Characterization of fus1 of Schizosaccharomyces pombe: a Developmentally Controlled Function Needed for Conjugation

JANNI PETERSEN, DIETMAR WEILGUNY,† RICHARD EGEI, AND OLAF NIELSEN*

Department of Genetics, Institute of Molecular Biology, University of Copenhagen, DK-1353 Copenhagen K, Denmark

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In Schizosaccharomyces pombe, the fus1 mutation blocks conjugation at a point after cell contact and agglutination. The cell walls separating the mating partners are not degraded, which prevents cytoplasmic fusion. In order to investigate the molecular mechanism of conjugation, we cloned the fus1 gene and found that it is capable of encoding a 1,372-amino-acid protein with no significant similarities to other known proteins. Expression of the fus1 gene is regulated by the developmental state of the cells. Transcription is induced by nitrogen starvation and requires a pheromone signal in both P and M cell types. Consequently, mutants defective in the pheromone response pathway fail to induce fus1 expression. The ste11 gene, which encodes a transcription factor controlling expression of many genes involved in sexual differentiation, is also required for transcription of fus1. Furthermore, deletion of two potential Ste11 recognition sites in the fus1 promoter region abolished transcription, and expression could be restored when we inserted a different Ste11 site from the mat1-P promoter. Since this element was inverted relative to the fus1 element, we conclude that activation of transcription by Ste11 is independent of orientation. Although the fus1 mutant has a phenotype very similar to that of Saccharomyces cerevisiae fus1 mutants, the two proteins appear to have different roles in the process of cell fusion. Budding yeast Fus1 is a typical membrane protein and contains an SH3 domain. Fission yeast Fus1 has no features of a membrane protein, yet it appears to localize to the projection tip. A characteristic proline-rich potential SH3 binding site may mediate interaction with other proteins.

Nutritional starvation is the major signal that activates sexual differentiation in the fission yeast Schizosaccharomyces pombe (11). As long as the nutritional conditions are favorable, haploid cells will propagate vegetatively, but under conditions of nitrogen starvation, the cells exit from the mitotic cycle and undergo a differentiation process, which requires sexual agglutination, conjugation, nuclear fusion, meiosis, and spore formation to occur in an orderly fashion (see reference 13). The process of conjugation involves the action of diffusible pheromones secreted by P and M cell types in order to attract each other. When exposed to the opposite pheromone, the cells form projections toward each other (18, 37) and fuse upon cell-cell contact. Attachment at the projection tips between paired cells culminates in localized cell wall degradation and plasma membrane fusion. Nuclear fusion is coordinated with these events, resulting in the formation of a zygote (see reference 53).

In the differentiation process, the pheromones act by binding to specific receptors on the surface of the opposite cell type (29, 60), thereby activating the pheromone response pathway. Transmission of the signal through the pathway involves the actions of the ras1 function and of three protein kinases encoded by byr2, byr1, and spk1 (19, 45, 47, 49, 58, 61, 65) and ultimately activates transcription of pheromone-controlled genes (see reference 48). The transcription factor Ste11 may be a target for this activation, since it is required for expression of many genes involved in sexual differentiation. It acts by binding to a 10-bp T-rich DNA element, the TR box (59). In the promoter of the pheromone-induced gene mat1-Pm, deletion of 21 bp containing a TR box abolishes transcription (1). Furthermore, studies of mutations in the TR box suggest that it is essential for pheromone-dependent expression.

The fus1 function acts during conjugation. Mutant fus1 cells are blocked at a step following cell contact but prior to cell wall fusion (5, 12, 33). The separating cell walls are not dissolved if both prezygotic partner cells carry this mutation, and only prezygotes will accumulate. The arrest occurs at the G1 stage of the cell cycle, and the cells can revert to vegetative growth if transferred to fresh medium (15). The fus1 phenotype is easily monitored in prezygotes as the conjugation tubes continue to grow, thereby creating horseshoe-shaped cell pairs. Meiosis is not affected in diploid fus1 strains.

In the budding yeast Saccharomyces cerevisiae, six FUS genes have been identified (17, 34, 40, 62). Mutations in these genes cause defects in cell fusion by interfering with the degradation of the cell wall during mating. The Fus1 gene, which is the best characterized, encodes a 512-amino-acid membrane protein (63). Transcription of Fus1 occurs only in haploid α and a cells and is strongly induced by exposure to pheromone (40, 62). Consistent with this is the observation that Fus1 transcription depends on the components in the pheromone response pathway (40); STE4, which encodes the β subunit of a G protein; STE7, STE11, and Fus53, which all encode protein kinases; and STE5, which has an unknown function (reviewed in reference 35). Transcription of Fus1 also requires Ste12, a transcription factor that binds to the pheromone response element (PRE) (10). In the Fus1 promoter, four PREs have been identified and deletion of all four PREs prevents Fus1 expression (22).
As part of a study aimed at understanding the molecular mechanism of conjugation in S. pombe, we further characterized the fus1 mutant and cloned the gene by complementation. Analysis of the regulation of the fus1 gene shows that pheromones cause a strong induction of the transcript. Consistently, we observed that transcription requires an intact pheromone response pathway. The fus1 upstream region contains two TR boxes. Our results suggest that these sequences are necessary for ste11-mediated transcription of fus1.

**MATERIALS AND METHODS**

**Strains, media, and genetic methods.** The S. pombe strains used are listed in Table 1. Standard classical and molecular genetic techniques for S. pombe were used as described previously (20, 44). Proteolysis of Fus1 was done essentially as described previously (46). The media PM and PM–N used for growing S. pombe cells were prepared as described in reference 3. Minimal sporulation agar (MSA) was prepared according to reference 16. Escherichia coli DH5(α) was used for routine plasmid and maintenance of plasmids. Recovery of plasmids from S. pombe into E. coli JA226 has been described previously (2). Standard procedures for manipulating DNA were used according to reference 57. PCR amplification was carried out in 50-μl reaction mixtures (31).

**Quantitative mating assay.** The cells were grown in PM to a density of 5 × 10^6 cells per ml, mixed in a 1:1 ratio. (Homothallic strains were grown to a density of 5 × 10^8 cells per ml.) Five microliters was spotted on an MS plate, and the cells were incubated for 48 h at 30°C. The efficiency of mating was calculated as (2^<M> - 1)×100, where M is the number of ascis and zygotes.

**Cloning of fus1.** S. pombe EG 382 (h^m^ fus1-B20 leu1 ade6-M210 ura4-D18) was transformed with a B. subtilis genomic DNA library in the vector pON163 (6). Ura^- transformants were selected and then replica plated to a lawn of strain EG 385 (h^m^ ade6-B10 fus1-B20 ade6-M216 ura4-D18) and then replica plated to MSA. The B102 mutation in the mal-1 Prn gene increases the number of zygotes that will resume diploid mitosis (12). The mutations ade6-M216 and ade6-M210 complement each other intragenically, so that only diploid cells resulting from conjugation can grow. Cloning of the ade6 gene was avoided because the gene bank was constructed from an ade6-M210 mutant (66). The plates were washed and the vegetative cells were killed with 30% ethanol (36). The ascospores were then spread on minimal plates from an iodine-positive colony containing only zygotic asci and therefore expected to be fus1^-^ ade6^-^ recombinant/parental was isolated and designated PDW220.

**Sequence analysis.** Sequencing was carried out by the dideoxy chain termination method with [α-32P]labelled nucleotides and a Sequenase kit (U.S. Biochemical Corp.). Double-stranded plasmid DNA inserted in pGEM plasmids was used as a template. Two sets of overlapping unidirectional deletions, generally 100 to 200 bp apart, were generated by treating PDW235, PDW234 (see Fig. 3A), and PDW372 (insert is identical to PDW236) with exonuclease III and nuclease S1 as described previously (24). The primers used were the standard sequencing primers homologous to the SP6 and T7 promoter sequences in the pGEM3 plasmid (Promega). Specific primers were also made. Both strands of the sequence presented in Fig. 4 were determined entirely from overlapping clones or specific primers.

**Northern (RNA) analysis.** Cells were grown at 30°C in PM to a density of 5 × 10^9 cells per ml, harvested, and resuspended in PM and PM–N at the same density. After 5 h of incubation at 30°C, RNA was isolated as described previously (50). The effect of mating pheromones on fus1 expression was monitored by adding 300 M of synthetic M factor (64) or P factor (27) per ml to mitotically growing cycl^-^ cells for 5 h (10^7 cells per ml).

As part of a study aimed at understanding the molecular mechanism of conjugation in S. pombe, we further characterized the fus1 mutant and cloned the gene by complementation. Analysis of the regulation of the fus1 gene shows that pheromones cause a strong induction of the transcript. Consistently, we observed that transcription requires an intact pheromone response pathway. The fus1 upstream region contains two TR boxes. Our results suggest that these sequences are necessary for ste11-mediated transcription of fus1.
TABLE 2. Mating efficiencies of mutants defective in fus1

<table>
<thead>
<tr>
<th>fus1 allele</th>
<th>h+ strain</th>
<th>Efficiency of mating (%) with h+ strainab</th>
</tr>
</thead>
<tbody>
<tr>
<td>fus1-20</td>
<td>EG 544 ( fus+ )</td>
<td>40.5 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>EG 754 ( fus- )</td>
<td>7.1 ± 0.2</td>
</tr>
</tbody>
</table>

* Values represent means of at least three separate trials ± standard deviations.
* The fus1-B20 mutation is slightly leaky. When it was present in a homothallic strain, we found a mating efficiency of 1.9% ± 0.3%. This higher value probably reflects the fact that mating-type switching leads to pairs of sister cells having opposite mating types (43) and hence improves pairing.

The experiment shows that mating between two fus1 mutants is completely blocked; only abnormal prezygotes are present. Mating of a wild-type cell with a fus1-20 partner produced normal zygotes, independent of which mating type was fus1-. This indicates that provision of fus1 activity from one side is sufficient for the breakdown of both separating cell walls, although the mating frequency is decreased compared with mating between two wild-type strains. This experiment suggests that the fus1 gene can be expressed in both mating types. We further examined the fus1 mutant for defects in other processes during sexual differentiation. The cells responded normally to pheromone by elongation and shmoo formation, they agglutinated normally, they produced normal amounts of pheromone, and, if they were made diploid by protoplast fusions, they sporulated as wild-type cells, suggesting that the mutation does not affect meiosis (data not shown). All of this is evidence that fus1 has a function only in cell fusion.

**Cloning of fus1 by rescue of the mutant phenotype.** We first attempted to clone the fus1 gene simply by restoration of sporulation to an h100 fus1-B20 mutant strain. This procedure, however, gave rise to a large number of false-positive sporulating diploid colonies (data not shown). We therefore designed a strategy that directly took advantage of the mating defect of the fus1 mutant. The experimental approach was based on intragenic complementation of two ade6 mutants and on the fact that provision of wild-type Fus1 activity from one side is sufficient for the breakdown of both separating cell walls (described above). The fus1-B20 mutant was transformed with a fission yeast genomic library with ura4 as a selective marker. Ura+ transformants were pregrown on minimal plates and mated to lawns of fus1- cells and then replica plated to medium on which only the fraction of the transformants that had formed diploids could grow. In order to eliminate false positives, due to the slight leakiness of fus1-B20, free ascospores of the selected diploids were plated and the descending haploid colonies were screened for the Fus+ phenotype. Several Ura+ and Fus+ clones were obtained. Vegetative progeny from these showed simultaneous loss of the Ura+ and Fus+ phenotypes when grown in nonselective medium, indicating that complementation of the two markers was caused by a single autonomously replicating plasmid. Plasmid DNA isolated from one of these transformants was recovered in E. coli. Upon retransformation, this plasmid (pDW220) was able to complement the fus1 mutation, suggesting that it contained the fus1 gene. Figure 2D shows the complementation observed with pDW220.

To define more precisely the boundaries of the complementing region of pDW220, different fragments of the nuclear DNA insert were cloned into the shuttle vector pDW232 (66), and the constructs were tested for complementation of the mating deficiency of the fus1-B20 allele (Fig. 3B). The region between the left HindIII site and the right XhoI site present in pDW375
proved to be sufficient for restoring the Fus+ function to fus1 mutant cells (Fig. 3B). Southern hybridization of total DNA restricted with EcoRV or XhoI showed that the cloned DNA insert was derived from a unique sequence in the S. pombe genome (data not shown).

Disruption of the fus1 gene and sequence analysis. To confirm genetically that pDW220 actually contained the fus1 gene and not an extragenic suppressor, the cloned segment was used to direct a disrupting selectable marker into the fus1 locus at the right arm of chromosome I (14). A disrupted fus1::ura4 allele was constructed by inserting a 1.7-kb ClaI fragment containing the S. pombe ura4 gene into the ClaI site of pDW234 (Fig. 3C). The resulting plasmid was digested with XhoI, and the fragment containing ura4 was used for transformation of a homothallic diploid strain carrying the ura4 mutation (EG 325-2n). Several transformants were obtained, and integration of this construct at the chromosomal fus1 locus was confirmed by Southern analysis (EcoRV-digested chromosomal DNA hybridized with a probe transcribed from pDW234 [data not shown]). Tetrad analysis of the hybrid diploids was dissected. Each ascus contained four viable spores, indicating that the fus1 gene is not essential for mitotic growth. The tetrad data showed regular 2:2 segregation for both the Fus+/Fus- and Ura+/Ura- phenotypes. As expected, close linkage between ura4 and fus1 was demonstrated (data not shown). The phenotype of transformants carrying the disrupted segment was found to be Fus-. This fus1 allele seems to be tight compared with fus1-B20, which is slightly leaky (as described above and shown in Fig. 8). Additionally, we made the disruption in a haploid strain and protoplast fused this strain (EG679) with the strain carrying the fus1-B20 allele (EG680). Sporulation of the resulting diploid strain gave rise to only Fus- progeny, which confirms that the cloned fragment actually is the fus1+ gene.

The DNA sequence of the 5-kb fus1-complementing HindIII-XhoI fragment from pDW375 was determined. Analysis of the sequence revealed the presence of a 1,372-amino-acid uninterrupted ORF (Fig. 4). A database search revealed no significant similarities to other known proteins, including S. cerevisiae FUS1 (62). We found an mRNA species corresponding to the fus1 coding strand (described below). The transcription start was determined by primer extension to be located at position -32 relative to the translation start (Fig. 4 [data not shown]). This means that the fus1 mRNA starts with AUG. Observation has shown that translation initiates at the first AUG codon present in 90% of eukaryotic mRNAs (32). However, it seems unlikely that translation can be initiated at the first codon in
FIG. 4. Nucleotide sequence of fus1 and the predicted amino acid sequence. The underlined sequences indicate two TR boxes, potential binding sites for the transcription factor Ste11. The transcriptional start site is marked by an arrowhead. In the amino acid sequence, a number of proline residues (a potential SH3 binding site) are printed in boldfaced type and marked with asterisks.
this messenger, and furthermore the product would be out of frame with the ORF. Therefore, translation is likely to initiate at the indicated second AUG codon, but it could also be initiated at the AUG codon just downstream of it. In that case, there would be an adenine residue at position –3, which has been shown to be important for efficient translation in S. cerevisiae (8).

**Induction of fus1 requires a pheromone signal.** Using strand-specific RNA probes, we performed Northern analysis to determine whether the level of fus1 mRNA was induced during conjugation. Figure 7 shows that four fus1-specific bands can be detected (h90 – N). The largest band has a size of approximately 4 kb, which corresponds to the size of the fus1 ORF. This band was highly induced in lanes with RNA prepared from starved homothallic cells, and we propose that it is the primary fus1 transcript. Two smaller bands presumably represented decay or processing products of the fus1 transcript, which were concentrated at the leading edge of the massive 18S and 25S rRNA bands (25). Their appearance seemed to correlate with that of the fus1 band. Another band, which showed no induction by starvation, was present below the 25S rRNA in all lanes. Presumably this was due to cross-hybridization to a transcript of an unknown gene, which appears to be more abundant in heterothallic strains. We have fused the HA epitope to the N terminus of the fus1 reading frame (see Materials and Methods). When expressed from the constitutive adh promoter, this construct fully complements fus1, and a Western blot of a strain transformed with it revealed only one band with a size predicted by the ORF (Fig. 5).

Thus, the 4-kb band observed in Northern blots appears to give rise to the Fus1 protein.

The fus1+-mediated degradation of cell walls is expected to function only during the process of mating. The activation of this function could be due to either transcriptional or translational induction or might be caused by posttranslational modifications of the gene product. We found that the fus1 gene is only very weakly expressed in mitotic cells (Fig. 6A, h90 + N). In a homostrain, activation of mating by nitrogen starvation caused a strong induction of fus1 transcription (Fig. 6A, h90 – N). Heterothallic strains failed to activate the fus1 gene upon nitrogen starvation (Fig. 6A, h– – N and h+ – N), which indicates that transcription of the fus1 gene may depend on a pheromone signal. To test this directly, we added purified pheromones to heterothallic cyr1 strains (Fig. 6B). Such strains can respond to pheromones in rich medium (9, 38, 68). The h– strain also carried the sxa2 mutation in order to prevent degradation of P factor (26). Addition of both P factor to the h– strain and M factor to the h+ strain caused a strong induction.

**FIG. 5.** (A) Identification of the HA-fus1 fusion protein by Western blot analysis. Primary antibody against HA was used for detection, and the only band that appeared had a size which corresponds to that of the fus1 ORF (about 160 kDa). The plasmid containing the fusion protein complements the fus1– strain EG 382. M, molecular mass standards (kilodaltons). (B) Complementation of the fus1– strain (EG 382) by HA-tagged Fus1.

**FIG. 6.** Transcriptional analysis of the fus1 gene. (A) A homothallic h90 strain (EG 282) and heterothallic h+ (EG 545) or h– (EG 544) strains were grown in liquid minimal medium with or without nitrogen present. The 4-kb fus1 transcript was only expressed in nitrogen-starved homothallic strains. (B) Addition of either P or M factor to heterothallic cyr1 strains (EG 794 and EG 796) caused a strong induction of the fus1 transcript. The same membranes were also hybridized to a cdc2-specific probe (bottom panels).
undersexual differentiation, and it is found in the promoter regions of many genes induced by G1-arrested cells. Expression of the Fus1 protein depends on an intact pheromone response pathway. Expression of pheromone-controlled genes, such as *mat1-Pm*, is abolished in cells defective in components of the pheromone response pathway (e.g., *map3*, which encodes the M factor receptor; *gpa1*, which encodes the α subunit of the G protein; *ras1*, which is a GTP-binding protein; or *byr2*, *byr1*, and *skp1*, which all encode protein kinases). Furthermore, transcription is abolished in a *ste11* strain, which is defective in a transcription factor (1, 19, 45, 49, 58–61, 65). It was therefore of interest to investigate whether defects in these genes affected Fus1 transcription. By Northern analysis of nitrogen-starved cells, we found that mutations in *byr2*, *byr1*, or *ste11* abolished transcription of the *fus1* gene (Fig. 7). The effect of *map3*, *gpa1*, and *ras1* mutations on fus1 transcription was also investigated, and all of these mutants failed to induce the transcript upon nitrogen starvation (data not shown). We therefore conclude that expression of *fus1* depends on an intact pheromone response pathway.

Function of the TR box is orientation independent. In the *fus1* 5′-upstream region, we noticed a potential positive regulator sequence (TR1) at position −238 relative to the translation start (Fig. 4). This element is a TR box (TTCTTTGTTY), which is the recognition site of the transcription factor Ste11, and it is found in the promoter regions of many genes induced under sexual differentiation: *mei2*, *mat1-P*, *matM*, and *ste11* itself (59). In the *mei2* promoter and in the *mat1-Pm* promoter, the TR boxes were shown to play a critical role in controlling expression of these genes (1, 59). Accordingly, we expected the TR box in the *fus1* 5′-upstream region to be involved in control of expression and therefore deleted it. In order to measure the effect of this deletion or others in the promoter region, we used an in vivo assay for *fus1* transcription (Fig. 8). The mutations were constructed in plasmids intended to integrate at the *fus1* locus at the right arm of chromosome I (14). They contained a truncated Fus1 gene under control of the mutated promoter and *ura4* as a selective marker. As a result of the integration event, the altered promoter will control the wild-type *fus1* reading frame, and a mating defect will result if transcription is substantially reduced. First, we showed that the unmodified promoter fragment (pJP20) was sufficient to give rise to wild-type Fus1 activity (Fig. 8B and C1). As a control (Fig. 8C2) we observed that a promoterless *fus1* gene (pJP36) could not give rise to Fus1 activity. Figure 8B shows a strong decrease in mating efficiency corresponding to that of the original *fus1-B20* mutant. Deletions in the promoter to a position just upstream of the TR box (pJP31) had no effect on the mating efficiency, and, surprisingly, a deletion of the TR box itself (pJP41) had no effect either. We therefore looked for additional TR elements, and at position −157 relative to the translation start, we found another TR box (TR2) situated in the opposite orientation. Deletion of one of these alone (pJP41) had no effect either, but when both TR boxes were removed simultaneously (pJP49), mating was strongly reduced (Fig. 8B and C3). To confirm that the reduction in mating was actually caused by deletion of TR1 and TR2, we tried to restore mating by insertion of a different TR box (pJP53). This TR box was identical to one from the *mat1-P* promoter. Figures 8B and C4 show that insertion of this TR box indeed restored mating to the same level as that seen in pJP41. Furthermore, the synthetic TR box was inserted in the opposite orientation compared with the wild-type TR box at this position. Hence, we conclude that at least one of the two TR boxes in the *fus1* promoter is required for expression.

The Fus1 protein associates with the projection tips. We wanted to determine the localization of the Fus1 protein within conjugating cells by indirect immunofluorescence microscopy with primary antibodies against the HA tag (67). First, we looked at nitrogen-starved homothallic cells expressing the HA-fus1 fusion protein from the strong constitutive *adh1* promoter on a plasmid. As mentioned above, these conditions fully restored conjugation in *fus1* mutant cells (Fig. 5B). The signal appeared to be mainly cytoplasmic, and we were unable to detect any asymmetrical distribution of the protein within the cells (data not shown). However, since these results were obtained with cells overexpressing the protein, we could not rule out the possibility that this obscured a specific localization of the protein. We therefore proceeded to express the fusion protein from the *fus1* promoter in single copy in the genome. In order to increase the sensitivity of detection, we used the triple HA tag (17a). To our surprise, the *fus1* mutant was no longer complemented under these conditions (data not shown). However, the protein now showed a specific association with the prezygotic projection tips in conjugating cells (Fig. 9). Presumably, the activity of the protein is reduced by the presence of the tag, and overexpression can compensate for this. The fact that the fusion protein localizes to the projection tip suggests that a specific mechanism that directs Fus1 to the point of cell fusion exists and that although the tagged protein can no longer mediate cell fusion, it has retained this ability.

DISCUSSION

At the biochemical level, very little is understood about the actual steps of zygote formation in *S. pombe*. Once in firm contact, the cell walls and plasma membranes separating the two G1-arrested cells must be removed to permit cell fusion.
**Figure 1**

**A**

A diagram showing the relationship between the fus1 locus and the Bla and Uni4 genes, with a scale of 1 Kbp. The diagram illustrates the insertion of different vectors at the fus1 locus to study their effects on mating efficiency.

**B**


<table>
<thead>
<tr>
<th>Vector</th>
<th>250 bp</th>
<th>Mating Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>pJP20</td>
<td>250 bp</td>
<td>79.1 ± 2.8</td>
</tr>
<tr>
<td>pJP31</td>
<td>250 bp</td>
<td>76.1 ± 3.1</td>
</tr>
<tr>
<td>pJP36</td>
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<td>1.8 ± 0.23</td>
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<td>pJP34</td>
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<td>75.1 ± 2.8</td>
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<td>pJP41</td>
<td></td>
<td>70.5 ± 2.7</td>
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<tr>
<td>pJP49</td>
<td></td>
<td>2.9 ± 0.9</td>
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<tr>
<td>pJP53</td>
<td></td>
<td>68.3 ± 0.74</td>
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<tr>
<td>Wildtype</td>
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<td>74.5 ± 5.4</td>
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<td>fus1-B20</td>
<td></td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>fus1::ura4+</td>
<td></td>
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</tr>
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</table>
FIG. 8. Deletion analysis of the fus1 5′-upstream region. (A) Strategy for integration of promoter-mutated plasmids, at the fus1 locus, in EG 235 (α::ura4-D18). (B) The two TR boxes in the fus1 promoter are shown (TR1 and TR2). Replacement of the TR boxes by restriction sites was done by PCR. K. KpnI; B. BamHI. The TR box inserted in pJP53 is identical to one from the mat1-Pm promoter. Wild type, EG 282; fus1-B20, EG 382; fus1::ura4*, EG 439. The mating efficiencies of the various constructs were calculated as described in Materials and Methods. Values represent means of at least three separate trials ± standard deviations. (C) Phenotypes of selected integrants.

The initial breakdown of the wall and membrane is presumably accomplished by enzymatic activities. From the genetic and physiological behavior of the fus1 mutant, we conclude that its gene product acts at the contact zone between mating pairs to regulate or participate in cell wall degradation, reorganization, and plasma membrane fusion, and this function is usually activated in both mating types. The phenotype conferred by the null mutation is almost identical to that of the original fus1 mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5). The slightly leaky phenotype of this mutant is probably due to some residual activity, since the disrupted mutant (5).

Fus1 protein sequence: EVPFPFFPFPAP

TABLE 3. Comparison of the proline-rich sequences in fus1 with SH3 binding sites

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
<th>Amino acids</th>
<th>Reference reporting binding to an SH3 domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pombe Fus1</td>
<td>PPFPAP</td>
<td>803-812</td>
<td></td>
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<tr>
<td>S. pombe Fus1</td>
<td>PPFPAP</td>
<td>809-818</td>
<td></td>
</tr>
<tr>
<td>3BP-1</td>
<td>APFMPFGP</td>
<td>266-275</td>
<td>7</td>
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<tr>
<td>Dynamin</td>
<td>PAAPGPGAP</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>mSOS1</td>
<td>EVPPPPVVP</td>
<td>1149-1158</td>
<td>56</td>
</tr>
<tr>
<td>mSOS1</td>
<td>PPPEPPPEP</td>
<td>1210-1219</td>
<td>56</td>
</tr>
<tr>
<td>m4maChr</td>
<td>PPAPAPPP</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>mFormin</td>
<td>APAPPPPP</td>
<td>872-881</td>
<td>52</td>
</tr>
</tbody>
</table>

a Binding of the sequences shown to an SH3 domain (a 50-amino-acid domain) has been demonstrated in all cases except fus1. The two fus1 sequences are partially overlapping (Fig. 4). The proline residues (P) are in boldface.

b m. mouse.
protein, and it has no obvious signal sequence, in contrast to budding yeast FUS1.

The DNA sequence of the fus1 gene revealed a 1,372-amino-acid ORF (Fig. 4) with no significant homology to other proteins in the databases. Hence, the structure of fus1 does not suggest that it encodes any known enzymatic activity (e.g., a glucanase), but one cannot exclude the possibility that it encodes a yet unknown enzymatic activity. Another possibility is that the fus1 gene product is involved in the process of controlling degradation of the cell walls during mating. Constitutive expression of the functional HA-tagged Fus1 protein did not cause cell death, indicating that the presence of Fus1 protein in itself does not directly cause cell wall degradation. This conclusion is also supported by the fact that heterothallic strains will induce fus1 already when exposed to pheromone (Fig. 6B). Rather, it would appear that the process of cell wall degradation is triggered after cell agglutination and successful active-pair formation and that fus1 mutants cannot receive this signal. Consistent with this idea is the observation that fus1 mutants fail to switch off the cell elongation process and hence produce horsehoe-shaped prezygotes (Fig. 2C).

Comparative studies of membrane proteins have revealed that S. cerevisiae Fus1 contains an Src homology 3 (SH3) domain (54). SH3 domains are small, 55- to 70-amino-acid protein motifs involved in protein-protein interaction through recognition of proline-rich sequences (7, 69). A large number of SH3-containing proteins participate in the control of cytoskeletal organization (39), suggesting that these domains are involved in regulating the interaction of signal molecules with the cytoskeleton. SH3-mediated interaction with membrane proteins has also been observed (55). Sequence analysis of S. pombe fus1 revealed the presence of a proline-rich motif (Fig. 4). Results from comparison of this sequence with other proline-rich sequences which have been shown to interact with an SH3 domain are summarized in Table 3. Perhaps S. pombe Fus1 and S. cerevisiae Fus1 regulate cell wall degradation during mating by interaction through, respectively, a proline-rich region (the SH3 binding sites) and an SH3 domain. One could then imagine that the other interacting proteins, containing the opposite part of the SH3 binding complex, could be the products of another fus gene. Our finding that S. pombe Fus1 appears to be located at the projection tip (Fig. 9) is compatible with this idea. Whether one of the five other S. cerevisiae genes contains a proline-rich region is not yet known.

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ADDENDUM IN PROOF

We have discovered that the Fus1 protein has sequence similarity to the S. cerevisiae bud site selection protein Bni1 (GenBank accession number L31766) and the Drosophila cytokinesis protein diaphanos (GenBank accession number U11288).

REFERENCES


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