Abstract. Zygote formation occurs through tightly co-ordinated cell and nuclear fusion events. Genetic evidence suggests that the FUS2 gene product promotes cell fusion during zygote formation in Saccharomyces cerevisiae, functioning with the Fus1 plasma membrane protein at or before cell wall and plasma membrane fusion. Here we report the sequence of the FUS2 gene, localization of Fus2 protein, and show that fus1 and fus2 mutants have distinct defects in cell fusion. FUS2 encodes a unique open reading frame of 617 residues that only is expressed in haploid cells in response to mating pheromone. Consistent with a role in cell fusion, Fus2 protein localizes with discrete structures that could be of cytoskeletal or vesicular origin that accumulate at the tip of pheromone-induced shmoos and at the junction of paired cells in zygotes. Fus2 is predicted to be a coiled-coil protein and fractionates with a 100,000 g pellet, suggesting that it is associated with cytoskeleton, membranes, or other macromolecular structures. Fus2 may interact with structures involved in the alignment of the nuclei during cell fusion, because fus2 mutants have strong defects in karyogamy and fail to orient microtubules between parental nuclei in zygotes. In contrast, fus1 mutants show no karyogamy defects. These, and other results suggest that Fus2 defines a novel cell fusion function and subcellular structure that is also required for the alignment of parental nuclei before nuclear fusion.

MEMBRANE fusion events govern many essential processes (for review see White, 1992); intracellular fusion events mediate secretion, endocytosis, and membrane recycling in all eukaryotic cells, whereas intercellular fusion events mediate viral invasion, myotube formation, fertilization, and mating in lower eukaryotes. ER to Golgi protein routing (Pryer et al., 1992; Rothman and Orci, 1992), exocytosis (Creutz, 1992), and viral invasion (White, 1992) each involve special fusion proteins that localize at the sites of fusion and promote fusion reactions between partner lipid bilayers (White, 1992; Rothman and Warren, 1994). In many cases these proteins are conserved (Baringa, 1993). With the exception of viral invasion, little is known about the molecules that catalyze intercellular fusion events, although candidate proteins that may directly participate in sperm–egg fusion (Blobel et al., 1992; White, 1992) and cell fusion during mating (Snell, 1990; Berlin et al., 1991) have been identified.

Mating in Saccharomyces cerevisiae involves the fusion of two haploid cells of opposite cell type (a and a) into a diploid zygote (a/a), providing a simple model for cell and nuclear fusion (Conde and Fink, 1976; Trueheart et al., 1987). The steps leading to zygote formation have been delineated cytologically (Byers and Goetsch, 1975) and by mutations that block zygote formation (for reviews see Cross, 1988; Sprague and Thorner, 1993). Mating is initiated by cell type–specific peptide pheromones that bind receptors on cells of opposite cell type to activate a common G protein–coupled signal transduction cascade. Cells initially stimulated by low levels of pheromone activate transcription of numerous genes involved in signal transduction and fusion, resulting in cell cycle synchronization in G1 phase and reversible attachment between cells of opposite type.

Cell attachment involves the combined effects of cell surface agglutinins (Lipke and Kurjan, 1992), cell polarization toward the highest gradient of pheromone secreted by a neighboring cell of opposite mating type (Jackson and Hartwell, 1990a,b; Segall, 1993), and a partner selection system involving the receptor (Jackson et al., 1991). Cell polarization is manifested as localized cell surface growth into a projection (or shmoo), accumulation of actin cables along the growth axis (Hasek et al., 1987; Baba et al., 1989), orientation of the cytoskeleton of partner cells to-
ward each other (Byers and Goetsch, 1975; Byers, 1981), new plasma membrane and cell wall at the shmoo tip (Lipke et al., 1976; Tkacz and MacKay, 1979; Field and Schekman, 1980), and enrichment of mating-specific proteins in the plasma membrane at the shmoo tip (i.e., Fus1, Trueheart et al., 1987; Ste2, Marsh and Herskowitz, 1988; Jackson et al., 1991; Ste6, Kuchler et al., 1993).

Contact between partner cells at the shmoo tip is followed by irreversible attachment and rapid fusion by coordinated cell and nuclear membrane fusion events. Cell fusion occurs between paired cell walls and plasma membranes to yield a transient heterokaryon (Conde and Finch, 1976), a step likely to involve cell wall degradation/reorganization and localized plasma membrane fusion (Trueheart et al., 1987). Nuclear fusion occurs rapidly after cell fusion between the nuclear envelopes of parental nuclei, once the spindle pole body and associated microtubules of each nucleus have oriented toward the shmoo tip, and each nucleus has migrated to the site of cell fusion. Nuclear migration occurs through the action of cytoplasmic microtubules that extend from the spindle pole bodies of the two nuclei (Byers and Goetsch, 1975; Rose, 1991).

Without stimulation by pheromone, both cell and nuclear fusion occur at very low frequency (Curran and Carter, 1986; Rose et al., 1986). Mutations that block cell and nuclear fusion have been identified (Conde and Finch, 1976; Trueheart et al., 1987; Berlin et al., 1991; Kurihara et al., 1994), supporting the existence of proteins that catalyze these events. Mutations that block nuclear fusion but not cell fusion define genes involved in the functioning of the spindle pole body and associated microtubules (Rose, 1991) as well as fusion between nuclear envelopes (Kurihara et al., 1994). Proteins with direct functions in nuclear fusion have recently been defined in vitro (Kurihara et al., 1994; Latterich and Schekman, 1994).

Mutations in cell fusion block zygote formation at a step after cell contact and generate morphologically aberrant zygotes that retain a septum at the intersection of the joined cells (Bresch et al., 1968; Trueheart et al., 1987). Electron micrographs of the partition in aberrant zygotes shows the presence of cell wall interrupting regions of close plasma membrane apposition as might be expected for a cell fusion block (Trueheart et al., 1987). Six genes (FUS1–3, and FUS5–7) are required for cell fusion on the basis of this mutant morphology (McCaffrey et al., 1987; Rose et al., 1986; Elion et al., 1990; Kurihara et al., 1994). Of these, only FUS1 and FUS3 have been characterized to date. FUS1 encodes an O-linked glycoprotein that spans the plasma membrane of the shmoo tip during mating, suggesting that Fus1 directly participates in cell fusion (Trueheart and Finch, 1989). FUS3 encodes a MAP kinesin with multiple functions required for signal transduction and mating (Elion et al., 1990, 1993), whose role in cell fusion is unknown.

Here, we show that Fus2 encodes a unique 617-residue protein that is expressed at a time and positioned at a site that is consistent with a role in cell fusion. Fus2 associates with novel structures that accumulate within the neck of the shmoo and near the plasma membrane at sites of cell fusion in pheromone-induced cells and in zygotes. The presence of Fus2 in zygotes is transient, and can be detected only before nuclear fusion, supporting an execution point at the time of cell fusion. Consistent with this immunolocalization pattern, Fus2 is associated tightly with cytoskeleton, membranes, or other large complexes. Although previous work suggests that Fus2 is functionally redundant with Fus1 (Trueheart et al., 1987), a comparative analysis of fus1 and fus2 mutants shows they have distinct defects in mating. Fus1 mutants are sensitive to low temperature and EGTA, whereas fus2 mutants are karyogamy defective and poorly align the two parental nuclei in zygotes, as judged by a defect in microtubule alignment. Fus2 may, therefore, define a cell fusion function that is also required for proper migration of nuclei before nuclear fusion.

**Materials and Methods**

**Microbiological Techniques**

Yeast strains are listed in Table I. Gene replacement (Rothstein, 1983) and eviction/transplacement (Winston et al., 1983) were used to construct fus derivatives as described (Trueheart et al., 1987; Trueheart, 1988). Yeast media were prepared as described (Sherman et al., 1986) containing 2% dextrose, glycerol, or ethanol as indicated. Yeast extract peptone and synthetic complete media were titrated to pH 4 with HCl where indicated. Yeast transformations were performed by the method of Ito et al., 1983. Standard methods were used for bacterial transformations, plasmid DNA preparation, and plasmid constructions (Maniatis et al., 1982) using Escherichia coli strains HB101, C600 (Bolivar et al., 1977), and JM109 (Messing, 1982).

**Plasmids Constructed**

pYEE52 (FUS2-lacZ URA3 2μ) has the BglII–Sall fragment of Fus2 from pSB265 (Trueheart et al., 1987) subcloned into the BamHI–Sall sites of Yerp35R (Myers et al., 1986). pYEE61 (FUS2-lacZ URA3 CEN4 ARS1) has the Sall–NcoI fragment of pYE52 encompassing Fus2-lacZ and a portion of the URA3 gene subcloned into the BamHI–NcoI sites of pYEE57, a derivative of YCP50 with the BamHI site converted to an Sall site by linker tailing (Lathe et al., 1984). pYEE63 (TRPE-FUS2) has the 1.2-kb HindIII–HindIII fragment of Fus2 subcloned into the HindIII site of pATH3 (Koerner et al., 1990).

**RNA Analysis**

Total RNA was isolated from S. cerevisiae as described (Elion and Warner, 1984). Northern analysis was performed as described (Elion et al., 1990). Fus2 mRNA was detected with a 1.1-kb HindIII–HindIII fragment from pSB265 and Fus1 and ORF1 mRNAs were detected with a 6.0-kb HindIII–HindIII fragment from pSB202 (Trueheart et al., 1987). Fus3 was detected with a 3.3-kb EcoRV–Sall fragment from pYEE94 (Elion et al., 1990). Act71 was detected with a 2.0-kb XhoI–HindIII fragment from pYEE15 (Elion et al., 1990). Double-stranded DNA probes were radiolabeled using random hexamers (Pharmacia Inc., Piscataway, NJ) and DNA polymerase I Knlocow fragment (New England Biolabs, Beverly, MA). The direction of transcription for Fus2 was determined by RNA dot blot analysis (Maniatis et al., 1982), using single-stranded DNA probes prepared by subcloning fragments of the Fus2 gene into M13, mp8, and mp19 (Fig. 1), isolating single (+) strand progeny (Viera and Messing, 1987), and radiolabeling as described (Elion and Warner, 1984).

**DNA Sequencing**

The entire sequence of both strands of the Scal–SalI Fus2 fragment was determined by the dideoxy method of Sanger (1977) using single-stranded M13 phage (Messing, 1982). Sequencing reactions were electrophoresed on gradient acrylamide gels as described (Biggin et al., 1983). All predicted six base restriction sites were confirmed by restriction digestions.

**Quantitative and Qualitative Mating Assays**

Yeast strains were mated quantitatively and qualitatively as described (Elion et al., 1990). The frequency of diploid formation is estimated as the
number of diploids formed per total cells mated. Each frequency is the average of two matings in which at least 100 diploids were recovered. Unless noted otherwise, all matings were performed at 30°C. The effect of EGTA and Ca²⁺ on mating efficiency was measured by spotting and drying varying amounts of EGTA and Ca²⁺ on YEPD plates before their use for the 4-h qualitative patch matings. Diploids were then selected on selective media of the appropriate dilution containing exogenous additions of EGTA or Ca²⁺. The effect of temperature on mating was measured by preincubating plates used for mating at the appropriate temperature and maintaining that temperature during the mating, then selecting for diploids at 30°C. The effect of polyethylene glycol (PEG)³ 3550 (Sigma Chemical Co., St. Louis, MO) on mating was determined in both liquid and solid medium, however, high concentrations of polyethylene glycol for an extended time precipitate in solid agar medium, precluding interpretation of the results.

**β-Galactosidase Assays**

Where indicated, yeast cells were induced with 5 μM α-factor for 90 min in media of pH 4 as described (Elion et al., 1990), before being assayed for β-galactosidase activity by the method of Craven et al., 1965. Cell extracts were prepared as described (Choi et al., 1994) and U of activity (nmol of o-nitrophenyl-galactoside cleaved/min per mg protein) were calculated by the formula: OD₄₂₅ × (377.8/time (min)) × vol extract (ml) × protein (mg/ml).

**Antibody Preparation**

Recombinant trpE-Fus2 protein (pYEE36) was expressed in E. coli strain RRI according to the method of Koerner et al., 1990. 0.2 ml of a fresh pre-culture grown in M9 media containing vitamin B1, ampicillin, and tryptophan was diluted into 100 ml of the same media and shaken for 2 h at 37°C, then induced with 20 μg/ml indoleacrylic acid for 4.5 h at 37°C. The culture was stored overnight on ice, pelleted, washed once in ice-cold 20 mM Tris-Cl, pH 7.4, and resuspended in 20 ml of 20 mM Tris-Cl, pH 7.4, 5 mM EDTA, 3 mg/ml bovine, and incubated for 2 h on ice. 1.4 ml 5 M NaCl and 1.5 ml 10% NP-40 were then added, the sample was incubated 30 min on ice, then centrifuged for 10 min at 10,000 rpm. The pellet was dispersed with a glass rod into 20 ml ice-cold 1 M NaCl, 10 mM Tris-Cl, pH 7.4, washed once with 10 mM Tris-Cl, pH 7.4, and then suspended in 0.4 ml 2x Laemmli buffer. Samples were sonicated and boiled before electrophoresis on preparative SDS–polyacrylamide gels (10% polyacrylamide, 30%/0.8% acrylamide/bisacrylamide; 3 mm thick). Gel slices containing trpE-Fus2 were excised after brief staining with 1% Coomassie blue, finely ground, and the protein was eluted from the gel by incubation at 24°C in electophoresis buffer. The elute was collected and concentrated with a microconcentrator 30, and protein concentration was estimated by SDS-PAGE using protein standards. Two rabbits (114 and 115, housed at the Whitehead Institute Animal Facility) were each injected three times with 0.1 mg of protein (in 0.25 ml PBS that was suspended in 0.5 ml complete Freund's adjuvant) following a standard injection and bleeding schedule. A portion of the antisera from one rabbit (115) was preadsorbed first to purified trpE protein affixed to nitrocellulose, then affinity purified to the original trpE-Fus2 fusion protein affixed to nitrocellulose exactly as described Smith and Fischer (1984).

**Preparation of Yeast Extracts**

Yeast strains containing plasmids were grown at 30°C in selective synthetic complete media with 2% dextrose to an A₆₀₀ of 0.4-0.8 and then induced (for 90 min at 30°C with a factor (5 μM α-factor for S71 strains, and 0.05 μM α-factor for strain sstLΔ strains) in media that were at pH 4 as described (Elion et al., 1990). Cells were disrupted by glass beads and proteins were precipitated with TCA as described (Osashi et al., 1982). Proteins were separated on 7.5% SDS–polyacrylamide gels (Laemmli, 1970). The distribution of Fus2 in whole-cell extracts was examined essentially as described (Franzusoff et al., 1991) with several modifications. Approximately 400 μl of logarithmically growing cells in SC media (YP957 MATa sstLΔ at OD₆₀₀ of 0.25) were induced with α-factor for 90 min, the cells were pelleted, washed once with water, then quick frozen in ethanol/dry ice. Cells were thawed on ice and resuspended in 2 ml buffer A (20 mM MES/Tris, pH 6.5, 100 mM NaCl, 5 mM MgCl₂, 0.7 M sorbitol, 10 mM DTT, 0.1 μM/ml PMSF, 5 μg/ml each of pepstatin A, chymostatin, leupeptin, antipain). 0.5 ml mzymoylease¹¹ (10 mg/ml) was added, and the samples were incubated for 30 min at 30°C. Samples were kept on ice and then washed twice with solution B (100 mM potassium phosphate, pH 7.5, 1.2 M sorbitol), and resuspended at a concentration of ~1 × 10⁶ cells/ml in solution B containing 30 μM B-mercaptoethanol, 0.1 μM/ml PMSF, 5 μg/ml each of pepstatin A, chymostatin, leupeptin, antipain. Lysate (0.25 ml) was added to 0.1 mg/ml and cells were digested for ~15 min at 30°C. The oxaolylcase was then diluted by adding three vol of ice-cold solution B, the spheroplasts were pelleted by centrifugation at 1,500 rpm at 4°C, washed twice with fresh solution B, and resuspended at 10⁶ cells/ml. 10 μl of spheroplasts were pipetted onto wells of microscope slides (Polyscience Corp., Niles, IL) that had been cold washed, dried, and coated with poly-lysine (1 mg/ml). Spheroplasts were allowed to settle for 10 min, then the slides were incubated at ~20°C in 100% methanol for 6 min, 100% acetone for 30 s. Samples were rehydrated with solution B, then incubated in solution B + protease inhibitors + 2% BSA for 1 h at 30°C. The primary antibody was added in the same buffer (affinity-purified Fus2 antisera diluted 1:5, β-galactosidase monoclonal diluted 1:50) and incubated for 2 h at 37°C, then rinsed twice with solution B. The samples were incubated in several dilutions of secondary antibody (1:50–1:250 dilutions of fluoroscein-conjugated affinity-purified goat anti-
Results

Fus2 mRNA Is Expressed Only in the Presence of Mating Pheromone

The Fus2 gene had been localized to a 2.5-kb SacI–SalI fragment by complementation of the mating defect of a MATa fus2Δ strain (Fig. 1; Trueheart et al., 1987). Northern analysis with a probe from this region shows that the Fus2 gene is not expressed in vegetatively growing haploid or diploid cells, but is expressed when MATa and MATa/MATa cells are treated with α factor (Fig. 2 A). The pattern of Fus2 transcription resembles that of Fus1 (McCaffrey et al., 1987; Trueheart et al., 1987), and contrasts with that of Fus3, which is expressed in vegetatively growing haploid cells (Elion et al., 1990). Fus2 appears to be more tightly regulated in vegetatively growing cells than is Fus1, because neither longer exposure of the autoradiogram, nor hybridization of more RNA reveal any contrasts with that of Fus1, because neither longer exposure of the autoradiogram, nor hybridization of more RNA reveal any

Fus2 Encodes a Unique Open Reading Frame

Detection of Fus2 mRNA with single-stranded DNA probes shows that the Fus2 gene is transcribed in the direction indicated in Fig. 1 A. Sequence analysis of the complementing region of DNA reveals a single open reading frame of 617 amino acids that encodes a protein of rabbit IgG, rhodamine-conjugated affinity-purified goat anti-rabbit IgG, fluorescein-conjugated affinity-purified rabbit anti-mouse IgG, all from Jackson ImmunoResearch Laboratories, Inc.) in solution B + protease inhibitors + 2% BSA for 2 h at room temperature in the dark. Samples were washed twice with solution B containing 0.3 M NaCl, three times with solution B. Coverslips were mounted with 90% glycerol containing 1 mg/ml p-phenylene diamine at pH 8.0, and 1 μg/ml 4,6-diamidino-2-phenylindole dihydrochloride. Photomicroscopy was performed with an Axioscope (Carl Zeiss, Inc., Thornwood, NY) and Tri-X 400, Technapan 2415, and T-MAX 400 film (Eastman Kodak Co., Rochester, NY).
Fus2 Localizes at the Site of Cell Fusion in Mating Yeast

Figure 2. Northern analysis of FUS2 transcription. (A) FUS2 transcription as a function of α factor induction in MATa, MATa/MATa, MATa/MATa, and MATα/MATα. Total RNA was isolated and analyzed by Northern blot analysis as described (Elion and Warner, 1985). 5 μg of total RNA was loaded in each lane. A single nitrocellulose blot was hybridized first with a FUS2 probe, then stripped and reprobed with an FUS1/ORF probe (Materials and Methods). — and + indicate whether strains were induced for 90 min with a factor (α F) as described in Materials and Methods. 25S and 18S indicate the positions of the corresponding rRNA in the top blot. Yeast strains are L3262 (MATa), L2501 (MATa/MATa), L2499 (MATa/MATa), and L2500 (MATa/MATa). (B) FUS2 transcription in fus3 ksl1 and ste12 strains. Northern analysis was performed exactly as described in A, with — and + indicating whether strains were induced for 90 min with α factor (α F) before RNA isolation. The nitrocellulose blot was first hybridized with FUS2 and FUS3 probes, then stripped and reprobed with an ACT1 probe. Yeast strains are: EY699 (WT), EY700 (fus3Δ), EY725 (ks1Δ), EY723 (fus3Δ ksl1Δ), EY718 (ste12Δ).

73,000 D. The open reading frame is on the same coding strand as that predicted by RNA analysis, and is of a size that agrees with the length of the FUS2 transcript (Fig. 1B). Two TGAAACA pheromone-response elements predicted to be bound by the STE12 protein (Dolan et al., 1989; Errede and Ammerer, 1989) are found upstream of the FUS2 open reading frame. The presence of the TGAAACA repeats is consistent with the pattern of FUS2 expression, which is dependent on Ste12 and pheromone (Fig. 2). The similar transcriptional regulation of FUS1 and FUS2 suggests that the two genes may share common promoter elements. Comparison of the 5′ regions of FUS1 and FUS2 reveals a 14-nucleotide stretch of identity (TATCTTTTTCTTT) between the two genes located at equivalent distances from the presumptive initiation codons.

Homology searches of standard public databases and of a private database (M. Goebl, personal communication) show that the Fus2 protein is unique. No homology is found between Fus1 and Fus2 in pairwise comparisons. Fus2 lacks an obvious signal sequence based on the parameters described by Kaiser et al. (1987) and does not have an obvious transmembrane domain according to the calculations of Eisenberg et al. (1984). Secondary structure predictions suggest that Fus2 is rich in amphipathic α-helical structure and contains regions likely to form coiled coils according to the algorithm of Lupas et al. (1991). Fus2 also shows weak homology (~23% identity, 45% similarity) to several cytoskeletal proteins including the yeast myosin-like protein, Mlp1 (Kolling et al., 1993), mouse dystrophin (Bies et al., 1992), and a human kinesin-related protein (Yen et al., 1992). These homologies may be significant because they extend across the entire protein and are consistent with the secondary structure predictions.

fus1 and fus2 Mutants Have Different Sensitivities to Polymyxin B, EGTA, and Low Temperature

The absence of homology between Fus1 and Fus2 suggests the two proteins perform different cell fusion functions. We therefore determined whether fus1 and fus2 mutants have any distinguishing phenotypes by assessing the effects of agents known to affect membranes either in vivo or in vitro (i.e., PEG, polymyxin B, temperature, Ca2+) on the ability of fus1 and fus2 mutants to form diploids. PEG is a potent fusogen of phospholipid vesicles (Wilschut and Hoekstra, 1984), intact mammalian cells (Pontecorvo, 1976), and yeast spheroplasts (van Solingen and van der Plaat, 1977). Polymyxin B alters membrane permeability of bacteria and yeast (Boguslawski, 1985) and interferes with agglutination during mating in yeast (Boguslawski, 1986). Temperature and Ca2+ affect phospholipid vesicle fusion in vitro, and Ca2+ is an important regulator of fusion in many systems (Stegmann et al., 1989; White, 1992).

We quantitated the ability of MATα fus+ and MATα fus− strains to form diploids under conditions in which the added reagent had minimal effects on the mating of Fus+ strains and little or no effect on cell viability. PEG stimulates prototroph formation two- to fourfold in matings between both Fus+ and Fus− parents (Table II), suggesting PEG affects FUS1- and FUS2-independent processes. This effect is detected when cells are mated in liquid culture, suggesting that PEG brings the mating yeast cells closer together by exclusion of water as it does with liposomes (Stegmann et al., 1989). In contrast, polymyxin B inhibits prototroph formation in both fus2 and fus1 matings (Table III). However, a distinct difference can be observed between fus1 and fus2 in the Fus+/×Fus− crosses: when only one parent is Fus+, fus2 strains are more sensitive than fus1 strains.

Matings between fus1 strains are much more cold sensitive than either wild-type or fus2 matings (38-fold inhibition for fus1 vs. twofold for fus2; Table IV). The effect of low temperature is most apparent when both parents lack FUS1. Likewise, the removal of Ca2+ and any other divalent cation by the addition of EGTA inhibits prototroph formation in crosses between fus1 mutants, but has no effect on either wild-type or fus2 matings (Fig. 3A; note that the effect is detected best in a qualitative patch mating assay). Furthermore, the inclusion of Ca2+ with the EGTA
### Table I. Yeast Strains Used in This Study

| Strain   | Genotype                                                                 | Source          |
|----------|---------------------------------------------------------------------------|-----------------|
| L2499    | MATa/MATa his4-Δ5his4-Δ9 arg11/arg11 cysl/cryl                           | G. Fink         |
| L2500    | MATa/MATa his4-Δ5his4-Δ9 arg11/arg11 cysl/cryl                           | G. Fink         |
| L2501    | MATa/MATa his4-Δ5his4-Δ9 arg11/arg11 cysl/cryl                           | G. Fink         |
| L3259    | MATa ura3-52 leu2-3,112 his4-34                                          | G. Fink         |
| EY699    | MATa ura3-1 trp1-1 leu2-3,112 ade2-1 his3-11,15 can1-l100                 | E. Elion        |
| EY700    | fus3-6::LEU2 derivative of EY699                                         | E. Elion        |
| EY707    | sst1Δ::URA3 derivative of EY699                                          | E. Elion        |
| EY723    | fus3-6::LEU2 kssl::URA3 derivative of EY699                               | E. Elion        |
| EY725    | kssl::URA3 derivative of EY699                                           | E. Elion        |
| EY957    | sst1Δ derivative of EY699                                                | E. Elion        |
| Isogenic derivatives of JY390 |                                                                 |                |
| JY390    | MATa kssl1- ura3-52 trp1Δ1 his4-34                                        | J. Trueheart    |
| JY387    | MATa kssl1- fus2Δ3 ura3-52 trp1Δ1 his4-34                                 | J. Trueheart    |
| JY417    | MATa kssl1- fus1Δ1 ura3-52 trp1Δ1                                        | J. Trueheart    |
| JY419    | MATa kssl1- fus1Δ1 fus2Δ3 ura3-52 trp1Δ1                                   | J. Trueheart    |
| JY428    | MATa kssl1- fus2Δ3 ura3-52 trp1Δ1 his4-34 con^a                           | J. Trueheart    |
| JY429    | MATa kssl1- fus1Δ1 fus2Δ3 ura3-52 trp1Δ1 con^a                             | J. Trueheart    |
| JY430    | MATa kssl1- fus1Δ1 ura3-52 trp1Δ1 con^a                                    | J. Trueheart    |
| JY431    | MATa kssl1- ura3-52 trp1Δ1 his4-34                                        | J. Trueheart    |
| Isogenic derivatives of JY396 |                                                                 |                |
| JY396    | MATa kssl1- ura3-52 leu2-3,112 his4-34 {K^*}                              | J. Trueheart    |
| JY395    | MATa kssl1- fus2Δ3 ura3-52 leu2-3,112 his4-34 {K^*}                        | J. Trueheart    |
| JY412    | MATa kssl1- fus1Δ1 fus2Δ3 ura3-52 leu2-3,112 {K^*}                        | J. Trueheart    |
| JY416    | MATa kssl1- fus1Δ1 ura3-52 leu2-3,112 {K^*}                               | J. Trueheart    |
| JY424    | MATa kssl1- fus2Δ3 ura3-52 leu2-3,112 his4-34                             | J. Trueheart    |
| JY425    | MATa kssl1- ura3-52 leu2-3,112 his4-34                                    | J. Trueheart    |
| JY426    | MATa kssl1- fus1Δ1 fus2Δ3 ura3-52 leu2-3,112                             | J. Trueheart    |
| JY427    | MATa kssl1- fus1Δ1 ura3-52 leu2-3,112                                    | J. Trueheart    |
| kssl-Δ derivatives of Fus strains |                                                                 |                |
| EY73     | JY396 kssl1-                                                               | E. Elion        |
| EY77     | JY416 kssl1-                                                               | E. Elion        |
| EY89     | JY395 kssl1-                                                               | E. Elion        |
| EY94     | JY425 kssl1-                                                               | E. Elion        |
| EY98     | JY427 kssl1-                                                               | E. Elion        |
| EY102    | JY424 kssl1-                                                               | E. Elion        |
| p° Cyh2^a derivatives of Fus strains |                                                                 |                |
| EY81     | JY396 p° Cyh2^a                                                           | E. Elion        |
| EY82     | JY395 p° Cyh2^a                                                           | E. Elion        |
| EY83     | JY412 p° Cyh2^a                                                           | E. Elion        |
| EY84     | JY416 p° Cyh2^a                                                           | E. Elion        |
| EY85     | JY424 p° Cyh2^a                                                           | E. Elion        |
| EY86     | JY425 p° Cyh2^a                                                           | E. Elion        |
| EY87     | JY426 p° Cyh2^a                                                           | E. Elion        |
| EY88     | JY427 p° Cyh2^a                                                           | E. Elion        |
| EY260    | JY421 p° Cyh2^a                                                           | E. Elion        |
| EY262    | JY425 p° Cyh2^a                                                           | E. Elion        |
| EY264    | JY426 p° Cyh2^a                                                           | E. Elion        |
| EY266    | JY427 p° Cyh2^a                                                           | E. Elion        |
| EY268    | JY395 p° Cyh2^a                                                           | E. Elion        |
| EY270    | JY412 p° Cyh2^a                                                           | E. Elion        |
| EY272    | JY416 p° Cyh2^a                                                           | E. Elion        |
| HO switched derivatives of JY132/JY133 |                                                                 |                |
| JY132    | MATa ura3-52 ly2-801 trplΔ1 his4-34                                       | Trueheart et al., 1987 |
| JY133    | MATa ura3-52 leu2-3,112 his4-34                                           | Trueheart et al., 1987 |
| EY310    | MATa/MATa diploid                                                         | E. Elion        |
| EY312    | MATa/MATa diploid                                                         | E. Elion        |
| EY324    | EY310 + pYEE52                                                           | E. Elion        |
| EY325    | EY310 + pYEE61                                                           | E. Elion        |
| EY326    | EY310 + pSB234                                                           | E. Elion        |
| EY327    | EY310 + B929                                                            | E. Elion        |
| EY338    | EY312 + pYEE52                                                           | E. Elion        |
| EY339    | EY310 + pYEE61                                                           | E. Elion        |
| EY340    | EY310 + pSB234                                                           | E. Elion        |
| EY341    | EY310 + B929                                                            | E. Elion        |
| Isogenic derivatives of JK103 |                                                                 |                |
| JK103    | MATa ura3-52 ade2-1 trplΔ1 fus1Δ1 ura3-52 fus2Δ1 fus3-6::LEU2 derivative of JK103 | J. Kim         |
| EY185    | fus1Δ1 derivative of JK103                                               | E. Elion        |
| EY195    | fus2Δ1 derivative of JK103                                               | E. Elion        |
| Congenic lys9 strains |                                                                 |                |
| JBY342   | MATa lys9                                                                | J. Brill        |
| JBY343   | MATa fus1Δ1 lys9                                                         | J. Brill        |
| JBY345   | MATa fus1Δ1::URA3 ura3-52 lys9                                           | J. Brill        |
| JBY347   | MATa fus1Δ1 fus2Δ1::URA3 ura3-52 lys9                                     | J. Brill        |
| JBY350   | MATa lys9                                                                | J. Brill        |
restores mating in fus1 crosses, suggesting that fus1 mutants are more sensitive to calcium levels for optimal cell fusion. Thus, fus1 strains are more temperature and Ca^{2+} dependent for efficient cell fusion than are wild-type and fus2Δ strains. These phenotypic differences suggest that FUS1 and FUS2 encode qualitatively different functions required for cell fusion.

**fus2 Mutants Display Karyogamy Defects**

The morphology of fus- and fus2-blocked zygotes suggests they could be defective in karyogamy as well as cell fusion (Trueheart et al., 1987). Zygotes defective in nuclear fusion give rise to stable haploid cytoductants containing the cytoplasm of one parent and the nucleus of the other, thus providing a convenient way to monitor nuclear fusion genetically (Conde and Fink, 1976). We quantitated the ability of fus mutants to transmit cytoplasmic particles while mating, by measuring the degree of transmittance of mitochondria from one parent to another in isogenic crosses. In each case, a MATα rho+ cyh2 parent was mated to a MATa rho+ CYH2 parent, and haploid exconjugants containing the Cyh2 nucleus and rho+ cytoplasm were selected. fus2 mutants exhibit a 150-fold higher frequency of cytoduction (percentage cytoductant/percentage diploid) compared with wild-type strains, whereas fus1 × fus1 crosses exhibit wild-type levels of cytoduction (Table V). The fus2 crosses exhibit a significant elevation in the transmission of mitochondria to haploid exconjugants, approximately one-tenth that found for the karl-1 mutant (Kim et al., 1991). The frequency of cytoduction increases even further in fus2 matings in which cell fusion is more tightly blocked (and the percentage diploids formed is decreased), to 260-fold greater for fus2 × fus2 matings in which only one parent fus2 and 2,700-fold greater for fus2 × fus1 fus2 matings in which both parents are fus2.

**Table II. Effect of PEG During Mating of Fus Mutants**

| Fus cross | YPD | 6.7% PEG | Fold stimulation |
|-----------|-----|----------|-----------------|
| + × +     | 8.6 | 15.5     | 1.9             |
| 1- × 1-   | 14.7| 44.0     | 3.0             |
| 2- × 2-   | 12.6| 56.7     | 4.5             |

*Cells were grown in YPD to an A600 of 0.5-0.8, then diluted to an A600 of 0.25 with either YPD, or YPD containing 6.7% polyethylene glycol (~mol wt of 3,350). Approximately 0.5 ml of each parent was mated in a 3 ml glass tube on a roller wheel for 6 h at 30°C. Samples were then diluted into ice-cold water, sonicated, and plated in duplicate onto YPD and minimal plates to determine the total number of cells and the number of prototrophs. The concentration of PEG used did not affect cell viability.

These surprisingly high cytoduction frequencies strongly suggest that fus2 mutants are defective at some step in nuclear fusion.

A second phenomenon associated with a block in nuclear fusion is chromo- or plasmiduction, the transmission of chromosomes or plasmid DNA from one parental nucleus to the other in the absence of nuclear fusion (Dutcher, 1981). For this experiment, a MATα ura3-52 parent harboring a URA3 CEN4 plasmid (YCP50) is mated to a MATa ura3-52 can18 cyh2 parent and MATα exconjugants containing YCP50 are selected. The frequency of Ura+ reversion of the ura3-52 locus in the MATα parent and mutation of both CAN1 and CYH2 to resistant alleles in the MATα parent is extremely low, ruling out these events as major sources of the colonies we observe. As shown in Fig. 3 C, plasmiduction is greatly enhanced in a fus2 × fus2 cross compared with FUS2 × FUS2 and fus1 × fus1 crosses. This increase in plasmiduction is similar to that seen in an isogenic karl-1 × KAR1 cross (Fig. 3 C), in which diploids form at ~10% wild-type levels. The effects of fus2 and karl on plasmiduction are not additive, as shown by the equivalent level of plasmiduction in a fus2 × fus2 karl cross. These results substantiate the cytoduction results and suggest further that fus2 mutants may perturb the same pathway required for nuclear fusion that is affected by a karl mutation.

**Parental Nuclei Misalign in fus2 Zygotes**

We examined the morphology of microtubules in defective fus2 zygotes, because nuclear fusion can be blocked by defects in the spindle pole body and associated microtubules (Rose, 1991), in addition to defects in the fusion of nuclear envelopes (Kurihara et al., 1994). Zygotes that had not yet undergone nuclear fusion were compared in Fus+ × Fus+ and Fus- × Fus- crosses by fixing populations of cells after they were mated for a brief time interval and then staining for microtubules and DNA. Random fields of cells containing occasional zygotes were photographed, and the zygotes were scored for position of parental nuclei and orientation of the spindle pole body and associated microtubules relative to the junction between the joined cells. In Fus+ × Fus+ zygotes, the parental nuclei migrate to the position of cell fusion and align with the spindle pole body and associated microtubules of each nucleus oriented toward the other (Rose and Fink, 1986). Misaligned nuclei are those in which the spindle pole bodies and associated microtubules fail to juxtapose. As shown in Table VI and Fig. 4, 100% of wild-type zygotes and 91% of fus1 × fus1 zygotes display an alignment of spindle pole
Table IV. Effect of Low Temperature During Mating of fus Mutants

| Fus cross | 30°C  | 14°C  | Fold inhibition |
|-----------|-------|-------|-----------------|
| + × +     | 47    | 28    | 1.7             |
| 1 × 1     | 13    | 0.34  | 38              |
| 2 × 2     | 11    | 3.5   | 3               |
| 1 × 2     | 0.6   | 0.02  | 30              |
| 1 × 1-2   | 5.3   | 0.5   | 10.6            |
| 1 × 1     | 29    | 11    | 2.6             |
| 1 × 1-2   | 16    | 4     | 4               |

*Prototroph formation was quantitated as described after a 3-h mating on YPD plates at the indicated temperature (Elion et al., 1990). The strains used in the experiment are: JBY342, JBY343, JBY345, EYL44, EYL45, EYL46, EYL47.

bodies and microtubules judged to be normal. By contrast, the majority (79%) of fus2 × fus2 zygotes have misaligned nuclei. This finding is consistent with the nuclear fusion defect of fus2A mutants and suggests that FUS2 is required for a function that affects proper alignment of the nuclei in addition to cell fusion.

Figure 3. Qualitative patch mating tests of fus1 and fus2 mutants. (A) Effect of EGTA and Ca²⁺. MATa yeast strains were grown overnight as patches on YPD plates, then mated to lawns of MATα fus1A cells for 2 h at 30°C on YPD plates with or without 7.5 mM EGTA and 7.5 mM Ca²⁺. Diploids were then selected on YNB plates containing uracil and histidine. Note that under these conditions of brief mating, it is possible to detect significant reductions in the mating efficiency of fus1 and fus2 single mutant crosses, in contrast to results obtained with 4 h matings (Trueheart et al., 1987). No effects were detected in parallel matings with lawns of MATα and MATα fus2A strains. Yeast strains are: JY387, JY390, JY417, JY416. (B) Measurement of plasmiduction in fus1, fus2, and kar1 strains. Patches of MATα fus- ura3-52 leu2-3,112 his4-24 strains harboring YCp50 were grown overnight on SC-ura plates and mated to lawns of MATα fus- ura3-52 leu2-3,112 ade2 trp1-289 can18 cyh28 strains for 4 h at 30°C. Plasmiducants were recovered by replica plating the mating cells to YNB plates containing adenine, leucine, tryptophan, canavanine, cycloheximide. Yeast strains patched are: JY424-JY427, EY94, EY98, EY102. Yeast strains used as lawns are: EY183, EY185, EY195.

Detection of Fus2 Protein in a Factor-induced Cells

To characterize Fus2 in vivo, we constructed a FUS2-β-galactosidase fusion (FUS2-lacZ) and raised an antiserum against an internal portion of Fus2 fused to the E. coli TRPE protein (Materials and Methods). The FUS2-lacZ fusion contains the entire FUS2 open reading frame and partially complements the mating defect of a fus2A mutant, but does not suppress a fus1A mutant, unlike native Fus2 (Trueheart et al., 1987). Immunoblot analysis of yeast whole-cell extracts shows that the Fus2 antiserum recognizes a protein of ~70 kD in cells that have been exposed to α factor, consistent with the predicted mass of Fus2 and the pattern of transcription of the FUS2 gene (Fig. 5, A and B). That this protein is Fus2 is supported by the fact that (a) it is not present in fus2A cells that have been induced by α factor, (b) its abundance increases in cells that harbor a multicopy plasmid containing the FUS2 gene, and (c) the FUS2-β-galactosidase fusion protein of the predicted size is recognized by both the Fus2 antiserum

Table V. Frequency of Cytoduction in fus Crosses

| Parents                  | Percent Diploid | Percent Cytoductant | Percent Cytoductant/Diploid |
|--------------------------|-----------------|---------------------|----------------------------|
| a rho⁺ Cyh5              | -               | -                   | -                          |
| X a rho⁺ Cyh5            | -               | -                   | -                          |
| 1 × 1                    | 50.3            | 0.211               | 0.00419                    |
| 1 × 1δ                   | 28.4            | 0.172               | 0.00606                    |
| 2 × 2                    | 31.8            | 19.4                | 0.610                      |
| 1 × 2                    | 0.0103          | 0.0115              | 1.117                      |
| 1 × 2δ                   | 0.0160          | 0.182               | 11.38                      |

*Prototroph and cytoductant formation were quantitated by mass matings as described (Elion et al., 1990) after a 4-h mating at 30°C.

Detection of Fus2 Protein in a Factor-induced Cells

To characterize Fus2 in vivo, we constructed a FUS2-β-galactosidase fusion (FUS2-lacZ) and raised an antisera against an internal portion of Fus2 fused to the E. coli TRPE protein (Materials and Methods). The FUS2-lacZ fusion contains the entire FUS2 open reading frame and partially complements the mating defect of a fus2A mutant, but does not suppress a fus1A mutant, unlike native Fus2 (Trueheart et al., 1987). Immunoblot analysis of yeast whole-cell extracts shows that the Fus2 antiserum recognizes a protein of ~70 kD in cells that have been exposed to α factor, consistent with the predicted mass of Fus2 and the pattern of transcription of the FUS2 gene (Fig. 5, A and B). That this protein is Fus2 is supported by the fact that (a) it is not present in fus2A cells that have been induced by α factor, (b) its abundance increases in cells that harbor a multicopy plasmid containing the FUS2 gene, and (c) the FUS2-β-galactosidase fusion protein of the predicted size is recognized by both the Fus2 antiserum

Table VI. Tally of Microtubule Distribution in Fus+ and Fus- Zygotes

| Genotype | Total zygotes scored | Number zygotes with unfused nuclei | Number aligned MTs in unfused nuclei | Percent misaligned nuclei |
|----------|----------------------|-----------------------------------|-------------------------------------|--------------------------|
| fus1 × fus1 | 25                   | 23                                | 21                                  | 8.7                      |
| fus2 × fus2 | 19                   | 19                                | 4                                   | 79                       |

*Microtubule distribution in zygotes was assessed as follows: yeast strains were grown exponentially at 30°C in YEPD to an OD₆₀₀ of 0.25-0.35. To mate, ~2.5 OD U of cells of parents were pelleted together, resuspended in 0.1 ml of supernatant, and transferred onto a 60 × 15 mm YEPD agar plate. Cells were mated briefly for 2 h at 30°C, then collected in 5 ml liquid YEPD, fixed with formaldehyde, and prepared for indirect immunofluorescence as described in Materials and Methods. Nuclear DNA was visualized by staining with the dye DAPI, microtubules were visualized with YOL1/34 mAb. Fields of cells containing zygotes were photographed at random for analysis. Microtubule alignment was defined as whether the spindle pole bodies of adjacent (paired) nuclei in zygotes were oriented towards each other in the same plane of focus. Misaligned parental microtubules were considered not to be oriented towards each other and/or in different planes of focus. The reduced number of fus1 × fus1 and fus2 × fus2 zygotes tallied may reflect the fact that these zygotes are hypersensitive to the zymolyase treatment used in preparation of the cells for indirect immunofluorescence, causing lysis at the septum between paired cells.

The strains used in the matings were: JY425 × JY396 (Fus × Fus); JY427 × JY416 (fus1 × fus1); JY395 × JY424 (fus2 × fus2).
Fus2 proteins, have not undergone nuclear fusion, and Fus-zygotes that have not undergone nuclear fusion, Figure 4. Morphology of nuclei and microtubules in wild-type and Fus-zygotes that have not undergone nuclear fusion. fus2Δ × fus2Δ zygotes display misaligned parental nuclei as evidenced by nonaligned spindle pole bodies and associated microtubules. Shown are a representative wild-type zygote after nuclear fusion (A–C) and prenuclear fusion (D–F), two fus1Δ × fus1Δ zygotes prenuclear fusion (G–I), and two fus2Δ × fus2Δ zygotes prenuclear fusion (J–L, M–O). Note that for both wild-type and fus1Δ × fus2Δ zygotes the spindle pole bodies have aligned towards each other in the same plane, whereas they are not pointed towards each other in the fus2Δ × fus2Δ zygotes. Panels display zygotes by DIC (A, D, G, J, M), microtubules by indirect immunofluorescence against β-tubulin (B, E, H, K, N), and nuclei by DAPI (C, F, I, L, O). Yeast strains used are: JY395, JY396, JY416, JY424, JY425, JY427. Cells were mated for 2 h before being fixed.

and a β-galactosidase mAb (Fig. 5, A and C). The similarity in mass between the predicted Fus2 protein and the protein recognized by the antiserum suggests that Fus2 is not grossly modified by asparagine-linked glycosylation. Consistent with this conclusion, tunicamycin treatment of cells harboring the His4-LacZ fusion exhibit diffuse cytoplasmic staining of an intensity that is proportional to cell volume (C). Furthermore, the punctate distribution of Fus2-lacZ contrasts sharply with that of Fus1-lacZ, which localizes in a sharp rim at the tip of projections, suggesting the two proteins do not colocalize (B).

The distribution of native Fus2 was also examined with the affinity-purified Fus2 antibodies, because the β-galactosidase segment of the Fus2-β-galactosidase fusion could interfere with proper localization of Fus2. Initial studies to detect native Fus2 with this antibody in haploid MATa/FUS2 strains were unsuccessful, despite the fact that the Fus2-lacZ protein could be readily visualized with the Fus2 antibody, even when the FUS2-lacZ gene was maintained on a centromeric plasmid. Since both FUS2-lacZ and FUS2 are expressed from identical promoters, the Fus2-lacZ fusion protein may be more stable than Fus2 (Fig. 5 A). However, we were able to detect Fus2 in diploid MATa/MATa FUS2/FUS2 cells after α factor induction and in populations of mating MATa/MATa FUS2/FUS2 and MATa/MATa FUS2/FUS2 cells (Fig. 7). Visualization was greatly enhanced when the cells contained extra copies of the FUS2 gene (on a multicopy plasmid). Fus2 distribution in these cells (Fig. 7 A, d–f) is very similar to that of Fus2-lacZ (Fig. 7 A, a–c) with one exception. The majority of native Fus2 is found at the projection tip, close to the plasma membrane, with a smaller fraction detected in the middle of the projection in occasional cells of strains harboring the FUS2 multicopy plasmid (d–f). In contrast, the Fus2-lacZ protein is more often detected in the middle of projections as well as at the tip (compare two cells in b and c). We conclude that native Fus2 associates with structures that accumulate at or near the plasma membrane of projection tips. The Fus2-lacZ protein may cause the accumulation of these structures within the neck of the projection (note the Nomarski micrograph which shows surface bumps that appear to superimpose over the Fus2-lacZ staining; Fig. 6 A, bottom two cells).

Fus2 Localizes at the Shmoo Tip in Pheromone-induced Cells

Fus2 was visualized in mating yeast cells by indirect immunofluorescence using the Fus2-lacZ fusion protein and a β-galactosidase mAb. Two additional β-galactosidase fusion proteins served as integral controls, a Fus1–LacZ fusion previously shown to localize to the plasma membrane at the projection tips of pheromone-induced cells (Trueheart et al., 1987; Trueheart and Fink, 1989) and a cytoplasmic β-galactosidase protein expressed from a HIS4 promoter. All three proteins are present in essentially equal abundance in α factor–induced cells (Fig. 5 C).

Fus2-lacZ localizes in punctate spots that resemble vesicles or other large structural elements such as the cytoskeleton. The spots accumulate within the projection neck at or near the projection tip of cells that have been exposed to α factor for 90 min (A). A small amount of cytoplasmic staining is also seen in addition to the staining within the projection. The asymmetric pattern of Fus2-lacZ distribution is readily visible in cells that have not yet undergone projection formation (Fig. 6 A, top row) indicating that the structure with which Fus2-lacZ associates is present before projection formation. In addition, the position of Fus2-lacZ does not appear to correlate with the position of the nucleus. The highly asymmetric distribution pattern of Fus2-lacZ is not an artifact of the heightened sensitivity of the projection tips to treatment by zymolyase (and thus better access to the antibody), because identically treated cells harboring the His4-LacZ fusion exhibit diffuse cytoplasmic staining of an intensity that is proportional to cell volume (C). Furthermore, the punctate distribution of Fus2-lacZ contrasts sharply with that of Fus1-lacZ, which localizes in a sharp rim at the tip of projections, suggesting the two proteins do not colocalize (B).

The distribution of native Fus2 was also examined with the affinity-purified Fus2 antibodies, because the β-galactosidase segment of the Fus2-β-galactosidase fusion could interfere with proper localization of Fus2. Initial studies to detect native Fus2 with this antibody in haploid MATa/FUS2 strains were unsuccessful, despite the fact that the Fus2-lacZ protein could be readily visualized with the Fus2 antibody, even when the FUS2-lacZ gene was maintained on a centromeric plasmid. Since both FUS2-lacZ and FUS2 are expressed from identical promoters, the Fus2-lacZ fusion protein may be more stable than Fus2 (Fig. 5 A). However, we were able to detect Fus2 in diploid MATa/MATa FUS2/FUS2 cells after α factor induction and in populations of mating MATa/MATa FUS2/FUS2 and MATa/MATa FUS2/FUS2 cells (Fig. 7). Visualization was greatly enhanced when the cells contained extra copies of the FUS2 gene (on a multicopy plasmid). Fus2 distribution in these cells (Fig. 7 A, d–f) is very similar to that of Fus2-lacZ (Fig. 7 A, a–c) with one exception. The majority of native Fus2 is found at the projection tip, close to the plasma membrane, with a smaller fraction detected in the middle of the projection in occasional cells of strains harboring the FUS2 multicopy plasmid (d–f). In contrast, the Fus2-lacZ protein is more often detected in the middle of projections as well as at the tip (compare two cells in b and c). We conclude that native Fus2 associates with structures that accumulate at or near the plasma membrane of projection tips. The Fus2-lacZ protein may cause the accumulation of these structures within the neck of the projection (note the Nomarski micrograph which shows surface bumps that appear to superimpose over the Fus2-lacZ staining; Fig. 6 A, bottom two cells).

Fus2 Localizes at the Junction of Paired Cells in Zygotes That Have Not yet Undergone Nuclear Fusion

Fus2 localization was also examined in zygotes at various stages after cell fusion in short-term matings. Strikingly, Fus2 was detected at the junction of joined cells in zygotes that had undergone cell fusion but not nuclear fusion, with similar results for wild-type zygotes and zygotes harboring
zygotes with fused nuclei, either because it is intrinsically
protein at the junction of joined cells, with little effect on
The timing and localization of Fus2 are thus highly consis-
membrane around the periphery of the zygote (Trueheart
et al., 1987; Elion and Fink, data not shown). Furthermore,
pheromone-induced cells. This distribution is different
from that found with Fus1–LacZ that decorates the plasma
membrane around the periphery of the zygote (Trueheart
et al., 1987; Elion and Fink, data not shown). Furthermore,
Fus2 could not be detected in zygotes that had undergone
nuclear fusion, suggesting that the protein is degraded in
zygotes with fused nuclei, either because it is intrinsically
unstable or degraded as a consequence of nuclear fusion.
The timing and localization of Fus2 are thus highly consist-
tent with a cell fusion execution point.

**Fus2 Is Enriched in a High Speed Pellet**

The immunolocalization patterns of both Fus2 and Fus2-
lacZ suggest that Fus2 is associated with a macromolecular
structure, such as large vesicles or cytoskeleton. Indeed,
preliminary attempts to assay Fus2–lacZ activity shows it is
enriched in an insoluble fraction, since 90% of the
β-galactosidase activity was detected in the pellet derived
from a 16,000 g centrifugation of glass-bead disrupted cells (Table VII).

The proportion of native Fus2 associated with soluble
and insoluble cell fractions was determined by separating
yeast extracts by a 100,000-g centrifugation into pellet and
supernatant and analyzing each fraction by immunoblot
analysis with the Fus2 antibody. The vast majority of Fus2
is found in the pellet, indicating it is not a soluble protein
(Fig. 8). The small amount of Fus2 in the supernatant may
represent the fraction of the protein not associated with
the structures seen by indirect immunofluorescence. As a
control, the same fractions were examined for the distribu-
tion of the ribosomal protein Tcm1, also predicted to be in the
pellet because of its association with ribosomes which
sediment at 100,000 g. As predicted, all of the Tcm1 pro-
tein is in the pellet. To determine whether Fus2 is loosely
associated with the insoluble fraction, extracts were treated
with salt, nonionic detergent, denaturant, or high pH be-
fore centrifugation, conditions typically used to distinguish
membrane-associated proteins (Fig. 8, Franzusoff et al.,
1990). Fus2 was very poorly extracted from the pellet under
all the conditions used, except for limited extraction with
1% Triton X-100, suggesting it is tightly associated
with an insoluble fraction that could either be membraneous
or cytoskeletal. Tcm1 was more readily extracted with
NaCl and sodium carbonate, consistent with an association
with ribosomes.

**Discussion**

**Fus2 Localizes to a Site Consistent with a Role
in Cell Fusion**

Several lines of evidence, taken together, are consistent
with a role for Fus2 in cell fusion. First, Fus2 is expressed
only in the presence of pheromone (Fig. 2), indicating that
Fus2 carries out a function required after signal transduc-
tion. Second, Fus2 localizes at or near the site of cell fusion
in mating cells. In shmoo cells, Fus2 associates with punctate
structures that accumulate at the plasma membrane of the
projection tip (Fig. 7), the site of cell fusion. In early
zygotes, Fus2 localizes at the interface between joined part-
er cells that have undergone cell fusion but not nuclear
fusion. Third, the presence of Fus2 is specific to early zyg-
gotes and is not found in late zygotes that have already
undergone nuclear fusion. Thus, Fus2 is expressed at a time
and positioned at a site that is consistent with a role in cell
fusion that occurs before the fusion of nuclei.

**Fus2 Identifies a Novel Structure at the Shmoo Tip**

Fus2 associates with punctate structures that resemble ves-
icles in that they appear spherical (Fig. 7). Preliminary
fractionation indicates Fus2 is largely insoluble, consistent
with an association with either membranes or cytoskeleton
(Fig. 8). The structures appear to be significantly larger
than the Fus1–LacZ-associated structures that accumulate
within the cytoplasm of cells treated for 2 h with α factor
Figure 6. Distribution of Fus2-lacZ, Fus1-lacZ, and His4-lacZ in α factor–induced cells. Three representative shmoos are shown in each panel. (A) Fus2-lacZ. (B) Fus1-lacZ. (C) His4-lacZ. Panels show shmoos by DIC and lacZ fusion proteins by indirect immunofluorescence with an mAb to β-galactosidase. Cells were induced for 90 min with α factor before being fixed. Yeast strains are JY132 containing either pYEE61 (FUS2-lacZ CEN), pSB234 (FUS1-lacZ 2µ), or B929 (HIS4-lacZ 2µ).

Fus2 Is Required for Nuclear Alignment in Addition to Cell Fusion

We find that fus2 mutants are clearly defective in nuclear fusion as measured by cytoduction and plasmiduction (Table V, Fig. 3), demonstrating that Fus2 has a second function required for diploid formation that is distinct from Fus1. The nuclear fusion defect may be due to the fact that fus2 zygotes improperly align their parental nuclei before nuclear fusion (as shown by nonaligned microtubules emanating from the spindle pole bodies of the parental nuclei; Fig. 4, Table VI), rather than a defect in fusion of the nuclear envelopes. fus2Δ thus defines a novel class of inefficient maters that shares features of both nuclear congression defective kar mutants (Kurihara et al., 1994) and cell fusion defective fus mutants (Trueheart et al., 1987; McCaffrey et al., 1987). Our results suggest the intriguing possibility that Fus2 operates at a step that intersects cell and nuclear fusion, events previously thought to be coordinated. Such coordination might involve attachment of the microtubules along contact points at the projection tip to ensure proper nuclear migration before or during cell fusion, and is consistent with the site of Fus2 localization. Fus2 could either be physically involved in microtubule alignment or catalyze a cell fusion step that must first take place in order for nuclear alignment to occur. We note that spa2 mutants that are severely defective in projection formation also exhibit a modest defect in nuclear fusion as measured by cytoduction (Gehrung and Snyder, 1992), suggesting that this phenotype may be shared by mutations affecting polarization at the projection tip.

FUS1 and FUS2 Reveal Different Cell Fusion Functions

Three lines of genetic evidence previously suggested that FUS1 and FUS2 are functionally redundant: (1) overexpression of FUS2 partially suppresses a fus1 mutant and (and are presumably secretory vesicles, Trueheart and Fink, 1989), suggesting they are distinct (Elion, E. A., and G. R. Fink, data not shown). Observation of Fus2 at different time points after α factor induction, suggests that these structures are distributed asymmetrically in projectionless cells, as well as cells that have formed a projection. Thus, they may identify a structure that either migrates to the projection tip or marks the point at which projection formation occurs. Ste6, the α factor transporter localizes in large structures resembling vesicles at the plasma membrane of the projection tip (Kuchler et al., 1993; Kolling and Hollenberg, 1994), suggesting a possible compartment for Fus2. However, these vesicles are found throughout the cell and are present constitutively. Furthermore, the relatively poor extraction of Fus2 with 1% Triton X-100 may point more to an association with a cytoskeletal element. Spa2 and Bem1, cell polarity determinants known to affect cytoskeletal structure and to be required for projection formation and mating (Gehrung and Snyder, 1990; Chenevert et al., 1992, 1994) also reside at the projection tip and could colocalize with Fus2 or be required for its localization. Interestingly, cells expressing the Fus2–lacZ fusion protein (which appears to be more stable than Fus2) often have somewhat more enlarged and elongated projections compared with wild-type cells (Figs. 6 and 7), raising the possibility that Fus2 and/or its associated structure affects projection formation. It will be of interest to determine whether Fus2 associates with a novel vesicle or cytoskeletal structure that plays a specific role in cell fusion.
Table VII. FUS2-β-galactosidase Activity by Chloroform or Glass Bead Disruption Method

| LacZ fusion  | Uninduced supernatant | Pellet | Induced supernatant | Pellet |
|--------------|------------------------|--------|---------------------|--------|
| FUS2-LacZ    | 0.49                   | 4.8    | 16.4                | 177.6  |
| FUS1-LacZ    | 0.24                   | 1.4    | 14.0                | 218.5  |
| HIS4-LacZ    | 43.8                   | 73.3   | 46.5                | 108.2  |

*MATa cells (strain JY425) harboring LacZ-fusion genes grown to an A600 of 0.4-0.6 were induced with 5 μM a factor for 90 min before being assayed as described in Materials and Methods. Uninduced cells were treated identically by the addition of an equal volume of methanol. Units of activity are determined as described in Materials and Methods.

Figure 7. Distribution of Fus2 in shmoos and zygotes from a mating mixture. (A) Representative shmoos of cells containing either Fus2-LacZ or Fus2 on a multicopy plasmid. Panels show Fus2-LacZ and Fus2 by indirect immunofluorescence using affinity-purified antisera to Fus2 (see Materials and Methods). Cells are recovered from a 2-h mating between MATa/MATa (EY310) and MATa/MATa (EY312) cells containing either pYEE61 (FUS2-LacZ CEN) or pSB257 (FUS2 2μ). a-c: Shmoos with Fus2-LacZ. d-f: Shmoos with excess Fus2. (B) Representative tetraploid zygotes. Panels show a zygote containing wild-type levels of Fus2 (a-b) and a zygote containing excess Fus2 (c-d). a and c show indirect immunofluorescence with affinity-purified Fus2 antisera, and b and c show the corresponding nuclei by DAPI. Cells are recovered from a 2-h mating between EY310 and EY312 with or without pSB257 in both parents.

vice versa, (2) fus1 and fus2 mutant zygotes are morphologically similar, and (3) a fus1fus2 double mutant is >1,000-fold more defective in mating than either single mutant (Trueheart et al., 1987). We show here that FUS1 and FUS2 most likely perform qualitatively different cell fusion functions and define distinct components of the cell fusion pathway. First, the predicted Fus2 protein bears no sequence similarity to the Fus1 protein. Second, as summarized in Table VIII, fus1 and fus2 null mutants have nonidentical phenotypes. fus2 mutants are defective in nuclear fusion and alignment of microtubules as well as more sensitive to polymyxin b, a compound that affects agglutination and membrane permeability. In contrast, fus1 mutants undergo normal nuclear fusion and are hypersensitive to low temperature and depletion of calcium during mating. Third, Fus1 and Fus2 appear to have different sites of localization at the shmoo tip, and neither protein is required for the other's localization (Elion, E. A., and G. R. Fink, data not shown).

Three different models can be compared as explanations for the functional similarity between FUS1 and FUS2 with respect to cell fusion. In the first model, FUS1 and FUS2 carry out the same step in cell fusion, and the different phenotypes reflect different properties of the Fus1 and Fus2 proteins. For example, Fus2 may be more calcium- and cold-sensitive than Fus1, whereas Fus1 may be more sensitive to agents that affect agglutination and/or membrane permeability than Fus2. However, this model does not explain the different localization of the two proteins and the fact that FUS1 is not required for nuclear fusion. In the second model, FUS1 and FUS2 function at different steps in a single cell fusion pathway. For example, FUS1 could define an earlier step (consistent with Fus1 localization across the cell membrane and the cell surface), whereas FUS2 defines a later step that is more dependent upon prior cell attachment and intersects with events required for nuclear fusion (consistent with the apparent localization of Fus2 inside the cell). However, if both genes are in the same pathway it is difficult to explain the finding that a fus1 fus2 mutant is far more defective in cell fusion than either single mutant. In the third model, FUS1 and FUS2 function in parallel cell fusion pathways, with FUS2 performing a second function required for nuclear fusion. This explanation best explains the phenotypes of single and double fus mutants, the different localization of Fus1 and Fus2, and the distinctive nuclear alignment defect of
**What is the Function of Fus2?**

The phenotypes of fus2 mutants coupled with the cytophilic localization of Fus2 protein argue strongly that Fus2 promotes some aspect of fusion at the projection tip and may have a direct physical role in cell fusion and karyogamy. One possibility is that Fus2 is associated with specialized vesicles that fuse with the plasma membrane to effect cell fusion and perhaps also coordinate nuclear fusion. For example, this type of vesicle might be analogous to the large exocytotic vesicles of chromaffin and neural cells or the acrosomal vesicles that fuse with sperm plasma membrane during the acrosome reaction of fertilization (Yanagimachi, 1988). Such specialized vesicles could either deliver enzymes that promote cell fusion or remove cell wall material to allow plasma membrane fusion. As yet there is no biochemical evidence for regulated secretory vesicles in yeast (Prior et al., 1992), although the subcellular distribution of Ste6 suggests the existence of a nonclassical vesicular pathway to the shmoop tip (Kolling and Hollingberg, 1994). A second possibility is that Fus2 is part of a cytoskeletal (or other) structural component that is required for both cell fusion and nuclear fusion, and is consistent with the predicted coiled coil nature of Fus2, its weak sequence homology to myosin-related proteins. Such a structure, assembled at the site of cell fusion at the shmoop tip, might organize the fusion machinery, prevent cytoplasmic leakage, and aid in the proper alignment of extranuclear microtubules required for an ensuing nuclear fusion event. Both interpretations posit that Fus2 interacts, directly or indirectly, with proteins required for nuclear fusion and projection formation. The identification of the proteins associated with Fus2 will help distinguish between alternative explanations of Fus2 function.

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**References**

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

Barney, M. 1993. Secrets of secretion revealed. Science (Wash. DC.) 260:487–489.

Berlin, V., J. A. Brill, J. Trueheart, J. D. Boeke, and G. R. Fink. 1991. Genetic screens and selections for cell and nuclear fusion mutants. Methods Enzymol. 194:774–792.

Bigrigg, M. D., T. J. Gibson, and G. F. Hong. 1983. Buffer gradient gels and S100 as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA. 80:3963–3965.

Biers, R., S. F. Philips, M. D. Cortez, R. Roberts, C. T. Caskey, and J. S. Chambers. 1992. Human and mouse dystrophin mRNA transcripts are differentially expressed during skeletal muscle, heart, and brain development. Nucleic Acids Res. 20:1725–1731.

Blobel, C., P. T. Wollsberg, C. W. Turck, D. G. Myles, P. Primakoff, and J. M. White. 1992. A potential fusion peptide and an integrin ligand domain in a protein active in sperm–egg fusion. Nature (Lond.). 356:248–252.

Boguslawski, G. 1985. Effects of polymyxin B sulfate and olyxmycin B nonapeptide on growth and permeability of the yeast Saccharomyces cerevisiae. Mol. Cell. Genet. 199:401–405.

Boguslawski, G. 1986. Polymyxin B nonapeptide inhibits mating in Saccharomyces cerevisiae. Antimicrob. Agents Chemother. 29:330–332.

Bolvazar, F., R. L. Rodriguez, T. J. Greene, M. C. Betlach, H. L. Heymeker, and H. W. Boyer. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene (Amst.). 295–113.

Brench, C., G. Müller, and R. Egel. 1998. Genes involved in meiosis and sporulation of a yeast. Mol. & Gen. Genet. 102:301–306.

Burnette, W. N. 1981. Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulfate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-labeled protein. Anal. Biochem. 121:195–203.

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

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

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

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

Choi, K.-Y., B. Satterberg, D. Lyons, and E. A. Elion. 1994. Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in S. cerevisiae. Cell. 78:499–512.

Conde, J., and G. R. Fink. 1976. A mutant of Saccharomyces cerevisiae defective for nuclear fusion. Proc. Natl. Acad. Sci. USA. 73:3651–3655.

Craven, G. R., E. J. Steers, J. L. Bethune, and C. B. Anfinsen. 1965. Purification, composition, and molecular weight of the β-galactosidase of Escherichia coli. J. Biol. Chem. 240:248–247.

Creutz, C. 1992. The annexins and exocytosis. Science (Wash. DC.). 258:924–930.

Cross, F., L. H. Hartwell, C. Jackson, and J. B. Koopeka. 1968. Conjugation in Saccharomyces cerevisiae. Annu. Rev. Cell Biol. 4:429–457.

Curran, B. P. G., and B. A. Carter. 1986. α-factor enhancement of hybrid formation by protoplast fusion in Saccharomyces cerevisiae II. Curr. Genet. 10:943–945.

Dolan, J. W., C. Kirkman, and S. Fields. 1988. The yeast STE12 protein binds to the DNA sequence mediating pheromone induction. Proc. Natl. Acad. Sci. USA. 86:5703–5707.

Dubin, S. K. 1981. Interspecies transfer of genetic information in the yeast KARI heterozygotes of Saccharomyces cerevisiae. Mol. Cell. Biol. 1:245–253.

Eisenberg, D., E. Schwarz, M. Konaromy, and R. Wall. 1984. Analysis of mem-
brane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol. 179:125–142.

Ellon, E. A. J., and J. R. Warner. 1984. The major promoter element of rRNA transcription lies 2 kb upstream. Mol. Cell. Biol. 4:2690–2699.

Ellon, E. A., P. L. Grisafi, and G. R. Fink. 1990. Fus3 encodes a P22DC28-related kinase required for the transition from mitosis into conjugation. Cell. 60:649–664.

Ellon, E. A., B. Satterberg, and J. A. Kranz. 1993. Fus3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAK1. Mol. Biol. Cell. 4:495–510.

Errede, B., and G. Ammerer. 1989. STE12, a protein involved in cell-type specific transcription and signal transduction in yeast is part of protein-DNA complexes. Genes & Dev. 3:1349–1361.

Field, C., and R. Schekman. 1980. Localized secretion of acid phosphatase reflects the pattern of cell surface growth in Saccharomyces cerevisiae. J. Cell Biol. 86:123–128.

Franzusoff, A., J. Rothblatt, and R. Schekman. 1991. Analysis of polypeptide transit through the secretory pathway. Methods Enzymol. 194:662–674.

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

Hasek, J., I. Rupe, J. Svobodova, and E. Streiblova. 1987. Tubulin and actin topology during zygote formation of Saccharomyces cerevisiae. J. Gen. Microbiol. 133:3355–3363.

Ito, H., Y. Fukada, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells with alkali cations. J. Bacteriol. 153:163–168.

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

Jackson, C. L., and L. H. Hartwell. 1990b. Courthip in Saccharomyces cerevisiae: an early cell-cell interaction during mating. Mol. Cell. Biol. 10:2203–2213.

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

Kaiser, C. A., D. Preuss, P. Grisafi, and D. Botstein. 1987. Many random sequences functionally replace the secretion signal sequence of yeast invertase. J. Cell Biol. 105:2203–2213.

Kurihara, L. J., C. T. Beh, M. Latterich, R. Schekman, and M. D. Rose. 1994. The karyogamy gene Brahe and surface protein sequences with the hydrophobic moment plot. J. Biol. 101:211–227.

Kurth, M., G. R. Fink, and J. Thorner. 1984. The Molecular and Cellular Biology of the Yeast Saccharomyces. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 657–744.

Kocher, D., E. A. Housholder, and R. Schekman. 1990. Nuclear congression and membrane fusion: two distinct events in the yeast mating process. Curr. Biol. 3:1349–1361.

van Solingen, P., and J. B. van der Plaat. 1977. Fusion of yeast spheroplasts. Z. Naturforsch. 32c:13042–13047.

McCaffrey, G., F. J. Clay, K. Kelsay, and G. F. Sprague. 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. 7:2690–2699.

Messing, J. 1982. Construction of improved m13 vectors using oligonucleotide-directed mutagenesis. In Genetic Engineering: Principles and Methods, Vol. 4. J. K. Sollow and A. Hollander, editors. Plenum Publishing Corp., New York. 19–34.

Myers, A. M., A. Tzagoloff, D. M. Kinnery, and C. J. Lusty. 1982. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of lacZ fusions. Gene (Amst.) 14:259–310.

Osaka, A., J. Gibson, I. Gregor, and G. Schatz. 1982. J. Biol. Chem. 262:13042–13047.

Pontececchi, G. 1976. Polyethylene glycol (PEG) in the production of mammalian somatic cell hybrids. Cytogenet. Cell Genet. 16:399–400.

Pringle, J. R., A. E. M. Adams, D. G. Drubin, and B. K. Haarer. 1991. Immunofluorescence methods for yeast. Methods Enzymol. 194:565–601.

Przyer, N. K., L. J. Wuestebuch, and R. Schekman. 1992. Vesicle-mediated protein sorting. Annu. Rev. Biochem. 61:471–516.

Rose, M. D. 1991. Nuclear fusion in yeast. Annu. Rev. Microbiol. 45:539–567.

Rothstein, R. 1983. One-step gene disruption in yeast. Methods Enzymol. 101:201–211.

Sanger, F., S. Nicklen, and A. R. Coulston. 1977. DNA sequencing with chain termination inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463–5467.

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

Sherman, F., G. R. Fink, and J. B. Hicks. 1986. Methods in Yeast Genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 120 pp.

Smith, D. E., and P. A. Fisher. 1984. Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in Drosophila embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J. Cell Biol. 99:20–28.

Snell, W. J. 1990. Adhesion and signalling during fertilization in multicellular and unicellular organisms. Curr. Opin. Cell Biol. 2:821–832.

Sprague, G. F., Jr., and J. W. Thorner. 1994. Pheromone response and signal transduction during the mating process of Saccharomyces cerevisiae. In The Molecular and Cellular Biology of the Yeast Saccharomyces. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 657–744.

Steinmann, H. 1988. Protein-mediated membrane fusion. Annu. Rev. Biophys. Biophys. Chem. 18:187–211.

Tanaka, Y. S., and L. M. MacKay. 1979. Sexual conjugation in yeast. J. Cell Biol. 100:326–333.

Trueheart, J. 1988. Molecular and genetic analysis of two genes required for yeast cell fusion. Ph.D. thesis. Massachusetts Institute of Technology, Cambridge, MA. 175 pp.

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

Trueheart, J., and G. R. Fink. 1989. The yeast cell fusion protein Fus1 is O-glycosylated and spans the plasma membrane. Proc. Natl. Acad. Sci. USA. 86:9916–9920.

van Solingen, P., and J. B. van der Plaat. 1977. Fusion of yeast spheroplasts. J. Bacteriol. 130:946–947.

Viera, J., and J. Messing. 1987. Production of single-stranded DNA. Methods Enzymol. 153:3–11.

White, J. M. 1992. Membrane fusion. Science (Wash. DC). 258:917–924.

White, J. M. 1992. Membrane fusion from liposomes to biological membranes. TINS (Trends Biochem. Sci.). 9:479–483.

Winston, F. C. 1983. Pheromone and mating to mutant genes in yeast. Methods Enzymol. 101:211–227.

Wolfsberg, T. G., F. J. Bazan, C. P. Blobel, D. G. Myles, P. Primakoff, and J. M. White. 1993. The precursor region of a protein active in sperm–egg fusion contains a metalloprotease and a disintegrin domain: structural, functional and evolutionary implications. Proc. Natl. Acad. Sci. USA. 90:10783–10787.

Yanagimachi, R. 1988. Sperm-egg fusion. Cell. 54:557–565.

Yen, R., T. J. Li, B. T. Schaar, I. Szilak, and D. W. Cleveland. 1992. CENP-E is a putative kinetochore motor that accumulates just before mitosis. Nature (Lon.). 359:536–539.