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Identification and Regulation of a Gene Required for Cell Fusion during Mating of the Yeast *Saccharomyces cerevisiae*

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We have devised a screen for genes from the yeast *Saccharomyces cerevisiae* whose expression is affected by cell type or by the mating pheromones. From this screen we identified a gene, *FUS1*, whose pattern of expression revealed interesting regulatory strategies and whose product was required for efficient cell fusion during mating. Transcription of *FUS1* occurred only in a and α cells, not in a/α cells, where it was repressed by a1 · α2, a regulatory activity present uniquely in a/α cells. Transcription of *FUS1* showed an absolute requirement for the products of five *STE* genes, *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*. Since the activators *STE4*, *STE5*, and *STE12* are themselves repressed by a1 · α2, the failure to express *FUS1* in a/α cells is probably the result of a cascade of regulatory activities; repression of the activators by a1 · α2 in turn precludes transcription of *FUS1*. In addition to regulation of *FUS1* by cell type, transcription from the locus increased 10-fold or more when a or α cells were exposed to the opposing mating pheromone. To investigate the function of the Fus1 protein, we created *fus1* null mutants. In *fus1* × *fus1* matings, the cells of a mating pair adhered tightly and appeared to form zygotes. However, the zygotes were abnormal. Within the conjugation bridge they contained a partition that prevented nuclear fusion and mixing of organelles. The predicted sequence of the Fus1 protein (deduced from the *FUS1* DNA sequence) and subcellular fractionation studies with Fus1-β-galactosidase hybrid proteins suggest that Fus1 is a membrane or secreted protein. Thus, Fus1 may be located at a position within the cell where it is poised to catalyze cell wall or plasma membrane fusion.

The yeast *Saccharomyces cerevisiae* exhibits three distinct cellular phenotypes: **a**, α, and **a**/α. The **a** and α cell types, which are typically haploid, can mate to yield the third cell type, an **a**/α diploid. These **a**/α diploids are not capable of mating but can be induced to undergo meiosis and sporulation, thereby regenerating haploid **a** and α cells (reviewed in references 18, 19, and 41). To mate efficiently, **a** and α cells must each secrete a specific peptide pheromone to which only the other cell type can respond. Thus, α cells secrete a 13-amino-acid-residue peptide, α factor, which binds to a specific receptor on the surface of **a** cells and triggers a physiological response in those cells (20, 21). Similarly, α cells display a surface receptor that enables them to respond to the **a**-factor pheromone secreted by **a** cells (3, 15, 31). The response of **a** and α cells to the pheromone of the other is similar and includes an increase in the transcription of a small set of genes (16, 17, 43), an increase in the ability to agglutinate with the other cell type (4, 13), and arrest of the cell division cycle in the G1 phase (8, 48). A mating pair whose cell cycles have been synchronized in this fashion can undergo controlled cell wall dissolution and membrane fusion to yield an **a**/α zygote.

Despite the complexity of the cellular phenotypes outlined above, the three cell types differ genotypically at a single locus, the mating type locus. Cells of the **a** type contain *MAT***a**, whereas cells of the α type contain *MAT*α. Diploid **a**/α cells contain both alleles. The mating type locus is a regulatory locus that controls the expression of unlinked genes required for mating and sporulation. This idea was originally proposed by MacKay and Manney (25) to explain the isolation of mating-deficient mutants that owed their phenotype to mutations in genes (designated *STE*) unlinked to *MAT*. As expected from the physiology of mating, mutations in some *STE* genes conferred a mating-deficient phenotype that was limited to one cell type (**a**- or α-specific *STE* genes). However, genes were also identified that were required for mating by both **a** and α cells (nonspecific *STE* genes). Even though mating clearly involves more than pheromone production and response, the *STE* genes identified thus far (14 total) are required only for this facet of mating; none has been found that is deficient for other aspects of mating, such as cell fusion.

From genetic (44) and biochemical analyses of mutations at the *MAT* locus, we know that the phenotypic difference between **a** and α cells is the consequence of the action of two regulatory proteins encoded by *MAT*α, referred to as α1 and α2. The regulator α1 activates transcription of α cell-specific genes (12, 14, 42; R. Jensen, K. Wilson, and I. Herskowitz, personal communication), whereas the regulator α2 represses transcription of **a** cell-specific genes (6, 14, 17; S. Michaelis and I. Herskowitz, personal communication). Since **a** cells lack both of the *MAT*α-encoded regulators, transcription of α-specific genes is prevented (no α1 is present) and transcription of **a**-specific genes is allowed (no α2 is present).

The regulatory circuitry in **a**/α cells is complex, but the net result is that many genes required for mating are not transcribed, whereas a new set of genes that confers the ability to sporulate is transcribed (23, 30, 34). This pattern of gene expression is achieved by the inclusion of a third regulatory

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protein, the a1 product of *MAT***a**, that acts in conjunction with α2. In particular, the combined action of a1 and α2 (referred to as a1 · α2) blocks transcription of the *MATα1* gene (24, 33, 39). Because this regulatory activity is coupled with repression of **a**-specific genes by α2 (or perhaps a1 · α2), neither α-specific nor **a**-specific genes are expressed in **a**/α cells. In addition, a1 · α2 blocks transcription of a set of genes that is transcribed in both haploid cell types. This set of haploid-specific genes includes several nonspecific *STE* genes (*STE4* [V. MacKay, personal communication], *STE5* [28; V. MacKay and J. Thorner, personal communication], and *STE12* [S. Fields and I. Herskowitz, personal communication]). Not all nonspecific *STE* genes are subject to this regulation, however, since *STE7* and *STE11* are transcribed in **a**/α cells (11).

The *MAT*-encoded regulators are not the only effectors of expression of the genes required for mating. Typically these genes are subject to one or two additional regulatory inputs. For example, transcription of the *STE3* gene, which encodes the **a**-factor receptor (3, 15, 31), not only requires the α1 product (42), but in addition is increased fivefold when α cells are exposed to **a** factor (16). Furthermore, the products of five nonspecific *STE* genes (*STE4*, *STE5*, *STE7*, *STE11*, and *STE12*) are required for efficient transcription: *STE3* mRNA levels are reduced 5- to 10-fold in cells carrying mutations at any of these genes (G. Sprague, K. Clark, S. Fields, and D. Chaleff, unpublished observations). This array of regulatory inputs complicates the investigation of the mechanism by which any one regulator affects transcription. We have therefore sought to identify other genes regulated by *MAT* with the expectation that some of these genes would be sensitive to a subset of these regulators and thus useful for dissecting the mechanism by which each regulator activates transcription. Moreover, we imagined that these newly identified genes might reveal steps in the mating process that had not been delineated by the study of existing *STE* genes.

In this paper we report the identification of a gene, *FUS1*, that is transcribed in **a** and α cells but not in **a**/α cells. The mechanisms that govern this pattern of regulation are investigated. We further show that *FUS1* is required for cell fusion during mating. Using an independent approach, Trueheart et al. (47) also recently identified the *FUS1* gene.

MATERIALS AND METHODS

Strains, plasmids, and media. Relevant strains are listed in Table 1. Strains YY751, YY752, YY754, and YY755 were constructed by the one-step gene replacement method of Rothstein (36). In these constructions the *URA3* gene substitutes for 1,390 base pairs (bp) of *FUS1*, including the *FUS1* promoter region and most of its coding sequence (the first 375 of 512 total *FUS1* codons are removed). The replacements were confirmed by Southern blot analysis (data not shown).

The media used were YEPD (same as YPD [38]), SD (38), SD-URA and SD-LEU (SD supplemented with adenine, L-histidine, L-arginine, L-methionine, L-tyrosine, L-tryptophan, L-phenylalanine, L-threonine, and L-uracil or L-leucine, respectively [38]), SD-ARG-CAN (similar to SD-URA and SD-LEU except lacking L-arginine and containing canavanine, 50 mg/liter), X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) indicator medium (35), and BBMB (40).

The plasmids used were pMC1585 (10), YEp13 (7), pJDB207 (2), pSL330 (15), and pSL555. Plasmid pSL555 was constructed by inserting the 2.2-kilobase-pair (kbp) *Hin*dIII-*Bam*HI fragment from pSL307 into pSL24 (15), a *LEU2* 2μm derivative of pJDB207 that contains *lacZ* sequences derived from pMC1403 (9).

Screen for cell type-regulated control sequences. A plasmid library that contains segments of yeast DNA adjacent to the *Escherichia coli lacZ* gene was provided by Stan Fields. The library, which is maintained in *E. coli*, was constructed by inserting short fragments, 0.2 to 2.2 kbp, of genomic yeast DNA from strain AB320, cut partially with *Sau*3AI, into the *Bam*HI site of pMC1585 (10), a 2μm *URA3* shuttle vector that contains a truncated form of *lacZ* (the first eight codons are missing). Plasmid DNA was isolated from the *E. coli* library and used to transform α strain SY816 to uracil prototrophy (Ura^+). Ura^+ colonies were plated onto X-gal plates, and blue colonies were removed with toothpicks to master plates. To form **a**/α cells whose β-galactosidase

TABLE 1. Yeast strains

Strain	Genotype	Source
DC5	*MAT***a** *leu2-3 leu-112 his3 can1 gal2*	J. Strathern
SY816	*MATα leu2-3 leu-112 his3 his4 trp1 ura3-52*	R. Jensen
HR125-5d	*MAT***a**; isogenic to SY816	R. Jensen
SY817	*MAT***a**/*MATα*; isogenic to SY816	R. Jensen
DC41	*MATα leu2 ura3 trp1 can his4-519*	D. Chaleff
246-1-1	*MATα leu2 ura3 trp1 can1 his4-519* Gal^-	K. Tatchell
EG123	*MAT***a**; isogenic to 246-1-1	K. Tatchell
1788	*MAT***a**/*MATα*; isogenic to 246-1-1	K. Tatchell
YY743	*ste4*::*LEU2*; isogenic to DC41	K. Clark
YY644	*ste5*::*LEU2*; isogenic to DC41	K. Clark
DC130	*ste7*; isogenic to DC41	D. Chaleff
DC39	*ste11*; isogenic to DC41	D. Chaleff
YY544	*ste12*::*LEU2*; isogenic to 246-1-1	S. Fields
XP8-4a	*MAT***a** *leu1 trp5 his6 met1*	P. Kushner
XP8-18b	*MATα*; other markers as in XP8-4a	P. Kushner
XP11	*MAT***a**/*MATα* (XP8-4a × XP8-18b)	P. Kushner
VC2	*matα1-2 ade6*; other markers as in XP8-4a	V. MacKay
VC73	*matα2-1 ade6*; other markers as in XP8-4a	V. MacKay
XT1172-S245c	*MATα ade6*; other markers as in XP8-4a	V. MacKay
320	*MAT***a** *ura3 ade2 leu1 can1-11 cyh2-1 rme1-1*	Y. Kassir
17-15	*mat***a***1-1*; other markers as in 320	Y. Kassir
MC18A	*mat***a***1-1*/*MATα* (17-15 × XT1172-S245c)	This work
XR28-29c	*MAT***a** *cryl-3 ade6 arg4 aro7*	J. Rine
SY235	*matα1-2*/*MAT***a** (XR28-29c × VC2)	Laboratory strain
SY229	*matα2-1*/*MAT***a** (XR28-29c × VC73)	Laboratory strain
YY754	*MAT***a** *fus1*::*URA3*; isogenic to 320	This work
YY752	*MAT***a** *fus1*::*URA3*; isogenic to HR125-5d	This work
YY751	*MATα fus1*::*URA3*; isogenic to SY816	This work

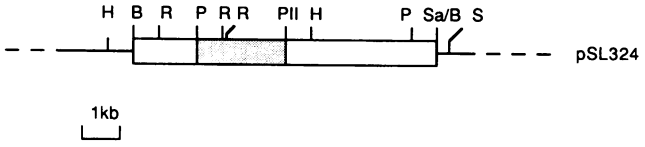

FIG. 1. Restriction map of the DNA segment containing the entire *FUS1* gene. Positions of *Hin*dIII (H), *Bam*HI (B), *Sal*I (S), *Pst*I (P), *Eco*RI (R), and *Pvu*II (PII) restriction endonuclease sites are drawn to physical scale. Sa/B is a hybrid *Bam*HI-*Sau*3A site at the end of the cloned fragment. The open bar represents the cloned DNA segment; lines indicate vector DNA. The *Sau*3AI site which is fused to the *lacZ* gene in pSL307 is located between the double *Eco*RI sites within the shaded region. The sequence of the shaded region is shown in Fig. 6.

phenotype could be assessed, the master plates were replica plated to SD plates to which $\sim 10^7$ **a** cells (strain MC-3a) suspended in YEPD broth had been applied. The **a** and α strains have complementary nutritional defects, and hence only **a**/α diploids formed by mating can grow on this SD plate. The **a**/α diploids were then replica plated to X-gal indicator plates, and the production of β-galactosidase by α cells and **a**/α cells bearing the same plasmid was compared.

Screen for a-factor-affected control sequences. The master plates containing the β-galactosidase-producing α transformants were replica plated to X-gal plates which had been spread with approximately 5×10^6 **a** cells (strain MC8-3a) in 0.2 ml of YEPD broth. The *MAT***a** cells provide a source of **a** factor, but are not able to grow on these plates once the YEPD is depleted.

Isolation of the entire *FUS1* gene. A clone bank in vector YEp13 of yeast DNA fragments generated by partial digestion with *Sau*3AI (average fragment size, 10 kbp) was provided by K. Nasymth (32). *E. coli* cells harboring the clone bank were screened by colony hybridization (26) for plasmids that contained the *FUS1* gene by using the 1.4-kbp *Eco*RI fragment from pSL307 as a probe. Three positive clones were isolated that contained overlapping restriction fragments and that were also homologous to the yeast sequences in pSL307. One of them, pSL324, is shown in Fig. 1.

Response and production of pheromones. The percentage of unbudded cells and morphologically altered cells (shmoos) present in a culture before and after exposure to **a** factor or α factor was determined by direct observation with phase-contrast microscopy as described previously (16). The response of wild-type and *fus1* mutant strains was compared for two different pheromone concentrations. At each concentration the percentage of unbudded cells and shmoos was equivalent for wild-type and *fus1* mutants. Pheromone production was measured by plate assay as described (40).

The **a**-factor preparation was simply the culture filtrate from a saturated culture of **a** cells (16). α Factor (Sigma Chemical Co.) was used at a concentration of 4.4×10^{-7} M. This concentration of α-factor consistently resulted in >75% unbudded **a** cells after 2 h of incubation. Likewise, the crude **a**-factor preparation consistently resulted in >75% unbudded α cells after 2 h of incubation. Moreover, the **a**-factor preparation caused induction of *FUS1* in α cells to the same level seen in **a** cells following treatment with 4.4×10^{-7} M α factor. In experiments in which the pheromone concentration was varied, the α-factor concentration was either 4.4×10^{-8} or 4.4×10^{-7} M and the **a**-factor concentration was either full-strength culture filtrate or 1/10-strength culture filtrate.

Mating tests. Cell-cell matings were done on YEPD medium by placing two cells of opposing mating types adjacent to each other by micromanipulation. Pairs that formed zygotes were moved to another part of the plate and allowed to form a colony. Colonies were replica plated to the appropriate plates to determine the mating and other phenotypes of the cells in the colony.

The quantitative efficiency of mating tests was determined as follows. From 10^6 to 10^7 cells (lawn) were mixed with 1,000 to 2,000 cells of the opposite mating type and immediately spread on plates that only allowed growth of diploid cells. The cells were mixed in either rich broth (YEPD) or diluted broth (1/10 YEPD in H_2O). No mating of wild-type cells was observed when the cells were plated only in H_2O. The efficiency of mating was calculated as the ratio of the titer of cells able to mate (form prototrophs) to the titer of cells whose mating efficiency was being measured (assayed on YEPD medium).

Transcript analysis. RNA was isolated from plasmid-free cells as described (42). Total RNA or poly(A)-enriched [poly(A)$^+$] RNA (prepared with Hybond mRNA affinity paper; Amersham Corp.) was fractionated by electrophoresis in a 1.5% agarose gel and transferred to nitrocellulose paper (46). The filters were hybridized at 54°C, using as probe ^{32}P-labeled RNAs generated in an SP6 polymerase/promoter system. The RNA-RNA hybrids were visualized by autoradiography.

DNA sequence analysis. DNA sequencing was performed by the dideoxy method of Sanger et al. (37) with phage M13 clones and a commercial synthetic oligonucleotide primer. Both strands were sequenced, and all sites used for cloning were overlapped by other cloned segments. Regions that showed compression were resequenced with 7′-deaza-dGTP (Boehringer Mannheim) in the reaction mixtures (1).

Cell fractionation and β-galactosidase assays. β-Galactosidase assays were performed on permeabilized plasmid-bearing cells as described (16). The localization of hybrid β-galactosidase molecules to the aqueous or detergent phase in Triton X-114 extracts (5) was determined as follows. Exponential cells harboring plasmids were harvested by centrifugation. Cells were washed with 0.15 M NaCl, centrifuged, and suspended at a density of about 10^9 cells per ml in lyticase buffer (50 mM potassium phosphate, pH 7.4, 10 mM 2-mercaptoethanol, 1 M sorbitol). Samples were withdrawn to create permeabilized cells and for turbidity measurements. The remaining cells were converted to spheroplasts by treatment with lyticase (provided by T. Stevens). The spheroplasts were washed by centrifugation and lysed by suspension on ice in TX buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-114). Samples were removed for assays (lysed-cell fraction), and the remainder was centrifuged in an Eppendorf Microfuge for 10 min at 4°C. The pellet from this step consisted of cell wall material and organelles and contained <4% of the β-galactosidase activity. The supernatant fraction, which contained soluble enzymes and solubilized membrane proteins in detergent micelles, was layered on a 6% sucrose plug (10 mM Tris, pH 7.4, 150 mM NaCl, 0.06% Triton X-114, 6% sucrose) and incubated at 30°C for 5 min. The supernatant fraction was then separated into aqueous and detergent phases by centrifugation at 450 × *g* for 5 min. The aqueous phase was reextracted by adding fresh Triton X-114, followed by centrifugation on the same sucrose plug as above. The composition of the aqueous and detergent phases was made comparable by adding appropriate amounts of H_2O, salt, and detergent to each phase. The four samples (permeabilized

cells, lysed cells, aqueous phase, and detergent phase) were assayed for β-galactosidase activity as described (16).

RESULTS

Isolation of the control region of the *FUS1* gene. To identify genes that are regulated by the alleles of *MAT* or by the mating pheromones, we screened a plasmid library in which segments of yeast DNA had been joined to a truncated *lacZ* gene that lacked signals required for transcription and translation initiation. Since synthesis of hybrid β-galactosidase from these fusion plasmids requires that these signals be provided by the yeast DNA fragment, regulation of β-galactosidase synthesis is expected to reflect regulation of the intact gene from which the segment is derived. For example, if the yeast sequences on a specific plasmid contain transcription and translation signals from a gene that is expressed in **a** and α cells but not in **a**/α diploid cells, then α strains bearing this plasmid will produce blue clonies on X-gal indicator plates. However, when these α colonies are mated with **a** cells, the resulting **a**/α diploids will be white on indicator plates. In principle, plasmids from which β-galactosidase production is either haploid specific, as in the example above, or α specific will be revealed by this test. Similarly, if a plasmid contains control sequences from a gene whose transcription is increased (induced) by **a**-factor treatment, then cells bearing this plasmid will be a darker blue on indicator plates that contain **a** factor than on indicator plates lacking **a** factor.

To screen for control sequences of genes expressed in α cells but not in **a**/α diploid cells, α cells were transformed with the plasmid library, and transformants containing functional hybrid genes were identified as blue colonies on X-gal indicator plates. These β-galactosidase-producing α transformants were picked to master plates and replicated to two types of plates—one that selected for **a**/α diploids and another that contained **a** factor (see Materials and Methods). β-Galactosidase production in α cells, **a**/α cells, and α cells exposed to **a** factor was then assessed on indicator plates. We screened 368,000 transformants by this method and identified more than 50 transformants that appeared to produce β-galactosidase as α cells but not when mated to create **a**/α cells. The plasmids were isolated from these 50 α transformants, amplified in *E. coli*, and reintroduced into isogenic α, **a**, and **a**/α strains. Quantitative *o*-nitrophenyl-β-D-galactopyranoside assays with permeabilized cells (liquid assays) showed that only two of these plasmids directed the synthesis of β-galactosidase in a manner that was dependent on cell type (see below). This discrepancy between the plate assay and the liquid assay may be due to permeability differences of cells on plates that are eliminated in liquid assays (27). The plate screen did not reveal any transformants whose β-galactosidase production was regulated by **a** factor, even though one of the plasmids that conferred cell type-specific production of β-galactosidase also yielded increased β-galactosidase activity (induction) when α cells were treated with **a** factor (see below). Thus, the plate assay is apparently not sufficiently reliable or sensitive to detect levels of induction of β-galactosidase that are 10-fold by liquid assay.

To determine whether the two plasmids that directed regulated synthesis of β-galactosidase contained α-specific or haploid-specific hybrid genes, quantitative liquid assays were performed with isogenic **a**, α and **a**/α strains harboring these plasmids. These assays revealed that **a** and α cells bearing plasmid pSL307 had comparable levels of β-galactosidase activity, whereas **a**/α cells bearing the same plasmid had very little β-galactosidase activity, indicating that expression of the hybrid gene is haploid specific (Table 2). This conclusion was confirmed by placing the hybrid gene in a different set of isogenic strains. Because the level of β-galactosidase present in pSL307-bearing cells was low, we first cloned the hybrid gene present in this plasmid into a different plasmid that is maintained at a higher copy number (pJDB207, a *LEU2* 2μm plasmid [2]). This new plasmid (pSL555) conferred the same pattern of β-galactosidase activity as was seen for pSL307 (Table 2). Although the plate assay did not show an effect of pheromone on expression of this hybrid gene, we also examined this in liquid assays. There was a 20-fold increase in β-galactosidase activity when **a** or α cells carrying pSL555 were exposed to the appropriate pheromone. As described below, we isolated the chromosomal gene from which the hybrid gene is derived. Stable RNA production from the chromosomal gene was regulated in the same manner as production of β-galactosidase from the hybrid gene. We named this gene *FUS1* for reasons that are justified below. In a previous report the gene was called *RPM1* (3).

TABLE 2. β-Galactosidase activity of cells harboring plasmids that contain cell type-regulated *lacZ* hybrid genes[a]

Plasmid	β-Galactosidase activity[b] (U)				
	α cells	α cells + **a** factor	**a** cells	**a** cells + α factor	**a**/α cells
pSL307	7	ND[c]	5	ND	0.2
pSL555	44	980	40	990	2
pSL311	8	ND	8	ND	1

[a] β-Galactosidase assays were performed on permeabilized whole cells as described in Materials and Methods. pSL307 and pSL311 present in the isogenic strians HR125-5d, SY816, and SY817. pSL555 was present in the isogenic strains 246-1-1, EG123, and 1788.

[b] Activities are reported as modified Miller units (16, 29) and are the average of two or three assays of the same transformed strains. Treatment with pheromone was for 2 h at 30°C.

[c] ND, Not done.

The second plasmid that directed regulated synthesis of β-galactosidase, pSL311, also contained a haploid-specific gene (Table 2). Restriction endonuclease analysis revealed that the yeast DNA fragment joined to *lacZ* in pSL311 was distinct from the fragment in pSL307 and therefore was derived from a different chromosomal gene. In this case, however, we were not able to show that regulation of the chromosomal gene occurred at the transcriptional level. There are many possible explanations for our inability to observe transcriptional regulation, and we have not explored this matter further. Rather, in this paper we focus on the regulation and function of *FUS1*.

Control of *FUS1* RNA synthesis by cell type. To determine whether the regulation of β-galactosidase synthesis from plasmid pSL307 reflected transcriptional regulation of a corresponding chromosomal gene, we used the yeast insert sequences from this plasmid as a probe in Northern blot (RNA blot) analysis (Fig. 2). A ~1.6-kilobase (kb) RNA species was present in *MAT***a** and *MAT*α strains but absent in *MAT***a**/*MAT*α diploids. This RNA species showed the same pattern of regulation in β-galactosidase production from *FUS1-lacZ* (see below also) and therefore must be the *FUS1* transcript. The pattern of regulation suggests that transcription of *FUS1* was blocked by the action of **a**1 · α2, as has been demonstrated for several other genes (24, 28, 33, 39). Alternatively, transcription of *FUS1* could be sensitive to

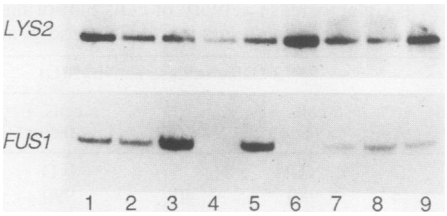

FIG. 2. Regulation of *FUS1* RNA production by the mating type locus. Poly(A)$^+$ RNA was isolated from strains differing in their *MAT* genotype. RNA (5 μg) from each strain was fractionated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with radioactively labeled RNA probe to *FUS1* sequences. Following autoradiography, the nitrocellulose filter was reprobed with a radioactive RNA probe to detect the *LYS2* transcript. The lanes were loaded with RNA from the following strains: 1, *MAT*α (XP8-18b); 2, *matα1* (VC2); 3, *matα2* (VC73); 4, *matα1*/*MAT***a** (G57A1); 5, *matα2*/*MAT***a** (G67A3); 6, *MAT*α/*MAT***a** (XP11); 7, *MAT*α/*mat***a***1* (MC18A); 8, *mat***a***1* (17-15); and 9, *MAT***a** (XP8-4a). The positions of the *FUS1* and *LYS2* transcripts are indicated.

the ploidy of the strain. To distinguish between these possibilities, we isolated RNA from haploid and diploid strains that differed in genotype only at *MAT* (Fig. 2). *FUS1* RNA was present in all haploid strains and in diploid strains containing *matα2* or *mat***a***1* mutations. *FUS1* RNA was not detectable in *MAT***a**/*MAT*α diploids or in *matα1*/*MAT***a** diploids. Thus, control of *FUS1* is at the level of transcription, and the **a**1 and α2 products are both needed for repression of this gene.

In an effort to detect low levels of *FUS1* transcript that might be present in **a**/α cells and to estimate the magnitude of repression in **a**/α cells, we varied the amount of RNA used in the Northern analysis. *FUS1* RNA was not detected when 20 μg of poly(A)$^+$ RNA from **a**/α cells was used for hybridization (Fig. 3). In contrast, *FUS1* transcript was readily detected when only 0.5 μg of poly(A)$^+$ RNA from α cells was used. Hence, there was at least a 40-fold difference in the level of expression of *FUS1* in α cells and **a**/α cells.

Control of *FUS1* RNA production by the peptide pheromones. We have shown that treatment of haploid cells bearing the *FUS1-lacZ* fusion plasmid with the opposing peptide pheromone resulted in an increase in the amount of β-galactosidase synthesized from the plasmid (pSL555, Table 2). To determine whether the mating factors affect transcript levels from the wild-type *FUS1* gene, we measured the amount of *FUS1* RNA present at various times after α cells were treated with **a** factor (Fig. 4). An increase in *FUS1* RNA was seen immediately after treatment with **a** factor, within the time it took to process an RNA sample (compare the lane marked α to the lane marked 0 min with **a** factor). By 15 min after **a**-factor treatment, the *FUS1* RNA levels reached a plateau, which persisted at least until 60 min. The 120-min sample showed a decrease in the *FUS1* RNA level, perhaps due to depletion of the factor and recovery of the cells. Similar increases were seen when **a** cells were treated with α factor (data not shown). In the experiment shown in Fig. 4, the magnitude of induction of *FUS1* by **a** factor was about 40-fold. The fold induction varied from experiment to experiment, however, due to fluctuation in the basal *FUS1* RNA level. This fluctuation may be a consequence of low and variable levels of pheromone (in this case, **a** factor) present in the medium. For example, the occasional failure of α2 to repress the **a**-factor structural genes could create a low (and variable) level of **a**-factor in a culture of α cells.

The rapid increase in *FUS1* RNA levels in cells treated with the mating factors suggested that induction may occur in the absence of new protein synthesis. We tested this interpretation by determining whether induction could occur in the presence of cycloheximide, a protein synthesis inhibitor. RNA was prepared from α cells treated with **a** factor in either the presence or absence of cycloheximide and hybridized with *FUS1* and *URA3* probes. *FUS1* RNA increased ninefold in α cells treated with **a** factor in both the presence and absence of cycloheximide (Fig. 5). To verify that protein synthesis was indeed inhibited in this experiment, the amount of β-galactosidase synthesized from the *FUS1-lacZ* fusion plasmid was measured when the plasmid-bearing α cells were treated with **a** factor in the presence or absence of cycloheximide. In the absence of cycloheximide, normal induction of β-galactosidase activity was seen following **a**-factor treatment. However, in the presence of cycloheximide, no increase in β-galactosidase activity was seen, confirming the effective inhibition of protein synthesis by cycloheximide (data not shown). Thus, induction of *FUS1* RNA by **a** factor is not dependent on new synthesis of protein regulators.

Control of *FUS1* RNA synthesis by the products of *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*. The products of the *STE4*, *STE5*, *STE7*, *STE11*, and *STE12* genes are required for efficient expression of **a**- and α-specific genes (14, 17; S. Fields, D. Chaleff, K. Clark, and G. Sprague, unpublished

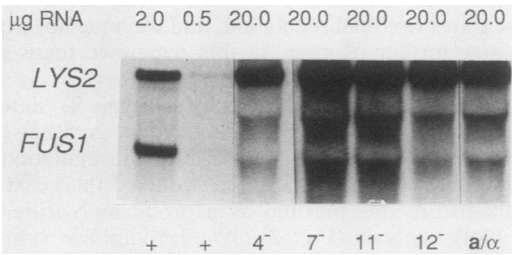

FIG. 3. Regulation of *FUS1* RNA production by *STE4*, *STE7*, *STE11*, and *STE12*. Poly(A)$^+$ RNA was isolated from (lanes, left to right) *MAT*α *STE* (DC41) (first two lanes), *ste4* (YY743), *ste7* (YY580), *ste11* (YY290), *ste12* (YY544), and *MAT***a**/*MAT*α (1788) strains and analyzed as described in Materials and Methods. All strains were isogenic except for the mutations in the *STE* genes. The amount of RNA loaded in each lane and the positions of *FUS1* and *LYS2* transcripts are indicated.

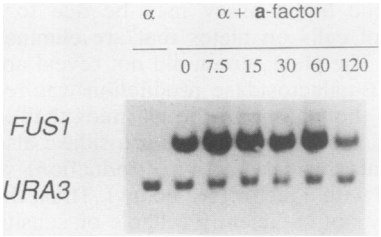

FIG. 4. Induction of *FUS1* RNA in α cells exposed to **a** factor. A culture of α cells (strain 246-1-1) was grown to a density of about 5 × 10^6 cells per ml. A sample was withdrawn for a zero-time point. To the remainder of the culture an equal volume of an **a**-factor preparation (16) was added, and samples were withdrawn at the indicated times (in minutes) for RNA preparation. A 20-μg amount of each RNA was analyzed as described in Materials and Methods. The positions of *FUS1* and *URA3* transcripts are indicated.

observations). To determine whether the products of these genes were also necessary for efficient expression of *FUS1*, poly(A)$^+$ RNA was isolated from strains that were isogenic except for a mutation at *ste4*, *ste5*, *ste7*, *ste11*, or *ste12*. RNA (20 μg) from each of these strains was used for Northern analysis (Fig. 3, *ste5* not shown); in no case was *FUS1* RNA detected. In contrast, *FUS1* transcript was detected when only 0.5 μg of RNA from the isogenic *STE* parent was used for hybridization. Hence, there was at least a 40-fold difference in the level of transcription of *FUS1* in α cells and in α cells carrying mutations at *ste4*, *ste5*, *ste7*, *ste11*, or *ste12*.

DNA sequence of *FUS1* and putative amino acid sequence of the gene product. The *FUS1* DNA fragment joined to *lacZ* was used as a probe to isolate the entire *FUS1* gene from a yeast genomic DNA plasmid library (see Materials and Methods). Plasmids containing the *FUS1* gene were identified by colony hybridization (26), and a plasmid that contained the entire *FUS1* gene was used as a source of fragments for DNA sequencing. The sequence of a 2,115-bp segment of yeast DNA containing *FUS1* was determined (Fig. 6). This segment contained an open reading frame of 512 codons. That this polypeptide corresponded to the *FUS1* gene product was indicated by two observations. First, it was the only extended open reading frame and its length agreed with the length of the *FUS1* transcript. Second, the site of fusion of *FUS1* to *lacZ* (the *Sau*3AI site at position 408) placed this open reading frame in frame with *lacZ* codons.

The deduced Fus1 protein had an interesting distribution of amino acid residues. The amino terminus (first 66 amino acids) was 23% serine and 24% threonine. Only 4 of the first 113 amino acids were charged, whereas the remainder of the protein was highly charged, 9.5% basic and 13.5% acidic. The amino terminus contained one cluster of hydrophobic amino acids that was of sufficient length to span a lipid bilayer (marked in Fig. 6) and may serve as a membrane-anchoring domain. There were a number of pairs of basic amino acid residues, specifically lysine-arginine pairs (marked in Fig. 6), which were potential sites for cleavage by the product of the *KEX2* gene (22). Of course, whether these sites are normally cleaved cannot be determined from the sequence. The predicted amino acid sequence of the Fus1 protein was not homologous to any of 2,500 known proteins from a variety of organisms.

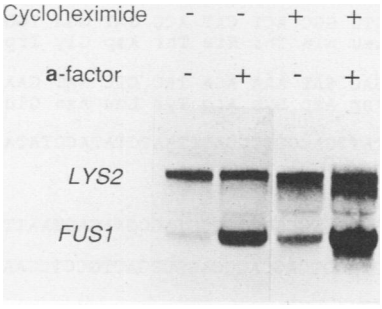

FIG. 5. Induction of *FUS1* RNA in α cells grown in the presence of cycloheximide. Total RNA was prepared from cultures of α cells (strain 246-1-1) grown in YEPD broth with or without cycloheximide (10 μg/ml) and exposed to **a** factor for 60 min or not exposed. The positions of *FUS1* and *LYS2* transcripts are indicated. The figure is a composite of two different exposures of the nitrocellulose filter to improve visibility of the samples prepared with cycloheximide, which apparently did not contain as great a ratio of mRNA to total RNA as did the samples prepared from cycloheximide-treated cells.

Subcellular location of the Fus1 protein. The hydrophobic stretch of amino acids near the amino terminus of the putative Fus1 protein suggested that this part of the protein may span a membrane. Knowledge of the cellular location of the Fus1 protein may provide insight into possible functions of the protein. Thus, we examined the subcellular location of the β-galactosidase activity produced from a plasmid-borne *FUS1-lacZ* fusion.

Two *lacZ* fusion plasmids were used: one, plasmid pSL555, contained 136 *FUS1* codons joined to *lacZ*, and the second plasmid, pSL330, contained a *CYC1-lacZ* fusion with only the initiator codon from *CYC1* fused to *lacZ*. The latter gene fusion served as a control for the localization of soluble β-galactosidase. Extracts of α cells containing either plasmid were prepared in the presence of the nonionic detergent Triton X-114. Triton X-114 forms small micelles when kept at 0°C. However, when extracts containing this detergent are shifted to 30°C, large heteromicelles that include integral membrane proteins are formed (5). The extracts were separated into detergent and aqueous phases at 30°C by centrifugation, and the fractions were assayed for β-galactosidase activity. More than 99% of the β-galactosidase activity in extracts of cells harboring the *FUS1-lacZ* fusion was found in the detergent fraction (Table 3). In contrast, essentially all the β-galactosidase activity from cells containing the *CYC1-lacZ* plasmid was in the aqueous fraction. Since only integral membrane proteins are thought to be found in the detergent fraction of extracts made with Triton X-114, these results suggest that the Fus1-β-galactosidase hybrid protein is an integral membrane protein.

Function of the Fus1 product. *FUS1* is transcribed only in **a** and α cells and transcript levels increase in response to exposure of these cells to the appropriate mating pheromone. This pattern of regulation suggests that the Fus1 gene product has a role in mating. To test this possibility, we created mutants carrying null alleles of the *FUS1* gene (*fus1::URA3*; see Materials and Methods) and examined the ability of these mutants to mate. In brief, we found that zygotes formed when *fus1* mutants mated contained a barrier or partition within the conjugation bridge joining the two cell bodies. This partition prevented nuclear fusion and mixing of cytoplasmic organelles. Eventually, the nuclei in the aberrant zygotes did fuse to create a true diploid, but this process was retarded compared with that in zygotes formed when wild-type cells mate. Thus, *fus1* mutants are defective in cell fusion of the mating pair (hence the gene name). This conclusion was supported by the following experimental observations.

Zygotes formed by mating two *fus1* mutant strains were examined microscopically and found to be abnormal (Fig. 7). Although the mated pairs adhered tightly (they could not be separated by sonication) and formed a classic dumbbell-shaped zygote, in many cases a readily visible structure was present in the conjugation bridge of the zygotes. This structure was sometimes seen in matings of *fus1* mutants with wild-type cells and was never seen in zygotes formed by making wild-type cells. The physical appearance of the structure suggested that it could serve as a barrier to nuclear fusion and to the exchange of other organelles. This supposition was tested by experiments in which the nuclear and cytoplasmic genotype of buds produced from *fus1* × *fus1* or *FUS1* × *FUS1* zygotes was determined. If *fus1* × *fus1* zygotes do not carry out nuclear fusion and exchange of organelles efficiently, then we expect that the initial buds

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                                        -267  CTGCAGGATCGCCCTTTTTGACGTATTGAATGGCATAATTGCACTGTCACTTTTCGCGCTGTCTCAT

-200  TTTGGTGCGATGATGAAACAAACATGAAACGTCTGTAATTTGAAACAAATAACGTAATTCTCGGGATTGGTTTTATTTAAATGACAATGTAAGAGTGGCT

-100  TTGTAAGGTATGTGTTGCTCTTAAAATATTTGGATACGACATCCTTTATCTTTTTTCCTTTAAGAGCAGGATATAAGCCATCAAGTTTCTGAAAATCAAA

   1  ATG GTA GCA ACA ATA ATG CAG ACG ACA ACA ACT GTG CTG ACG ACA GTC GCC GCA ATG TCT ACT ACC TTA GCA TCA
   1  Met Val Ala Thr Ile Met Gln Thr Thr Thr Thr Val Leu Thr Thr Val Ala Ala Met Ser Thr Thr Leu Ala Ser

  76  AAT TAC ATA TCT TCG CAA GCT AGT TCC TCG ACG AGT GTA ACA ACA GTA ACG ACA ATA GCG ACA TCA ATA CGC TCT
  26  Asn Tyr Ile Ser Ser Gln Ala Ser Ser Ser Thr Ser Val Thr Thr Val Thr Thr Ile Ala Thr Ser Ile Arg Ser

 151  ACA CCG TCT AAT CTA CTC TTT TCT AAT GTG GCG GCT CAG CCA AAA TCA TCT TCA GCA AGC ACA ATT GGG CTT TCA
  51  Thr Pro Ser Asn Leu Leu Phe Ser Asn Val Ala Ala Gln Pro Lys Ser Ser Ser Ala Ser Thr Ile Gly Leu Ser

 226  ATC GGA CTT CCC ATC GGA ATA TTC TGT TTC GGA TTA CTT ATC CTT TTG TGT TAT TTC TAC CTT AAA AGG AAT TCG
  76  Ile Gly Leu Pro Ile Gly Ile Phe Cys Phe Gly Leu Leu Ile Leu Leu Cys Tyr Phe Tyr Leu Lys Arg Asn Ser
                                                                                        +   +

 301  GTG TCC ATT TCA AAT CCA CCC ATG TCA GCT ACG ATT CCA AGG GAA GAG GAA TAT TGT CGC CGC ACT AAT TGG TTC
 101  Val Ser Ile Ser Asn Pro Pro Met Ser Ala Thr Ile Pro Arg Glu Glu Glu Tyr Cys Arg Arg Thr Asn Trp Phe

 376  TCA CGG TTA TTT TGG CAG AGT AAG TGT GAG GAT CAG AAT TCA TAT TCT AAT CGT GAT ATT GAG AAG TAT AAC GAC
 126  Ser Arg Leu Phe Trp Gln Ser Lys Cys Glu Asp Gln Asn Ser Tyr Ser Asn Arg Asp Ile Glu Lys Tyr Asn Asp
                                                                                            ♦

 451  ACC CAG TGG ACC TCG GGT GAT AAC ATG TCT TCA AAA ATA CAG TAC AAA ATT TCC AAA CCC ATA ATA CCG CAG CAT
 151  Thr Gln Trp Thr Ser Gly Asp Asn Met Ser Ser Lys Ile Gln Tyr Lys Ile Ser Lys Pro Ile Ile Pro Gln His
                                  ♦

 526  ATA CTG ACA CCT AAG AAA ACG GTG AAG AAC CCA TAT GCT TGG TCT GGT AAA AAC ATT TCG TTA GAC CCC AAA GTG
 176  Ile Leu Thr Pro Lys Lys Thr Val Lys Asn Pro Tyr Ala Trp Ser Gly Lys Asn Ile Ser Leu Asp Pro Lys Val
                                                                            ♦

 601  AAC GAA ATG GAG GAA GAG AAA GTT GTG GAT GCA TTC CTG TAT ACT AAA CCA CCG AAT ATT GTC CAT ATT GAA TCC
 201  Asn Glu Met Glu Glu Glu Lys Val Val Asp Ala Phe Leu Tyr Thr Lys Pro Pro Asn Ile Val His Ile Glu Ser

 676  AGC ATG CCC TCG TAT AAT GAT TTA CCT TCT CAA AAA ACG GTG TCC TCA AAG AAA ACT GCG TTA AAA ACG AGT GAG
 226  Ser Met Pro Ser Tyr Asn Asp Leu Pro Ser Gln Lys Thr Val Ser Ser Lys Lys Thr Ala Leu Lys Thr Ser Glu

 751  AAA TGG AGT TAC GAA TCT CCA CTA TCT CGA TGG TTC TTG AGG GGT TCT ACA TAC TTT AAG GAT TAT GGC TTA TCA
 251  Lys Trp Ser Tyr Glu Ser Pro Leu Ser Arg Trp Phe Leu Arg Gly Ser Thr Tyr Phe Lys Asp Tyr Gly Leu Ser

 826  AAG ACC TCT TTA AAG ACC CCA ACT GGG GCT CCA CAA CTG AAG CAA ATG AAA ATG CTC TCC CGG ATA AGT AAG GGT
 276  Lys Thr Ser Leu Lys Thr Pro Thr Gly Ala Pro Gln Leu Lys Gln Met Lys Met Leu Ser Arg Ile Ser Lys Gly

 901  TAC TTC AAT GAG TCA GAT ATA ATG CCT GAC GAA CGA TCG CCC ATC TTG GAG TAT AAT AAC ACG CCT CTG GAT GCA
 301  Tyr Phe Asn Glu Ser Asp Ile Met Pro Asp Glu Arg Ser Pro Ile Leu Glu Tyr Asn Asn Thr Pro Leu Asp Ala
              ♦                                                                 ♦

 976  AAT GAC AGT GTG AAT AAC TTG GGT AAT ACC ACG CCA GAT TCA CAA ATC ACA TCT TAT CGC AAC AAT AAC ATC GAT
 326  Asn Asp Ser Val Asn Asn Leu Gly Asn Thr Thr Pro Asp Ser Gln Ile Thr Ser Tyr Arg Asn Asn Asn Ile Asp
      ♦                           ♦

1051  CTA ATC ACG GCA AGA CCC CAT TCA GTG ATA TAC GGT ACT ACT GCA CAA CAA ACT TTG GAA ACC AAC TTC AAT GAT
 351  Leu Ile Thr Ala Arg Pro His Ser Val Ile Tyr Gly Thr Thr Ala Gln Gln Thr Leu Glu Thr Asn Phe Asn Asp

1126  CAT CAT GAC TGC AAT AAA AGC ACT GAG AAA CAC GAG TTG ATA ATA CCC ACC CCA TCA AAA CCA CTA AAG AAA AGG
 376  His His Asp Cys Asn Lys Ser Thr Glu Lys His Glu Leu Ile Ile Pro Thr Pro Ser Lys Pro Leu Lys Lys Arg
                                                                                        +   +

1201  AAA AAA AGA AGA CAA AGT AAA ATG TAT CAG CAT TTA CAA CAT TTG TCA CGT TCT AAA CCA TTG CCG CTT ACT CCA
 401  Lys Lys Arg Arg Gln Ser Lys Met Tyr Gln His Leu Gln His Leu Ser Arg Ser Lys Pro Leu Pro Leu Thr Pro
          +   +

1276  AAC TCC AAA TAT AAT GGG GAG GCT AGC GTC CAA TTA GGG AAG ACA TAT ACA GTT ATT CAG GAT TAC GAG CCT AGA
 426  Asn Ser Lys Tyr Asn Gly Glu Ala Ser Val Gln Leu Gly Lys Thr Tyr Thr Val Ile Gln Asp Tyr Glu Pro Arg

1351  TTG ACA GAC GAA ATA AGA ATC TCG CTG GGT GAA AAA GTT AAA ATT CTG GCC ACT CAT ACC GAT GGA TGG TGT CTG
 451  Leu Thr Asp Glu Ile Arg Ile Ser Leu Gly Glu Lys Val Lys Ile Leu Ala Thr His Thr Asp Gly Trp Cys Leu

1426  GTA GAA AAG TGT AAT ACA CAA AAG GGT TCT ATT CAC GTC AGT GTT GAC GAT AAA AGA TAC CTC AAT GAA GAT AGA
 476  Val Glu Lys Cys Asn Thr Gln Lys Gly Ser Ile His Val Ser Val Asp Asp Lys Arg Tyr Leu Asn Glu Asp Arg
                                                                          +   +

1501  GGC ATT GTG CCT GGT GAC TGT CTC CAA GAA TAC GAC TGATGAAAATAATATTGACGTTCGCATTTAATCTATACCTATAATTCTGTAC
 501  Gly Ile Val Pro Gly Asp Cys Leu Gln Glu Tyr Asp  512

1588  TTATATACTGTTCCTTAATTGAAGATTTCAACATCGTTTTTGATGTAGGTCTTTTCACCTGGAGGTGCGGCTGGGGTACCGAAGACTAATTGAGCTTGTA

1688  CGGTCCAAGACTCAGGGATTTTGCTTGGCAAAGCAGCTTTTATGTAACCATTGTAGTGTTGTAGGTGACCACCCAGGCCGATTGCCTCCAAGGCAACCCA

1788  CGAGTTGATTTGAGCGGCACCAGAGGTATGGTCCGGGAAACTAGGGAATGCAGCTG  1847
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FIG. 6. Nucleotide sequence of the coding strand of *FUS1*. The proposed coding region is shown with the corresponding amino acids below. Numbers to the left of the nucleotide sequence indicate the coordinate of the first nucleotide on each line, relative to the first nucleotide of the initiation codon. Numbers to the left of the amino acid sequence indicate the number of the first amino acid on each line. Arrows underline a sequence that may be important for increased transcription from genes that respond to pheromone (J. Thorner, J. Kronstad, and V. MacKay, personal communication) (see Discussion). The cluster of hydrophobic amino acid residues is boxed. Potential glycosylation sites (Asn-X-Thr or Asn-X-Ser, where X is any amino acid residue) are indicated (◆). Pairs of basic residues are marked with + below the line.

TABLE 3. Localization of hybrid β-galactosidase proteins[a]

Hybrid gene	a Factor	β-Galactosidase activity[b] (U)			
		Permeabilized cells	Lysed cells	Aqueous phase	Detergent phase
FUS1-lacZ	−	39	57	<1	14
	+	1,760	1,970	7	790
CYC1-lacZ	−	330	230	160	<1

[a] In all cases, the strain used was SY816. Treatment with **a** factor was for 2 h at 30°C.

[b] β-Galactosidase activity (modified Miller units [16, 29]) was measured in permeabilized whole cells, in lysed cells, or in the aqueous and detergent phases of cell extracts prepared in Triton X-114 (see Materials and Methods). The concentration of detergent was similar in all assay samples. Less than 1 U of β-galactosidase activity was detected from cells bearing plasmid pJDB207.

formed by such zygotes would be haploid and have a mitochondrial genotype identical to that of the parent that contributed the haploid nucleus present in the bud. Twenty-three *fus1* × *fus1* zygotes were isolated by micromanipulation and allowed to grow into colonies on YEPD plates. Fourteen of these colonies contained a mixture of both haploids and diploid cells, as expected. The other nine colonies were homogeneous, either totally diploid or totally haploid, the latter type suggesting that the zygote had died before diploid formation was complete. In contrast, all 14 zygotes isolated from a *FUS1* × *FUS1* mating gave rise to pure colonies that contained only diploid cells. We interpret this to indicate that *fus1* × *fus1* zygotes can form true diploids, but that haploid buds are often produced before diploid formation is complete.

To determine whether organelles other than nuclei also are not readily mixed in *fus1* × *fus1* zygotes, we repeated these experiments and assessed the ability of mitochondria to be transferred from one cell body to the other. A *MAT***a** *fus1* [*rho*°] strain, which lacks mitochondrial DNA, was mated to a *MAT*α *fus1* [*rho*$^+$] strain, and zygotes were isolated by micromanipulation. Colonies obtained from these zygotes contained a mixture of diploid and haploid cells as before. The striking observation was that the haploid cells that had inherited the *MAT***a** nucleus were still [*rho*°]; mitochondria had not migrated from the *MAT*α half of the zygote to the cytoplasm that was enclosed in the *MAT***a** haploid buds. Thus, in *fus1* × *fus1* zygotes, there was a barrier to the mixing of cytoplasmic components of the mated pair, and it is reasonable to suppose that the structure observed microscopically is the physical manifestation of this barrier.

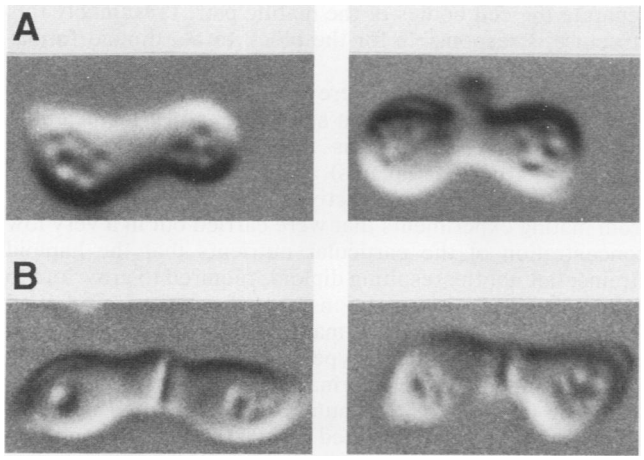

FIG. 7. Wild-type and mutant zygotes. Cells are allowed to mate for 6.5 h on YEPD plates. Samples were then washed off, fixed in 0.15 M NaCl plus 3.7% (vol/vol) formaldehyde, and viewed with Nomarski optics. (A) *FUS1* (strain SY816) × *FUS1* (strain 320); (B) *fus1* (strain YY751) × *fus1* (strain YY754).

TABLE 4. Mating efficiencies of *fus1* mutants[a]

Strain[b]	Lawn	Mean mating efficiency (%) ± 1 SD
*MAT*α *FUS1*	*MAT***a** *FUS1*	100
*MAT*α *FUS1*	*MAT***a** *fus1*	33 ± 14
*MAT*α *fus1*	*MAT***a** *FUS1*	27 ± 16
*MAT*α *fus1*	*MAT***a** *fus1*	0.5 ± 0.3
*MAT***a** *FUS1*	*MAT*α *FUS1*	100
*MAT***a** *FUS1*	*MAT*α *fus1*	19 ± 9
*MAT***a** *fus1*	*MAT*α *FUS1*	34 ± 22
*MAT***a** *fus1*	*MAT*α *fus1*	2 ± 2

[a] The strains whose mating efficiency was tested were mixed with a vast excess of *FUS1* or *fus1* cells of the opposite mating type (lawn) in diluted YEPD broth (1/10 strength in H_2O). The mix was then plated on synthetic minimal agar plates (SD), on which only prototrophic diploids could grow. Mating efficiencies were calculated as described in Materials and Methods, and the values were normalized to the efficiency of *FUS1* × *FUS1* matings. The absolute efficiencies for these wild-type matings were 7 ± 4% for α cells mated to an excess of **a** cells and 21 ± 16% for **a** cells mated to an excess of α cells. The efficiencies reported are the mean of four repetitions of each mating.

[b] The strains used were SY816 (*MAT*α *FUS1*), 320 (*MAT***a** *FUS1*), YY751 (*MAT*α *fus1*::*URA3*), and YY754 (*MAT***a** *fus1*::*URA3*).

The finding that *fus1* × *fus1* zygotes do yield diploid cells suggests that the barrier is eventually overcome and that nuclear fusion then ensues. We sought a quantitative measure of the failure in cell fusion and reasoned that this failure might be most apparent if we performed mating assays under conditions that provided only very small amounts of the nutrients that the haploid strains require to grow and to mate. Under these conditions, the incomplete zygote would be metabolically active and therefore capable of overcoming the barrier to cell and nuclear fusion for only a brief time. Accordingly, we mixed **a** *fus1* and α *fus1* mutants carrying complementary nutritional defects in a dilute YEPD solution (see Materials and Methods) and plated the mix on synthetic minimal medium, which permits the growth of only **a**/α diploids. Under these conditions *fus1* × *fus1* matings had an efficiency that was approximately 1% of that of *FUS1* × *FUS1* matings (Table 4). *FUS1* × *fus1* matings had an intermediate efficiency, 20% to 50% of that of wild-type matings.

Support for the idea that the barrier to cell fusion present in *fus1* × *fus1* zygotes is eventually overcome if the zygotes are metabolically active was provided by a slight variation of the quantitative mating experiments, this time done with full-strength YEPD broth to mix and plate the two strains. Under these conditions, no difference in mating efficiency was seen when *FUS1* × *FUS1* and *fus1* × *fus1* matings were compared (data not shown).

fus1 mutants were examined for deficiencies in other mating functions, but none was found. *fus1* mutants produced normal amounts of pheromone, agglutinated normally, and responded normally to pheromone as judged by cell cycle arrest and the appearance of morphologicaly altered cells (shmoos). We conclude that *fus1* mutants are specifically defective for cell fusion.

DISCUSSION

We have identified a gene, *FUS1*, whose expression is subject to three regulatory inputs. Transcription of this gene is limited to the **a** and α cell types, requires the products of several *STE* genes, and increases when **a** or α cells are treated with the appropriate mating factor. Although this appears to be a complex collection of regulatory inputs, two of these inputs—cell type and the *STE* genes—are almost certainly different manifestations of the same underlying mechanism. The regulation of *FUS1* expression suggests that the Fus1 product has a role in mating. Indeed, we found that *fus1* mutant cells did not complete zygote formation efficiently—they were defective for cell wall or plasma membrane fusion.

Regulation of *FUS1*. *FUS1* transcript was undetectable in **a**/α cells but readily detectable in **a** and α haploids. We estimate that there is at least a 40-fold difference in *FUS1* transcript levels in haploids compared with **a**/α diploids. Expression of *FUS1* in **a**/α cells was prevented in the presence of the repressor **a**1 · α2, and on this basis we consider *FUS1* a haploid-specific gene. As discussed below, however, repression of *FUS1* by **a**1 · α2 is probably indirect, the consequence of repression of activators of *FUS1* transcription. Our experiments did not reveal whether it is synthesis or stability of *FUS1* RNA that is regulated. We believe, however, that regulation is at the level of transcript synthesis, because a 140-bp segment from the control region (upstream activation sequence [UAS]) of *FUS1* was sufficient to impart regulated expression to a heterologous gene (*CYC1-lacZ*) that is not normally controlled by cell type (unpublished observations).

Transcription of *FUS1* in haploid cells required the products of five *STE* genes, *STE4*, *STE5*, *STE7*, *STE11*, and *STE12*. These *STE* genes are known to be required for efficient expression of several α- and **a**-specific genes (14; G. Sprague, K. Clark, S. Fields, and D. Chaleff, unpublished observations), and we therefore determined whether *FUS1* transcription also exhibited a requirement for the *STE* gene products. In the case of α- and **a**-specific genes, expression was typically reduced 5- to 10-fold in mutants defective at any one of the *STE* loci. *FUS1* transcription showed a more stringent requirement for the *STE* gene products, however. In each mutant strain *FUS1* RNA was undetectable, indicating that expression was reduced at least 40-fold. The role(s) that these *STE* gene products play in gene expression is unknown, but recent evidence suggests that *STE7* and *STE11* may encode protein kinases (45; B. Errede and D. Chaleff, personal communication). Perhaps α- and **a**-specific genes, as well as some haploid-specific genes such as *FUS1*, share a transcription factor whose activity is determined by its phosphorylation state. *STE4*, *STE5*, or *STE12* may encode such a transcription factor or, like *STE7* and *STE11*, they may affect its activity.

Although the two regulatory inputs to *FUS1* transcription discussed thus far—repression by **a**1 · α2 in **a**/α cells and activation by the *STE* gene products—would seem to occur by distinct mechanisms, studies of the expression of the *STE* genes suggest a more economical view. *STE5* (14, 28; V. MacKay and J. Thorner, personal communication) and *STE4* (V. MacKay, personal communication) transcripts are not detected in **a**/α cells, and *STE12* transcripts are present at much reduced levels compared with the amount present in **a** or α cells (S. Fields and I. Herskowitz, personal communication). Thus, the absence of these activators of *FUS1* transcription in **a**/α cells is sufficient to account for the failure of those cells to express *FUS1*; direct repression by **a**1 · α2 need not be invoked. In support of this view, the *FUS1* UAS element does not contain a version of the sequence that is believed to be the site of action of **a**1 · α2 (28). Hence, *FUS1* may be indirectly repressed in **a**/α cells, in a manner analogous to the late genes of a λ prophage—these genes are silent not because of the direct action of a negative regulator, but because synthesis of a positive regulator is repressed.

Expression of *FUS1* is also subject to a third regulatory input; transcript levels increase 10- to 30-fold in response to the intracellular signal(s) that is generated when a mating pheromone binds to its cell surface receptor. It is tempting to imagine that one or more of the *STE* gene products required for *FUS1* transcription is a target of that signal. In this view, the pheromone-generated signal interacts with an *STE* gene product to create a product that is better able to activate transcription from the *FUS1* locus. A DNA sequence has been identified that may serve as the site of action of the regulator that ultimately stimulates transcription. That this sequence is important for increased transcription from genes that respond to pheromone was suggested by sequence homology comparisons among several such genes (J. Thorner, personal communication). Deletion analysis of the upstream control region of one pheromone-inducible gene also points to the sequence as being particularly important for pheromone-stimulated transcription (J. Kronstad and V. MacKay, personal communication). It is therefore intriguing that the induction of *FUS1* transcription by the pheromones was unusually high and that this gene contained four copies of the critical sequence within its UAS (see Fig. 6).

Function of the Fus1 product. To investigate the role of the Fus1 product, we created substitution mutations in which the UAS and essentially all codons from *FUS1* were deleted and replaced with the *URA3* gene. Strains bearing the *fus1::URA3* mutation had a subtle but significant defect in mating that was most apparent when two *fus1* mutants were mated. The zygotes formed by these mutants initially contained an impediment or barrier to mixing of organelles and fusion of nuclei so that the first buds generated from the zygote were often haploid. Microscopic examination of the zygotes formed by *fus1* mutants revealed that they were abnormal or incomplete. Specifically, at the position of the conjugation bridge a structure existed that appeared to separate the cell bodies of the mating pair. Presumably this structure is responsible for the block to **a**/α diploid formation.

That true **a**/α diploids were eventually formed suggests that other gene products can substitute, albeit inefficiently, for the Fus1 product. This transition (from incomplete zygotes to true **a**/α diploids) apparently requires that the zygotes be metabolically active. This conclusion follows from mating experiments that were carried out in a very low concentration of the particular nutrients that the haploid strains, but not the resulting diploid, required to grow and to mate. Under these conditions, the frequency of diploid formation in *fus1* × *fus1* matings was reduced 100-fold compared with that in wild-type matings. We interpret this to indicate that true diploid formation occurs more quickly in wild-type matings than in mutant matings. When nutrients are limiting, they are depleted before the *fus1* mutants can complete formation of an **a**/α diploid. These experiments also suggest that the barrier or impediment present in *fus1* zygotes prevents not only intermingling of organelles, but also transfer of enzymes (or small molecules) between the two cell bodies present in the incomplete zygote. If enzymes

were shared by the two cell bodies, the incomplete zygote would effectively be a dikaryon and the nutritional defects would be complemented. Hence, the dikaryon would be metabolically active, which would allow true diploid formation to occur.

The biochemical role of Fus1 in mating is not known. However, the deduced Fus1 amino acid seuqence does contain a stretch of hydrophobic amino acids near the amino terminus, which suggests that Fus1 is a membrane or secreted protein. The existence of numerous pairs of basic residues (e.g., Lys-Arg; see Fig. 6) suggests in addition that the primary Fus1 translation product may be processed to yield several different polypeptides. A membrane locale for the Fus1 product is supported by our finding that a Fus1-β-galactosidase hybrid protein fractionated as a membrane protein in cell extracts. Trueheart et al. (47) used a slightly different Fus1-β-galactosidase hybrid to demonstrate by indirect immunofluorescence that the hybrid protein is preferentially located at the tips of the morphologically aberrant cells (shmoos) that arise after prolonged exposure to α-factor. Shmoos are thought to be the exaggerated consequence of morphological changes that occur in cells that are preparing to mate but have no mating partners. In normal matings these changes occur at the site where the mating pair will join and form a conjugation bridge. Thus, the Fus1 product is apparently delivered to the region where the mating cells will adjoin, at which location Fus1 is poised to catalyze cell fusion.

In summary, the phenotype of *fus1* mutants has defined a heretofore uncharacterized step in mating that is subsequent to pheromone response and agglutination. The mating pair adhere extremely tightly—they cannot be separated by sonication—but zygote formation is arrested, at least temporarily, at a position prior to cell wall and plasma membrane fusion.

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