his4-34* mutation, which lies within the region of *his4* that is duplicated upon integration of these plasmids, we subsequently selected for His$^+$ recombinants to obtain the appropriate loop-out excision event. Those His$^+$ derivatives that had simultaneously become Ura$^-$ invariably harbored the appropriate linker insertion (or frameshifted restriction site) and no plasmid sequences. Complementation tests (data not shown) with these strains transformed with the original set of pJEF423 derivatives confirmed our previous conclusion that two genes required for efficient mating reside on the *Hin*dIII fragment immediately 5′ to *HIS4*. The *fus1-483* allele (harboring linker insertion 483) was chosen for subsequent tests as the canonical allele; it displays a phenotype equivalent to that seen with the linker deletions 514 and 543 (Fig. 1).

The *fus2-1* mutation was uncovered in a cross between C52a and JY146 (*fus1-483*). Several tetrads from this cross produced one ascospore which when mated to Δ453 lawns displayed a drastic defect as compared with the response with wild-type lawns. (*fus1* alone displays only a partial defect when mated to Δ453). These segregants were outcrossed to wild-type strains, and the segregation of wild-type

(good mating with all lawns), partially defective (poor mating with mutant parent lawns, but partial mating with *fus1* lawns), and completely defective (poor mating with *fus1* lawns and with mutant parent lawns) phenotypes among the spore clones suggested the presence of two unlinked *fus* mutations in the mutant parent. Single *fus* mutants mate poorly with double mutants, but partially with single mutants, and conversely, double mutants mate poorly with single mutants and double mutants (but mate well with the wild type). The mating type of mutants does not affect their phenotype as assayed by replica plating.

Both *fus1* and *fus2* are recessive and can complement one another in *MAT*α/*mat***a***1* diploids, which mate as α. In such *MAT*α/*mat***a***1* backgrounds, *fus1*/*FUS1*, *fus2*/*FUS2*, and *fus1*/*FUS1 FUS2*/*fus2* strains are able to give wild-type mating responses with a *MAT***a** *fus1 fus2* lawn (JY284). On the other hand, *fus1*/*fus1* and *fus2*/*fus2* strains retain the same mating defect as their haploid counterparts. Recently, we have mapped *fus2* to chromosome XIII, closely linked to *rna1* (data not shown).

Induction by mating pheromone. For induction by α factor, cells were grown to 40 Klett units in YPD or SC-ura (for selection of plasmids) which had been previously titrated to pH 4 with hydrochloric acid. α Factor (Sigma) was added to a concentration of 5 μM (1:100 dilution of a 0.5 mM solution in methanol); methanol was added to control cultures to a concentration of 1%. Cells were grown for two additional hours at 30°C at which time >90% of the α factor-treated population had arrested as shmoos or unbudded cells.

For induction by **a** factor, cells were pregrown in untitrated SC-ura to 40 to 50 Klett units and then pelleted and suspended in an equal volume of YPD medium in which JY132 cells (*MAT***a**) or EEX8 cells (**a**/α) were growing at a similar density, or in an equal volume of unconditioned YPD. The cells were then grown for two more hours at 30°C.

Northern analysis. Total RNA was isolated from cells treated for 2 h with α factor or from control cells essentially by the method of Carlson and Botstein (5). The RNA was separated by electrophoresis through a 1.5% agarose denaturing gel, transferred to the nylon membrane Gene Screen Plus according to the instructions of the manufacturer (New England Nuclear Research Products, Boston, Mass.), and hybridized either with labeled RNA obtained from in vitro SP6 transcription (Promega Biotec, Madison, Wis.) or with DNA labeled by nick translation (24). The RNA probe extended from nucleotides 3635 to 4030 (see Fig. 7) of the *FUS1* gene. The DNA probe consisted of the *BIK1-FUS1* *Hin*dIII fragment inserted into the *URA3* vector YIp5.

β-Galactosidase assays. Cells were permeabilized by vigorous agitation in Z buffer (25) supplemented with 0.0075% sodium dodecyl sulfate and 60 μl of chloroform per ml, and the assays were performed essentially as described previously (25). Units of β-galactosidase activity are expressed by the formula (1,000 × OD_{420} of centrifuged reaction mixture)/(OD_{600} of culture × volume of culture × minutes of assay), where OD_{420} is the optical density at 420 nm and OD_{600} is the optical density at 600 nm.

RESULTS

Isolation of mutants. The *fus1* mutation was identified in strains carrying a deletion extending from *HIS4* to *LEU2* (30) (Fig. 1). This large deletion (~15 centimorgans, 25 to 40 kb), designated Δ453, had no obvious effect on the vegetative growth of the cells, but subsequent genetic manipulations revealed that Δ453 strains form diploids at greatly reduced rates when crossed with strains carrying the same deletion, but at relatively normal rates when crossed with wild-type strains. A centromere plasmid bearing the 6-kb *Hin*dIII fragment beginning in *HIS4* and extending toward *LEU2*, subcloned from a 15-kb *HIS4* *Bam*HI-*Eco*RI fragment (isolated by "eviction" [10]), restored normal mating to a Δ453 strain (L1052), suggesting that this segment contains the mating functions missing in Δ453.

Extensive deletion analysis and random linker insertion mutagenesis (a subset of which is presented in Fig. 1) revealed that the phenotype of Δ453 results from deletion of two separate genes, both of which are located on the *Hin*dIII fragment. The genes were further defined by introducing the linker insertions into the chromosome of wild-type cells and analyzing the behavior of the resulting strains in crosses (see Materials and Methods). The linker insertions fall into two groups based on complementation tests. Insertions in one of these groups lead to a block in cellular fusion in crosses between members of the same group. The gene defined by this complementation group is called *FUS1*. Mutants in the second group mate normally with the wild type and *fus1* mutants; however, in crosses with members of the same complementation group, the cells fuse normally, but their nuclei fail to fuse. The gene defined by this complementation group is called *BIK1* (bilateral karyogamy defect, in contrast to the unilateral defect seen in *kar* mutants [6]; a complete description of the function of the *BIK1* gene will be presented in a future report). These experiments show that the mating defect of Δ453 results from the deletion of two genes: *BIK1* and *FUS1* (Fig. 1), each of which has its own unique function.

The *fus2* mutation was uncovered in crosses of *fus1* by strain C52a (obtained from C. Nombela). The phenotype of *fus1* mutants is rather leaky; many pairs in a *fus1* × *fus1* cross fuse their nuclei despite the abnormal bridge between the pair (see below). In crosses of JY146 (α *fus1-483 ura3-52 leu2-3,112*) by C52a (**a** *exb1-1*), several ascospore segregants were obtained which displayed a much more severe fusion defect than that of the *fus1* mutant. These segregants were shown by genetic analysis to be double mutants, *fus1 fus2* (see Materials and Methods). The *fus2* mutation was apparently present in C52a, although it is not linked to the *exb1-1* mutation described by Santos et al. (as assayed by the methylumbelliferyl-β-D-glucoside overlay technique) (35). As shown by subsequent tests (see below), *fus2* has a phenotype very similar to that of *fus1*.

FUS2* suppresses *fus1*; *FUS1* suppresses *fus2*.** Isolation of the *FUS2* gene permitted an analysis of the functional interactions between *FUS1* and *FUS2* during conjugation. To clone the *FUS2* gene, we took advantage of the severe mating defect of a *fus2* mutant when mated with a *fus1 fus2* double mutant. Since a FUS^+ strain can readily mate with a *fus* double mutant in the replica plating assay, and since the *fus2* defect is recessive, cloning by complementation was straightforward. A *MATa** *fus2* strain (JY306) was used as a recipient in transformation with the YEp24 library of Carlson and Botstein (5), and a clone was identified (pSB257) (of ~2,000 screened) which restored normal mating function to *fus2* when it was mated to *fus1 fus2* (JY217). Genetic analysis demonstrated that the 9-kb DNA segment we isolated corresponds to *FUS2*: crosses between FUS^+ strains and a *fus2* strain harboring a $FUS2^+$-$URA3^+$ plasmid integrated at *fus2* yielded 19 of 21 tetrads which contained four FUS^+ spores. One-step gene disruption (32) by the substitution of an internal 1.1-kb *Hin*dIII fragment with the *URA3* gene resulted in a phenotype identical to that of *fus2-1*: a

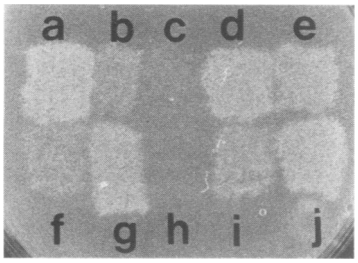


FIG. 2. *FUS1* in high copy numbers suppresses *fus2*; *FUS2* in high copy numbers suppresses *fus1*. a through e, JY138 (**a** *fus1*) transformants; f through j, JY306 (**a** *fus2*) transformants. Transforming plasmids: a and f, pJEF423 (*CEN4-FUS1*$^+$); b and g, pSB265 (*CEN4-FUS2*$^+$); c and h, YCp50; d and i, pSB273 (2μ-*FUS1*$^+$); e and j, pSB257 (2μ-*FUS2*$^+$). All plasmids carry the *URA3*$^+$ selectable marker; single-copy *CEN4* plasmids are derived from YCp50, and 2μ plasmids are derived from YEp24, a high-copy-number vector. Patches of transformants were mated to lawns of JY217 (α *fus1 fus2*) on YPD agar for 4 h and replica plated to YNB medium.

gross mating defect with *fus1 fus2* strains, but only slightly reduced diploid formation with either *fus2-1* or *fus2::URA3* strains. (A more detailed account of the structure and function of the *FUS2* gene will appear in a future report.)

FUS1 in high copy numbers suppressed the *fus2* defect, and *FUS2* in high copy numbers suppressed *fus1* (Fig. 2). There was even a slight suppression of the Fus$^-$ mating defect when the heterologous genes were carried on centromere vectors, when the copy number of the heterologous gene is expected to be only one or two additional copies per cell. These suppression studies suggest some overlap of *FUS1* and *FUS2* function consistent with the leakiness of *fus1* and *fus2* null mutations. However, low-stringency Southern analysis reveals no physical homology between *FUS1* and *FUS2* (data not shown). This observation, together with the incomplete suppression, suggests that *FUS1* and *FUS2* encode nonidentical functions.

Mating defects in *fus* mutants. Light micrographs of *fus* zygotes (Fig. 3) show an unusual plate which persists between the two would-be parents, suggesting that they have failed to degrade or reorganize the cell wall that lies between them. In matings between *fus1 fus2* double mutants (Fig. 3C and D), DAPI staining of the nuclei demonstrates that these structures have not fused (<1% of zygotes) and are located on opposite sides of the undegraded partition and at random positions within their respective half of the zygote. (We will use the term prezygote to refer to such pairs, while the term zygote will specify both zygotes and prezygotes.) Nuclear fusion is not completely abolished in crosses between *fus* single mutants (Fig. 3B); some 30 to 50% of such zygotes display a single fused nucleus (versus >99% in wild-type matings), despite the apparent persistence of the aberrant junction. *fus2* × *fus2* and *fus2* × *fus1* zygotes display a microscopic phenotype very similar to that of *fus1* × *fus1* zygotes, in that the partition is unmistakable but not as pronounced as that seen in the double mutants. *FUS*$^+$ × *fus*$^-$ zygotes also occasionally show this abnormal interparental junction, although to an even lesser degree (data not shown). Another unusual feature of such matings, tripartite conjugation, is the association of more than two cells in a stable mating complex. These complexes can be seen at a high frequency (~0.5% of all zygotes) in all bilaterally mutant pairing configurations (Fig. 3C, arrowhead). This abnormality might result from a failure in cytoplasmic mixing (see Discussion).

Electron micrographs of *fus1 fus2* prezygotes confirmed that no membrane fusion occurred; indeed, remnants of undegraded cell wall remained between the mating pair (Fig.

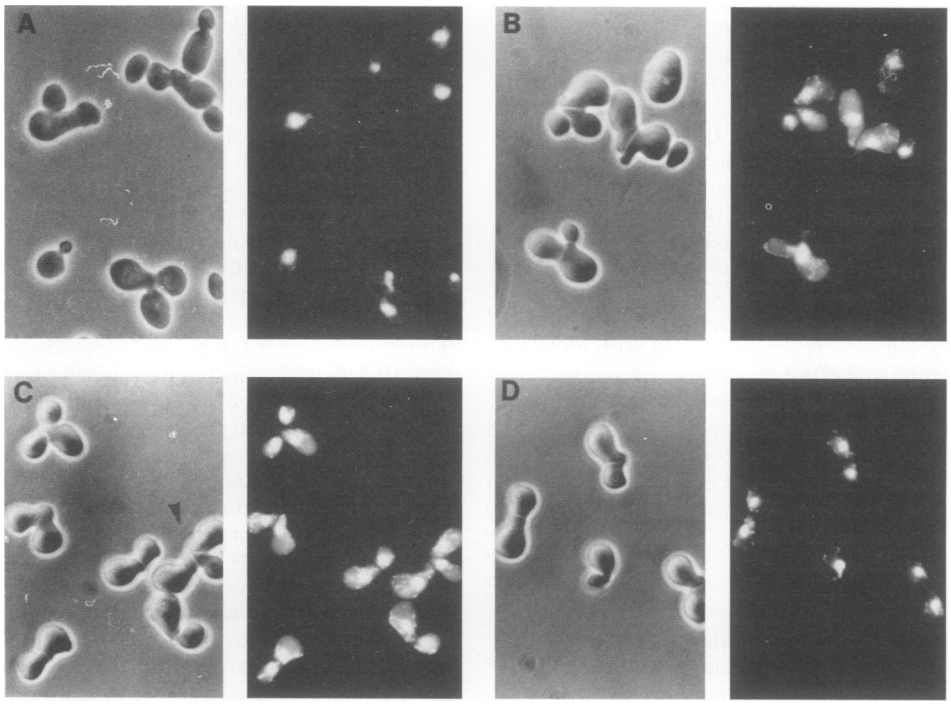


FIG. 3. (A) *FUS*$^+$ × *FUS*$^+$ (JY132 × JY133) after 4 h. (B) *fus1* × *fus1* (JY138 × JY245); after 3 h. (C and D) *fus1 fus2* × *fus1 fus2* (JY240 × JY283) after 3 h. Fluorescence micrographs of DAPI-stained cells appear to the right of the respective phase-contrast images. The arrowhead in panel C indicates a tripartite conjugation.

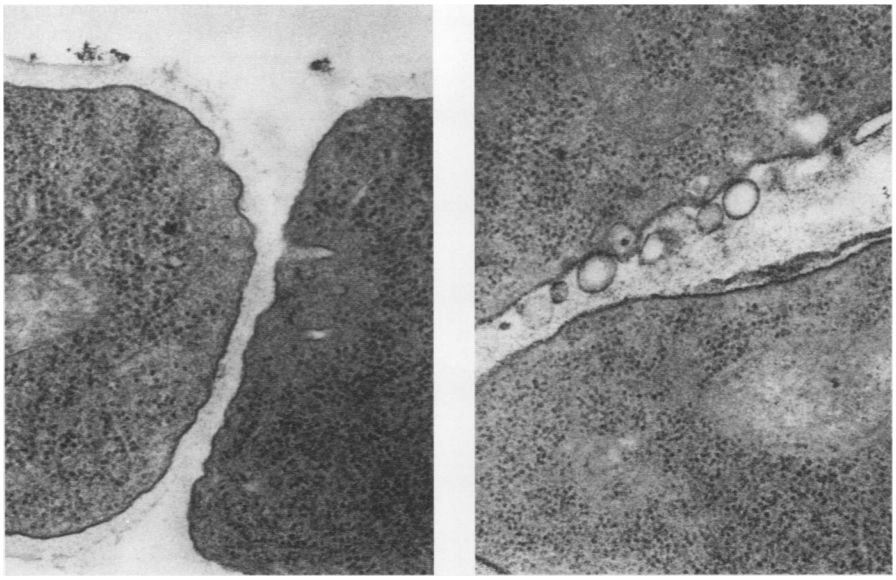


FIG. 4. Electron micrographs of the interparental junctions of *fus1 fus2* × *fus1 fus2* (JY215 × JY217) prezygotes after 4 h of mating. Standard procedures for preparing thin sections of yeast cells were used (4).

4). However, unlike cells in the early stages of conjugation, whose reversible association with many other individuals is due simply to nonexclusive agglutination, the paired cells in *fus1 fus2* × *fus1 fus2* prezygotes were not dispersed from one another by vigorous sonication. Zymolyase digestion separated the pairs into single units which occasionally appeared to have a flattened end, perhaps reflecting the site of their association in the prezygote.

The conjugation defects in our mutants were initially identified by the lawn replica plating technique (see Materials and Methods). To verify the results of those experiments, we assayed diploid formation quantitatively in mass matings on nitrocellulose filters. In the experiment shown in Table 2, a *MATα FUS*$^+$ strain was compared with a *MATα fus1 fus2* strain when mated against a panel of *MAT***a** strains: *FUS*$^+$, *fus1*, *fus2*, and *fus1 fus2*. It was apparent that matings between the *fus* double mutant and any *fus*$^-$ partner produced a greatly reduced number of diploids, whereas mating a wild-type strain with a *fus*$^-$ partner produced almost normal frequencies of diploids.

Although there is a slight reduction (2.5-fold) in diploid formation in matings between *FUS*$^+$ and *fus1 fus2* (as well as between *fus* single mutants [data not shown]), we were reluctant to attach much significance to this reduction since this assay is subject to variations in zygote formation owing to cell density, growth rates, and agglutination of the parents. Since the mating defect in our mutants does not stem from an inability to form zygotes, but rather an inability to undergo a subsequent process, a more revealing assay would be one that follows the fate of individual zygotes or prezygotes. By micromanipulation of such cells it is possible to study the results of a single conjugation event by analysis of the zygotic clone that arises by mitotic division of the exconjugants.

Table 3 summarizes the results of a series of these micromanipulations, which confirm the gross defects of *fus1 fus2* × *fus*$^-$ matings and the relatively normal mating behavior of *FUS*$^+$ × *fus1* or *FUS*$^+$ × *fus2*. Despite the somewhat frequent formation of diploids in matings between *fus* single mutants and between *FUS*$^+$ and *fus1 fus2* cells, it is apparent that such matings display significant abnormalities when compared with the *FUS*$^+$ × *FUS*$^+$ mating. In addition, all the crosses in Table 3 which involved at least one *fus* mutant generated inviable zygotes (unable to form a colony) at a moderate to high frequency (5 to 53%), while the *FUS*$^+$ × *FUS*$^+$ cross produced no inviable zygotes (of 40 manipulated). The frequency of diploid formation in these experiments appeared to depend on the mating types of the parents (compare, in Table 3, row 1, column 4 with row 4, column 1; and row 2, column 3, with row 3, column 2). We are currently constructing the appropriate isogenic strains to address this possibility more rigorously.

Microscopic examination of *fus* zygotes suggested that cellular fusion is defective, but could not reveal whether the cytoplasmic contents of the parental cells are constrained or are free to mix. Zygotes resulting from *fus* crosses (Table 3) were analyzed for their ability to transfer the cytoplasmic killer particle from one parent to the other in those instances in which nuclear fusion (as expressed by diploid formation) fails. The data in Table 4 demonstrate that the killer particle can be freely transmitted to a [K$^-$] parent in a *fus1* × *kar1* cross, in which cytoplasmic fusion is apparently permitted but nuclear fusion is blocked (6). However, in a *fus* × *fus* cross, those zygotes that failed to become diploid failed to undergo cytoplasmic fusion. These data show that the defect

TABLE 2. Efficiency of diploid formation in crosses of *fus* mutants as measured by mass mating (see Materials and Methods)[a]

*MAT***a**	*MATα*	
	FUS$^+$ (XT300-3A)	*fus1 fus2* (JY283)
FUS$^+$ (JY132)	56.7	22.9
fus1 (JY138)	57.5	1.3
fus2 (JY306)	54.8	0.26
fus1 fus2 (JY240)	22.8	0.059

[a] The numbers in the table represent the percentage of diploids formed after 4 hr.

TABLE 3. Diploid formation in micromanipulated zygotes[a]

MATa	MATα			
	FUS⁺ (XT300-3A)	*fus1* (JY245)	*fus2* (JY248)	*fus1 fus2* (JY283)
FUS⁺ (JY132)	100 (40)	97 (37)	100 (36)	42 (19)
fus1 (JY138)	100 (38)	21 (68)	85 (54)	0 (29)
fus2 (JY306, JY317)	89 (27)	25 (59)	54 (84)	1.5 (66)
fus1 fus2 (JY240)	86 (35)	0 (33)	7 (27)	0 (26)

[a] The numbers in the table represent the percentage of diploids among the manipulated zygotes (the actual number of zygotic clones is in parentheses, e.g., for *FUS*⁺ × *FUS*⁺, all 40 viable zygotes gave rise to diploid clones).

in cell wall-membrane dissolution observed microscopically reflects not only an apparent block in the process, but an actual defect in cytoplasmic mixing of the killer particles. An apparent exception is the *fus2* × *fus2* cross, in which three of six haploid exconjugants acquired the killer trait. The low numbers in this experiment reflect the high rate of diploid formation and the bias of such zygotes toward forming a colony consisting entirely of the parental [K⁺] donor.

Cytoduction experiments, which assay the transmission of mitochondria from the parents to the zygotic bud, confirmed our conclusion that cytoplasmic mixing is blocked in *fus*⁻ zygotes. A cytoductant is a haploid exconjugant possessing the nuclear genotype of one parent and the cytoplasmic genotype of the other; the existence of such zygotic products is an indication of cytoplasmic fusion without concomitant nuclear fusion (6). Typically, one mates a Cyh^r [*rho*^{0}] strain with a Cyh^s [*rho*$^+$] strain and selects for Cyh^r [*rho*$^+$] cytoductants on a nonfermentable carbon source containing cycloheximide (although any recessive drug resistance marker will theoretically suffice). Table 5 summarizes experiments in which we mated α *fus*⁻ *cyh2* [*rho*^{0}] to a panel of **a** *CYH2* [*rho*$^+$] strains; only when the cross involved the *kar1-1* mutation, which unilaterally blocks nuclear fusion but does not affect cytoplasmic mixing, were there an appreciable number of cytoductants. (The mating between the *fus2* single mutants showed a somewhat higher frequency of cytoductants than did other *fus* crosses, although not nearly as high as that seen when a parent harbored the *kar1-1* mutation.) These data show that the drastic defect seen in *fus* × *fus1 fus2* zygotes manifests itself not only in the failure of nuclear fusion, but also in the prohibition of transmission of two cytoplasmic elements, the mitochondrion and the much smaller killer particle.

Induction by mating pheromones. The level of *FUS1* mRNA was assayed to determine whether its expression was altered during conjugation. Figure 5 shows two autoradiograms derived from Northern analysis (see Materials and Methods), which probed the transcripts of *FUS1*, *BIK1*, *ORF*, and *URA3* (A) or that of *FUS1* exclusively (B). The addition of α factor to **a**/**a** cells, but not to isogenic **a**/α cells, caused the induction of a 1.6-kb *FUS1* message not observed in cells that were not exposed to the pheromone.

This pheromone effect was analyzed further by the construction of a *fus1-LACZ* fusion that possessed the promoter region of *FUS1* and sequences which encoded the first 254 amino acids of FUS1 fused to β-galactosidase. Addition of α factor to wild-type **a** cells containing this fusion on a high-copy-number 2μ vector led to at least a 1,000-fold induction of β-galactosidase (Table 6). (An equivalent induction ratio was seen for single-copy derivatives of this fusion.) Incubation of α cells containing the same *fus1-LACZ* plasmid with wild-type **a** cells (a source of **a** factor) also caused a substantial induction, although the uninduced level appeared to be significantly higher than in **a** cells.

Localization of *fus1-LACZ*. The cellular location of the *FUS1* gene product was determined by immunofluorescence microscopy (22) on pheromone-treated cells containing the *fus1-LACZ* fusion on a high-copy-number vector. As a probe we used a mouse monoclonal anti-β-galactosidase antibody (generously provided by T. Mason), which was subsequently

TABLE 4. Transmission of the killer trait [K⁺] in *fus* matings

[K⁺] (*MAT*α)	[K⁻] (*MAT***a**)	[K⁺] *MAT***a**/total *MAT***a** exconjugants
FUS⁺ *kar1* (L483)	*fus1* (JY138)	22/23
fus1 (JY245)	*fus1*	1[a]/30
fus2 (JY248)	*fus1*	0/5
fus1 fus2 (JY283)	*fus1*	0/20
fus1	*fus2* (JY317)	0/4
fus2	*fus2*	3/6
fus1 fus2	*fus2*	0/32

[a] This zygote produced a mixed colony of [K⁺] and [K⁻] *MAT***a** exconjugants.

TABLE 5. Frequency of cytoduction in *fus* crosses, as measured by mass matings (see Materials and Methods)

α [*rho*^{0}] Cyh^r parent	**a** [*rho*$^+$] Cyh^s parent	% Diploid[a]	% Cytoductant[a]
fus1 (JY186)	*FUS*⁺	47.3	0.048
	fus1	46.4	0.20
	fus2	33.9	0.18
	fus1 fus2	6.4	0.24
	kar1 (8964-9C)	2.1	7.0
fus2 (JY336)	*FUS*⁺	71.6	0.022
	fus1	51.4	0.021
	fus2	53.3	0.48
	fus1 fus2	7.2	0.12
	kar1	7.3	6.5

[a] The numbers in the table represent the percentages of diploids and cytoductants formed after 5.5 h. *MAT***a** strains are as in Table 2.

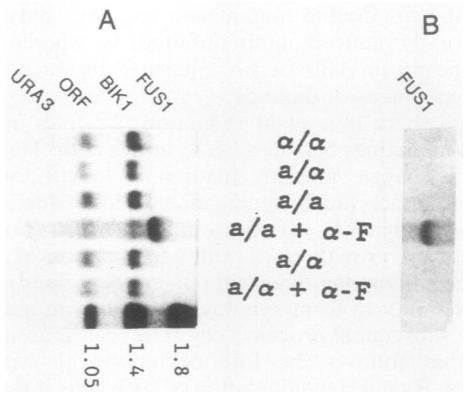


FIG. 5. Northern hybridizations of total RNA isolated from strains treated with α factor (α-F) or with solvent minus α factor for 2 h. **a**/**a**, L2501; α/α, L2499. **a**/α, L2498 (above) or L2500 (below). (A) Probe is nick-translated pSB202 containing *URA3*, *BIK1*, *FUS1*, and *ORF* (Fig. 1). The identity of the *BIK1* transcript was determined from a subsequent experiment (data not shown); that of the *ORF* transcript is inferred. (B) Probe is ^{32}P-labeled RNA transcribed from pSB227 (which contains only an internal *FUS1* fragment) by SP6 RNA polymerase. The numbers refer to the sizes (in kilobases) of the molecular weight markers.

TABLE 6. Induction of *fus1-LACZ* by mating pheromone

Strain[a]	Units of activity[b]	
	Uninduced	Induced
JY132(pSB234) (*MAT***a**)	0.5	740
JY133(pSB234) (*MAT*α)	4.7	650

[a] pSB234 is a 2μ-*URA3*-based plasmid which encodes the *fus1-LACZ* gene product (the fusion contains the first 254 amino acids of FUS1 fused to β-galactosidase).
[b] Units of activity are determined as described in Materials and Methods.
[c] *MAT***a** cells were induced with 5 μM α factor, and *MAT*α cells were induced by exposure to *MAT***a** cells and their supernatant (see text).

visualized with a fluorescein isothiocyanate-conjugated goat antimouse antibody (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Figure 6 shows a series of micrographs from such cells, with a *CYC1-LACZ* fusion in similarly treated cells acting as a control fusion that should be cytoplasmic. In a large fraction of the cells, the FUS1-LacZ protein can be seen exclusively at the tip of the shmoo. In cells exposed to α factor for longer periods of time (150 versus 90 min), the staining appeared to become somewhat delocalized, although still remaining at the surface of the cells. This latter observation is in accord with the observation that cells exposed to pheromone for long periods of time begin to form secondary shmoo tips at different sites (S. White and J. Pringle, personal communication; unpublished data). Wild-type zygotes which harbor the fusion plasmid show staining at the neck (data not shown), consistent with the hypothesis that the shmoo tip is the site of cell fusion in conjugation.

Sequence of *FUS1-BIK1* region. The precise structure of the cloned *FUS1* gene was determined by dideoxy sequencing of a collection of M13mp18 and M13mp19 phage containing different segments of the entire region, including *BIK1* sequences (Fig. 7). Except for small stretches of the intervening open reading frame, sequencing was done on both strands of the DNA. The sequence disagrees with that described in a previous publication (11) at four positions between nucleotides 519 and 540; in three cases, we found an extra T, and at one position we found a T rather than an A (GTAGCTGTTCATTCTCAGCGTC). Interestingly, the *BIK1* coding region extends to within 14 bases of the first upstream repeat (TGACTC) of the *HIS4* gene (11). The proximity of these genes, and the organization of the region in general, underscore the compactness of the yeast genome.

The sequence of *FUS1* reveals the presence of three copies of the hexamer TGAAAC in the 5′ noncoding region, at positions 3038, 3049, and 3065. This sequence has been found in at least four other genes regulated by α factor: *MFa1*, *STE2*, *BAR1* (S. W. Van Arsdell and J. Thorner, ICN-UCLA Symp. Mol. Cell. Biol., in press), and *CHS1* (3). Deletion of these repeats through position 3130 abolished the ability of a YCp50 plasmid containing the *FUS1* gene to complement the *fus1-483* mutation. However, linker 570 (Fig. 1), which deletes sequences from nucleotides 3077 to 3130 but does not remove any repeats, retains full complementing activity (data not shown).

The amino-terminal end of the deduced amino acid sequence of the *FUS1* gene product contains a striking concentration of serine and threonine residues (33 of 71). Following this segment is a 25-amino acid hydrophobic stretch, a likely candidate for a membrane-spanning or -anchoring domain. The rest of the protein is predominantly hydrophilic, with a high proportion of proline residues (28 of 416). The amino acid sequence also contains an unusual heptapeptide of four lysines and three arginines near the carboxy terminus. Eight potential sites for N-glycosylation (Asn-X-Ser/Thr) are found downstream from the presumed membrane-spanning domain. Preliminary evidence suggests that the protein is glycosylated (unpublished observations).

The open reading frame located between *BIK1* and *FUS1*

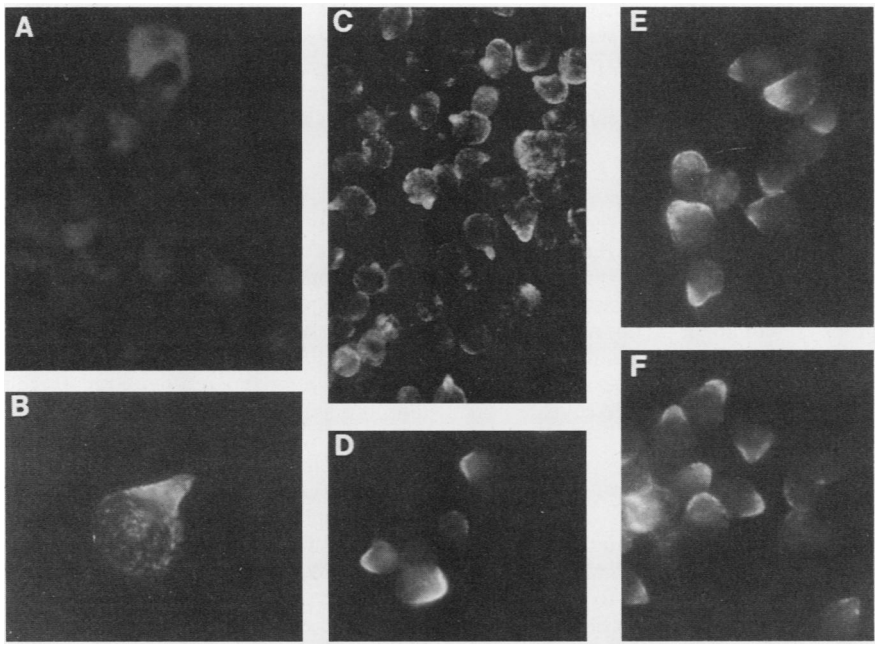


FIG. 6. Immunofluorescence micrographs of α factor-induced shmoos fixed in formaldehyde, digested with Zymolyase, and subsequently incubated with anti-β-galactosidase antibody and fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin (22). All cells are transformants of JY132 (**a** *FUS*$^+$). (A) *CYC1-LACZ*(pBL101) (17) after 150-min induction. (B and C) *fus1-LACZ*(pSB234) after 150-min induction. (D, E, and F) *fus1-LACZ* after 90-min induction.

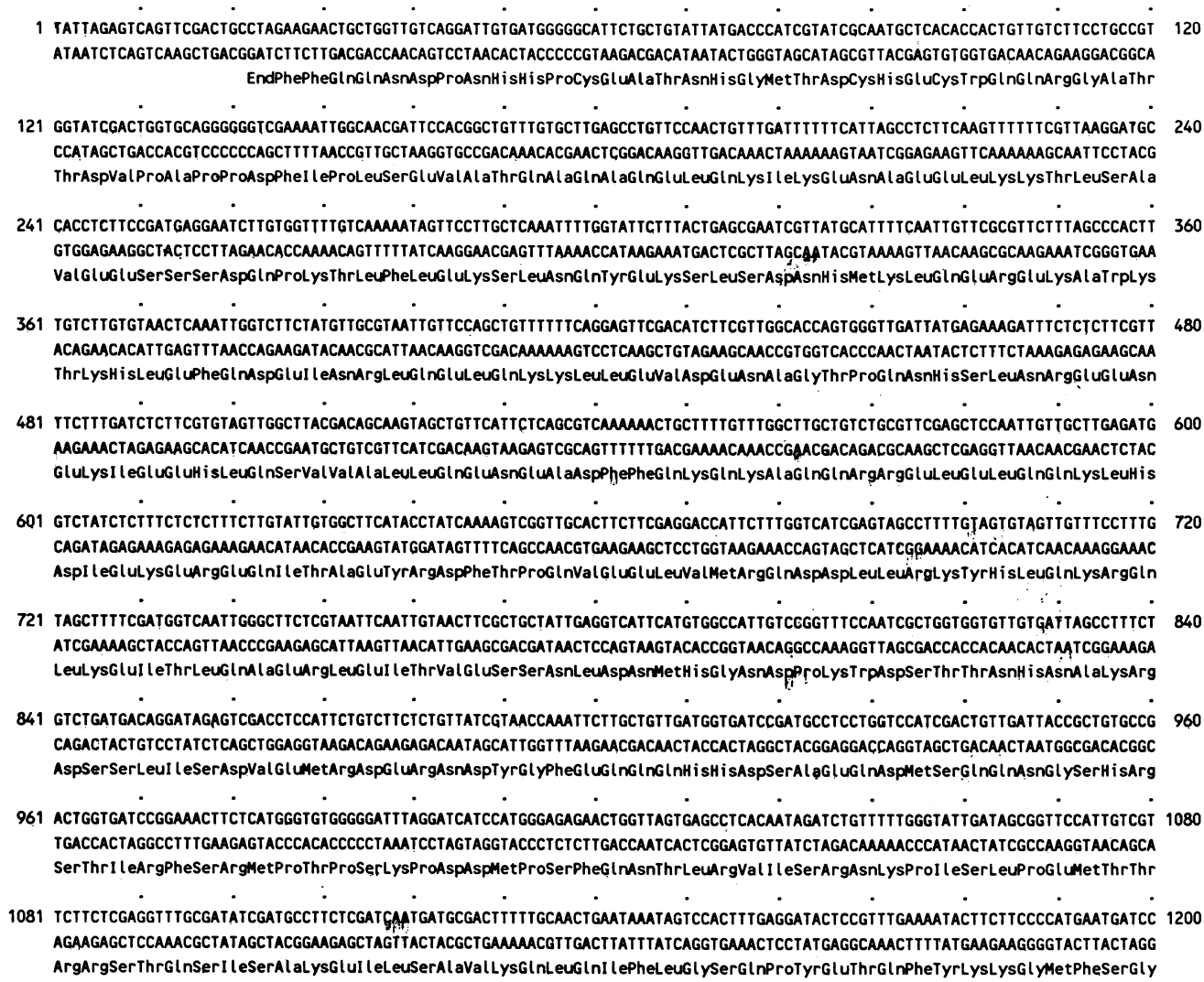


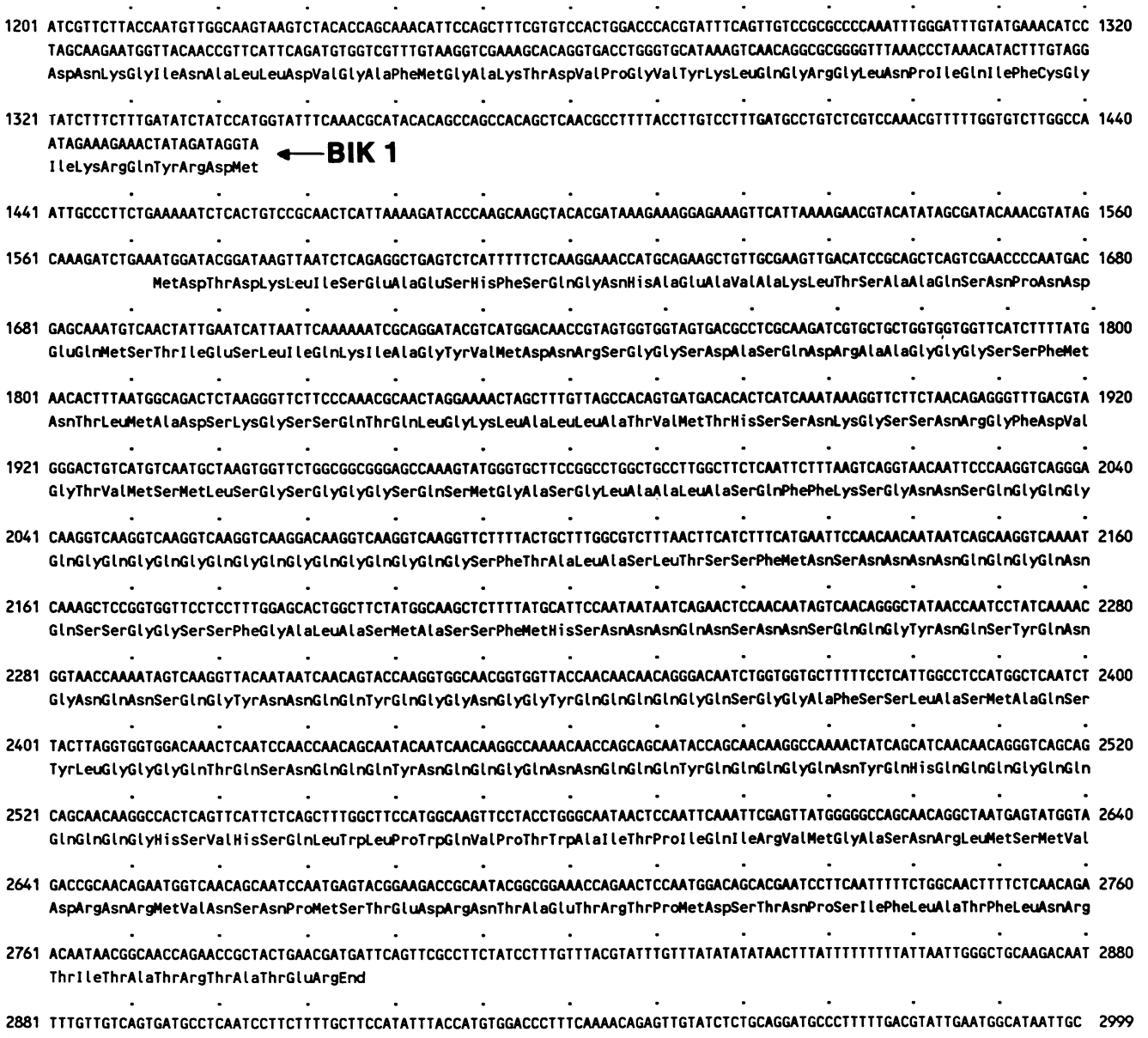
FIG. 7. Sequence of *BIK1-FUS1* region. Position 1 corresponds to position −245 of the *HIS4* gene (10). The coding strand of *BIK1* reads from right to left and is printed under the antisense strand. Double lines appear above the TGAAAC hexamers thought to be required for regulation (Van Arsdell and Thorner, in press). Threonine and serine residues which occur before the putative membrane-spanning domain (underlined) are indicated with asterisks.

has no function in mating that we could detect with our assays; strains carrying mutated versions of the gene that encodes it had no mating defect with wild-type or Δ453 strains. The deduced amino acid sequence consists largely of glutamines and asparagines (106 of 407 total amino acids), as well as an astonishing stretch of 20 amino acids entirely composed of alternating glutamine and glycine residues. A computer search of proteins sequenced to date revealed no other stretches with this unique composition.

DISCUSSION

We isolated two genes, *FUS1* and *FUS2*, required for the efficient formation of diploids in *S. cerevisiae*. Mutations in these genes do not affect the ability of a cell to respond to mating pheromones or its ability to pair irreversibly with the appropriate partner. The block in *fus*⁻ mutants occurs at a point soon after cell-cell contact, before or during the surface reorganization events necessary for fusion and diploid formation. The *FUS1* gene product is expressed at high levels only in the presence of mating pheromone, exposure to which constitutes the first step of the conjugation process. In addition, it appears that the protein is directed to the cell surface, specifically, to the tip of the shmoo. The following evidence supports the contention that the site of cell fusion correlates with the site of shmoo formation: the shape of the zygote, whose tapered neck recalls the distorted shmoo tip; and the localization of concanavalin A-binding activity (42), of actin (S. White and J. Pringle, personal communication), and of FUS1-LacZ protein at the shmoo tip as well as at the neck of zygotes.

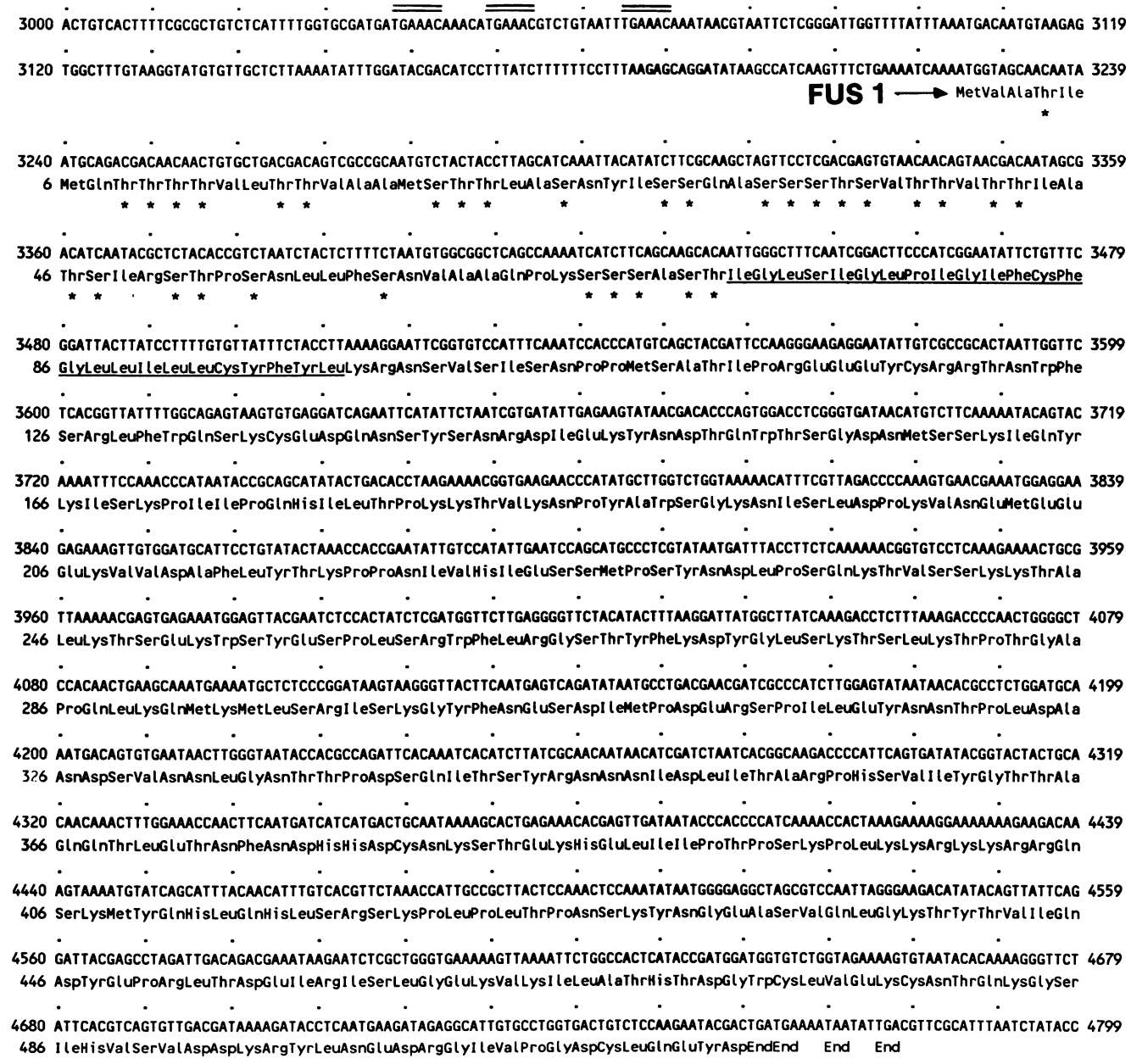
Although it is possible that the β-galactosidase moiety results in abnormal localization of the fusion protein, the fact that β-galactosidase itself and β-galactosidase fused to known cytoplasmic proteins end up in the cytoplasm supports our contention that the amino terminus of the FUS1 fusion protein is responsible for its eventual appearance at the cell surface. In addition, preliminary experiments show that a *fus1-SUC2* fusion (which lacks the normal signal sequence for invertase) directs invertase to the surface of



cells in the presence of the appropriate pheromone, suggesting that the amino terminus of FUS1 is capable of localizing another protein to the cell surface. The presence of the β-galactosidase moiety of the FUS1-LacZ protein could prevent localization events subsequent to membrane insertion, such as a proteolytic cleavage that would release the native protein into the lumen of the endoplasmic reticulum, and subsequently into the periplasmic space. The presence of a Lys-Arg dipeptide immediately following the putative membrane-spanning domain, which would subject the native protein to processing by the KEX2 protease (21), strengthens this possibility. Immunofluorescence and cell fractionation studies using antibodies against the FUS1 protein should allow us to address more directly the question of FUS1 localization.

The assertion that yeast cell fusion during conjugation involves the restructuring and fusion of the cell wall and plasma membrane requires little empirical support; it follows naturally from elementary topological considerations. Nonetheless, electron microscopic studies (28) have described this process as the result of degradation of intervening cell walls and vesiculation of the membrane at the point of contact of the mating pair. In addition, it seems likely that these functions should be specific to the mating process; their activation during vegetative growth would presumably be deleterious, if not lethal. Therefore, one would conclude that the proteins responsible for this event would be (i) localized to the cell surface and (ii) induced by the conjugation pathway. For FUS1, we showed that both of these expectations are realized.

One interpretation of the high concentration of serines and threonines at the amino terminus of the deduced FUS1 protein sequence is that the protein is O-glycosylated on these amino acids. Such sequences have been shown to be substrates for O-glycosylation in animal cells (8). For yeasts, one group (26) has reported the detection of a surface



glycoprotein whose mannan residues are predominantly O-linked; this protein appears only in α factor-induced a cells. If the FUS1 protein also contains O-linked sugar residues, it is curious that the potential site of this glycosylation is on the other side of the presumed membrane-spanning hydrophobic stretch from the potential N-glycosylation sites. This arrangement could be explained if the hydrophobic stretch anchors the protein in the membrane with both termini facing the lumen, or if O-glycosylation occurs on the cytoplasmic face. Resolution of questions concerning the localization and processing of the wild-type FUS1 protein awaits further analysis.

The leaky phenotype of *fus* single mutants as compared with the tight phenotype of *fus* double mutants raises the possibility that *FUS1* and *FUS2* encode redundant or overlapping functions, examples of which abound in *S. cerevisiae*: α-tubulin (36), histones H2A and H2B (18), *RAS* (9, 39), α factor (38), a factor (A. J. Brake, personal communication), cytochrome *c* (23), enolase (20), and glyceraldehyde phosphate dehydrogenase (19). Since leakiness is a property of *fus1* and *fus2* null mutants when they are mated to single mutants or to the wild type, the presence of one wild-type *FUS* gene partially compensates for the absence of the other. Along these same lines, it is interesting to note that for cell fusion to be severely defective, not only must one parent be doubly mutant, but the other parent must also harbor at least one mutant *fus* gene. This result implies that the apparatus which mediates plasmogamy need only act from one side of the prezygote or that this apparatus need only be supplied by one of the two parents. This apparatus might be an activity that functions in cell wall autolysis, or is perhaps a membrane fusogen of the type described in enveloped animal viruses (43).

The observation that *FUS1* in multiple copies can partially suppress the *fus2* defect (the inability to mate normally with *fus1 fus2*), and vice versa, suggests a specific model for *FUS*

function. *FUS1* and *FUS2* might encode distinct degradative enzymatic activities against different components of the cell wall, neither of which is sufficient for complete hydrolysis of its respective component. There may be a synergistic relationship between these two enzymes, either of whose action alone is inefficient. Inactivation of one or the other activity leads to an inefficient hydrolysis of one component, and no hydrolysis of the other. However, a large amount of one enzyme might increase its efficiency with respect to its cell wall component, and heightened activity against a single component might make up for the lack of activity against the other. These components might be, for example, different types of linkages in the cross-linked glucan chains. Alternatively, perhaps these two genes encode rate-limiting functions in two different pathways, whose relationship is similar to that suggested in the first model. A third model depicts both gene products encoding different auxiliary proteins, inactivation of both of which leads to a drastic phenotype simply because of an additive effect of two compromising lesions. Again, these models should be modified to account for the high-copy cross-suppression observed.

The observation that *fus*$^-$ prezygotes do not exclude a third parent implies that these pairs have not yet reached a step in conjugation that prohibits such interactions. Wild-type haploid cells rarely ($<10^{-6}$ [29]) mate to form triploid progeny, so some mechanism must exist that prevents tripartite conjugation. We postulate that the cytoplasmic mixing that occurs rapidly in wild-type matings is the condition that prevents further interaction; presumably the state induced by an a/α cytoplasm leads to surface alterations that make the new zygote refractory to subsequent conjugal attempts. By contrast, these conditions are not met in *fus*$^-$ prezygotes because they fail to achieve cytoplasmic mixing rapidly (or at all), and therefore do not exclude secondary attachments.

The ability, although not pronounced, of *fus2* × *fus2* zygotes to transfer cytoplasmic elements without concomitant nuclear fusion raises questions about the nature of the *fus2* defect. It is possible that the *fus2-1* allele is very slightly leaky and that in a fraction of the zygotes some cytoplasmic mixing is permitted. In these cases the cell wall-membrane fusion was perhaps not extensive enough to permit the passage of the nucleus. It is also formally possible that the mutation affects nuclear fusion per se as well, that even in those cases in which cell fusion is fully realized, there exists a secondary block at the level of the nucleus. The construction of new mutations in *FUS2*, as well as an examination of the behavior of *fus2 kar1* double mutants, should enable us to address these questions more directly.

The abnormal septum or partition seen in *fus*$^-$ prezygotes appears to reflect an inability to degrade the cell wall separating the two would-be parents. Electron micrographs show apparent cell wall material between the parental plasma membranes, although we have not yet used staining techniques that would reveal the exact composition of this material. Using light microscopy, we saw this partition even in those singly mutant zygotes in which the nuclei had already fused (Fig. 3B), implying that this abnormality persists even when the plasma membranes have certainly fused. These observations lead us to suspect that the partition seen in the light microscope is a manifestation of the invagination of the cell walls of the adjoined parents; when fusion finally occurs in the single mutant, the invagination remains, obscuring what must be an attenuated membrane fusion perhaps limited to the central region of the junction. The fact that in the double mutant the prezygotes are resistant to sonic disruption leads to the further conclusion

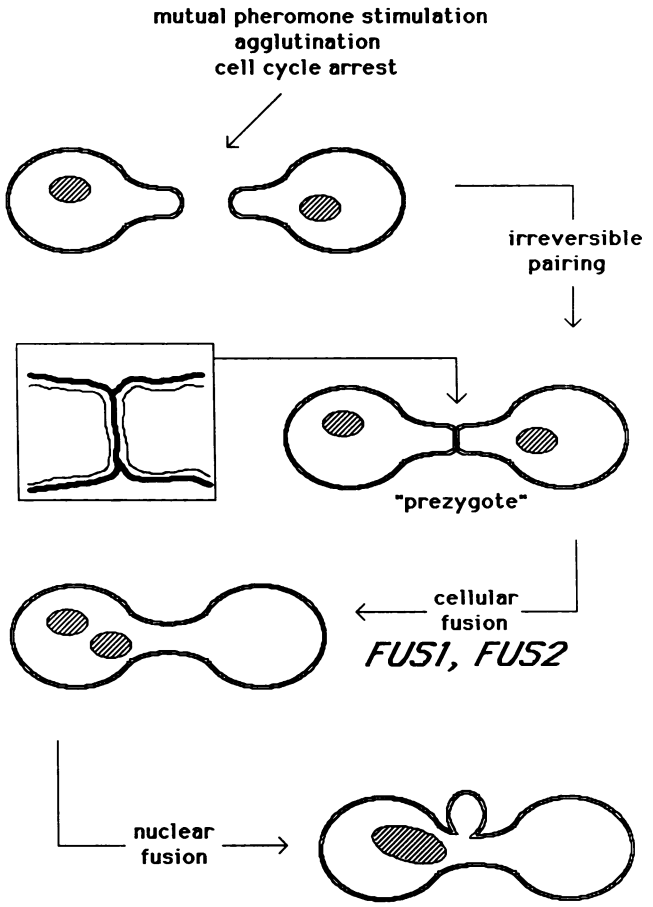


FIG. 8. Conjugation pathway in *S. cerevisiae*. Hatched circles represent nuclei. The inset depicts the proposed organization of cell wall (heavy line) and plasma membrane (thin line) at the interparental junction of the prezygote. Under the conditions used in this laboratory, an early mating mixture contains many different cell types: isolated shmoos, zygotes which appear to have arisen from the fusion of elongated shmoos, and zygotes which appear to have resulted from the fusion of cells that have not first become shmoos. All types of cells are generally found in large, clumped aggregates, the result of agglutination. We depict only one type, a pair of unagglutinated shmoos.

that the partition is not merely the junction of two agglutinated cells but that the surfaces of the cells have become irreversibly enmeshed, perhaps by enzymatic cross-linking of the glucan chains. Nevertheless, since the doubly mutant prezygotes are dissociated by the action of Zymolyase on the cell wall, membrane fusion has certainly not been achieved in these mutants.

The genetic and physiological analysis of *fus* mutants permitted us to expand our current understanding of the conjugation pathway in *S. cerevisiae* (Fig. 8). The elucidation of the details of cellular fusion awaits the further characterization of *fus1* and *fus2* and perhaps of additional mutants defective in the process.

ACKNOWLEDGMENTS

We thank M. Rose, R. Gaber, E. Elion, and V. Berlin for their advice and assistance and K. Murphy and C. Riser for the electron microscopy. We are also grateful to C. Nombela for providing us with strain C52a. J.B. was a Helen Hay Whitney fellow, and G.R.F. is an American Cancer Society Professor of Genetics. This work

was supported by a grant to G.R.F. from the National Institutes of Health.

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