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NOTES

Regulation by the Yeast Mating-Type Locus of *STE12*, a Gene Required for Cell-Type-Specific Expression

STANLEY FIELDS[1]* AND IRA HERSKOWITZ[2]

Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794,[1] and Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California 94143[2]

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The *STE12* gene of the yeast *Saccharomyces cerevisiae* is necessary for RNA synthesis from two sets of cell-type-specific genes. We isolated a recombinant plasmid that carries the *STE12* gene by complementation of the mating defect of *ste12* cells. The DNA of the cloned gene was used to disrupt the chromosomal *STE12* gene and to identify the *STE12* transcript. We show that the *STE12* transcript level is repressed 5- to 10-fold in a/α cells. The *STE12* product thus acts to promote diploidy by activating expression of the two sets of genes necessary for mating, and then its synthesis is repressed by products unique to the diploid cell type.

Haploid cells of the yeast *Saccharomyces cerevisiae* exist as cell type **a** or α, which are able to mate with each other to form the third cell type, the **a**/α diploid. Cell type in the haploid is determined by the expression of one of two alternative sets of genes (reviewed in reference 7). In **a** cells, the **a**-specific genes are uniquely active, whereas in α cells the α-specific genes are uniquely active. The two haploid cell types are genetically identical except at *MAT*, with α cells carrying the *MAT*α allele, **a** cells the *MAT***a** allele, and **a**/α cells both alleles. *MAT*α encodes an activator of the α-specific genes (α1 product) and a repressor of the **a**-specific genes (α2 product). *MAT***a** encodes the **a**1 product, which has no known role in the **a** cell. In the **a**/α cell, **a**1 in conjunction with α2 represses the α1 gene as well as a set of genes active in both **a** cells and α cells (the haploid-specific genes). In contrast to the products encoded by *MAT*, the product of the *STE12* gene is required for mating by both α and **a** cells (6) and is necessary for expression of both α- and **a**-specific genes (5). We report here the isolation of the *STE12* gene, the construction of a null allele, and the repression of *STE12* expression by products of the mating-type locus.

To obtain the *STE12* gene, we transformed strain SF101-1c (*MAT*α *ste12-51 mfα1-lacZ ade2 his3 trp1 ura3-52*) with a clone bank of yeast fragments in a centromere-containing vector (M. Rose, J. Thomas, P. Novick, and D. Botstein, personal communication). We plated transformants for uracil prototrophy, selecting for the *URA3* gene carried on the plasmid. Approximately 24,000 transformants were then screened for their ability to mate with a tester strain. Seven colonies tested as mating proficient, and plasmid DNA was isolated from three such transformants. Preliminary restriction mapping of the three inserts showed that two of these, with inserts of 5.5 and 12.5 kilobases (kb), were related. The smaller of these, pSC4, was completely contained within the larger and thus was characterized further. Figure 1 (top) shows a restriction map of the insert from pSC4.

We demonstrated that pSC4 carried yeast DNA present at the *STE12* locus by showing that it could direct integration into the yeast genome at this site. The *Cla*I fragment of approximately 5.5 kb (Fig. 1), containing essentially the entire insert, was transferred into the yeast integrating vector YIp5 (containing the yeast *URA3* gene [16]). The resultant plasmid, pSI14-12, was linearized at a unique *Hpa*I site in the insert and used to transform the *ste12* strain SF101-1c. Transformants were selected for uracil prototrophy; all transformants tested were mating proficient. In addition, transformants were stably Ura^+ after passage on nonselective medium, indicating that the plasmid had integrated into the yeast genome and that the *Cla*I fragment can complement in single copy.

One integrant was crossed to an Ste^+ *ura3* strain, and the diploid was sporulated. Of 10 tetrads, all segregated 2 Ura^+:2 Ura^- and 4 Ste^+:0 Ste^-. The results of the *ura3* segregation indicated that the DNA had integrated at a single chromosomal site. Integration must have occurred at the *STE12* locus, because both homologs in the diploid must carry a wild-type *STE12* allele in order to observe 4:0 segregation for mating in all tetrads.

We sought to delimit the region of DNA required to complement the *ste12* mutation in order to construct a null mutation of the *STE12* gene. As indicated in Fig. 1, we generated subclones containing various restriction fragments of the *STE12* region in the vector YCp50 (described in reference 10). The subclones were transformed into strain SF101-1c, selecting for the *URA3* gene. Transformants were tested for complementation of the *ste12* mutation by scoring the ability of a patch of cells to mate to a tester strain carrying complementary auxotrophic mutations. All subclones of pSC4 failed to complement except pSAC1, which contained an insert of approximately 2.5 kb. This plasmid could restore mating ability, although at a reduced level from the wild type, as scored by the number of diploids produced per patch. The results from the other subclones indicated that the *STE12* gene must cross the leftward *Xba*I site, since the *Sac*I-*Xba*I fragment (pXSC2) and *Xba*I-*Xba*I fragment (pXBA2) that make up the 2.5-kb *Sac*I fragment both failed

* Corresponding author.

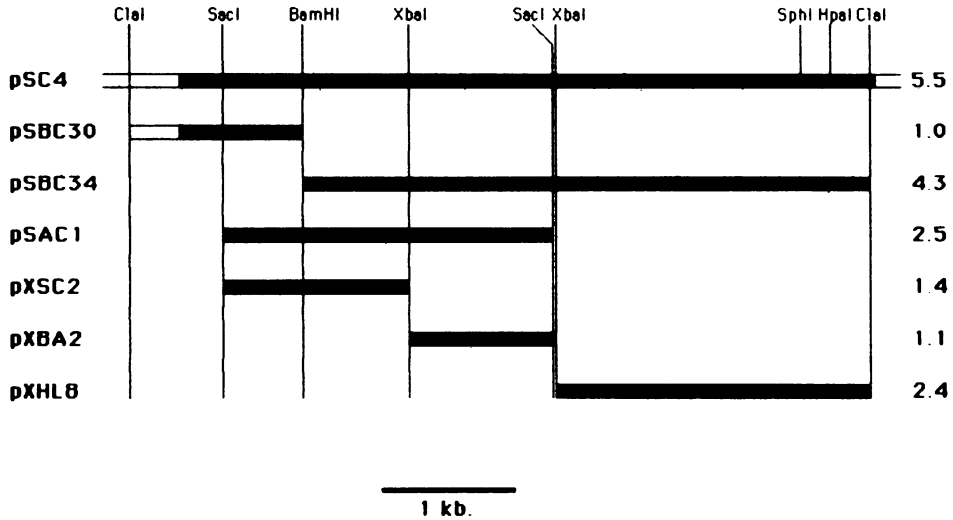

FIG. 1. Restriction map and subclones of the *STE12* region. Designations of plasmids are given on the left and the size of the yeast insert (in kilobases) on the right. The black bar indicates sequences present in the *STE12* region, and the white bar indicates vector sequences. pSC4 was isolated from a clone bank in the vector YCp50, and all subclones were made in this vector.

to complement. Similarly, the *STE12* gene must extend to the left of the *Bam*HI site, because pSBC34 failed to complement. In addition, we considered it likely that one or both ends of the gene lay close to the *Sac*I sites, because of the failure to obtain full complementation by the pSAC1 plasmid.

The complementation data suggested that the *Xba*I fragment of 1.1 kb contained part of the *STE12* gene. We replaced this DNA with a *Bgl*II fragment carrying the *LEU2* gene from the plasmid YEp13 (1) to generate plasmid pSUL16. This plasmid was cut with *Sac*I and *Sph*I to produce a fragment that had DNA from the *STE12* locus flanking the *LEU2* gene. We then used the one-step gene disruption technique (13) to replace a copy of the *STE12* gene by this *ste12*::*LEU2* construction. We transformed strain AM1788 (11), an **a**/α *STE*$^+$/*STE*$^+$ *leu2*/*leu2* strain, to leucine prototrophy. One Leu$^+$ transformant, SF167, was sporulated, and the resulting tetrads were analyzed. All 15 tetrads exhibited 2 Leu$^+$ mating-defective spores and 2 Leu$^-$ mating-proficient spores. This result indicated that the gene disruption had destroyed *STE12* function, leading to a mating defect. In addition, all spores colonies were of equal size. The equal spore viability and growth of Leu$^+$ and Leu$^-$ colonies demonstrated that the *STE12* gene does not encode an essential function. We confirmed that the gene disruption was a single replacement at the *STE12* locus by Southern blot analysis (data not shown).

TABLE 1. Mating efficiency of strains carrying *STE12* or *ste12*::*LEU2* alleles[a]

Strain	Relevant genotype	Mating efficiency
246-1-1	*MAT*α STE$^+$	0.40
SF167-1c	*MAT*α *ste12*::*LEU2*	$<3.3 \times 10^{-7}$
EG123	*MAT***a** STE$^+$	0.47
SF167-5a	*MAT***a** *ste12*::*LEU2*	$<7.7 \times 10^{-6}$

[a] Mating assays were performed as described in reference 15. Strains were grown to mid-logarithmic phase in YEPD. Samples of serial dilutions were plated both on SD minimal plates previously spread with an excess of an STE$^+$ strain of opposite mating type carrying complementary auxotrophic mutations and on YEPD plates. The mating efficiency is the ratio of colonies on SD (diploids resulting from mating) to colonies on YEPD (total cells tested). Strains were isogenic except at *MAT* or *STE12*.

The ability of *ste12*::*LEU2* segregants of SF167 to mate was assayed by a quantitative mating. As shown in Table 1, the α Ste$^+$ and **a** Ste$^+$ strains (14) mated at a high efficiency. The *ste12*::LEU2 strains produced diploids at a frequency of less than 1 in 10^5 or 10^6 cells; in fact, we failed to obtain any diploids through mating of these strains. The fact that null mutants were completely mating defective suggests that the less severe mating deficiency (approximately 10^{-4}) observed for the *ste12-51* allele we isolated previously (5) was due to leakiness of this allele.

We identified the *STE12* transcript by comparison of the RNA from an Ste$^+$ strain to one carrying the *ste12*::*LEU2* allele. Figure 2 shows a Northern blot (RNA blot) of an **a** Ste$^+$ strain (lane 1) and an **a** *ste12*::*LEU2* strain (lane 2), probed with pSI14-12. This plasmid detected a transcript of approximately 2.5 kb that was present only in the Ste$^+$ strain and absent from the *ste12* mutant, and which we thus conclude derived from the *STE12* gene. The plasmid also detected the *URA3* transcript (since the plasmid carried the *URA3* gene) and a transcript just slightly larger than that from *URA3* which were present in both strains. This other

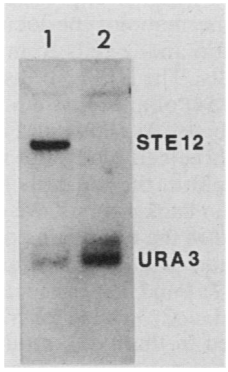

FIG. 2. Identification of the *STE12* transcript. Total RNA was isolated from EG123, **a** STE$^+$ (lane 1), and SF167-5a, **a** *ste12*::*LEU2* (lane 2), fractionated by agarose gel electrophoresis, and transferred to nitrocellulose (17). Hybridization was with nick-translated pSI14-12 DNA, which detects the *STE12* transcript, the *URA3* transcript, and another transcript just above that from *URA3* which may be encoded by a gene adjacent to *STE12*.

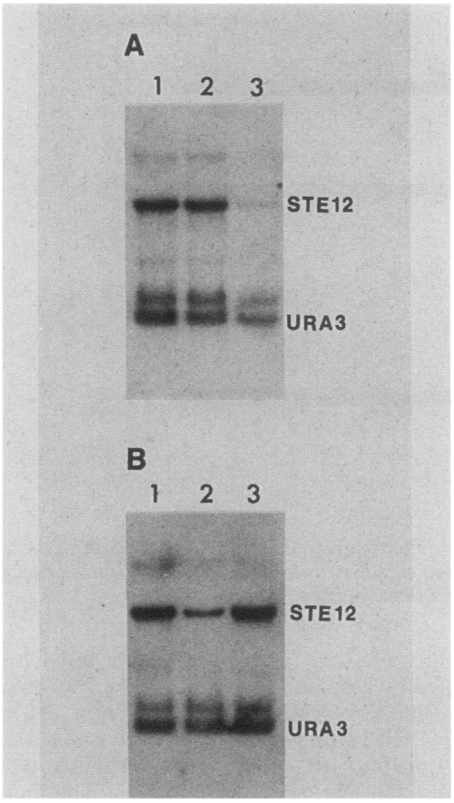

FIG. 3. Regulation of *STE12* RNA by the mating-type locus. (A) Strains carrying wild-type *MAT* alleles were analyzed for the *STE12* transcript as indicated in the Fig. 2 legend. RNA was isolated from 246-1-1, *MAT*α (lane 1); EG123, *MAT***a** (lane 2); and AM1788, *MAT***a**/*MAT*α (lane 3). (B) Strains carrying mutations at *MAT* were analyzed: AM1789, *mat***a***1*/*MAT*α (lane 1); AM1790, *MAT***a**/*mat*α*1* (lane 2); and AM1791, *MAT***a**/*mat*α*2* (lane 3).

transcript presumably is encoded by a gene adjacent to *STE12*. (In addition, we detected in some experiments high-molecular-weight material whose presence also did not correlate with whether the RNA derived from an Ste$^+$ or *ste12* strain.) A probe containing only the 1.1-kb *Xba*I fragment detected only the 2.5-kb transcript (data not shown).

We next analyzed whether expression of the *STE12* gene was regulated by the mating type locus. *STE12* activity is required by haploid **a** and α cells to mate and may thus be repressed in **a**/α cells. The *STE12* transcript was assayed in an isogenic set (11, 14) of **a**, α, and **a**/α strains differing only at the mating type locus. As shown in Fig. 3A, this transcript was present in all three cell types, but its level was reduced approximately 10-fold in the **a**/α cell. In other experiments and in different strain backgrounds, we observed a reduction of from 5- to 10-fold in the **a**/α strain compared with **a** and α strains. To determine whether this repression required the products of the *MAT***a***1* and *MAT*α*2* loci, we probed a set (11) of **a**1$^-$/α, **a**/α1$^-$, and **a**/α2$^-$ strains for *STE12* RNA (Fig. 3B). Repression occurred in the **a**/α1$^-$ strain (lane 2) but not in strains carrying a mutation in either the *MAT*α*2* gene (lane 3) or *MAT***a***1* gene (lane 1). Here the reduction in the **a**/α1$^-$ strain was only approximately half as severe as observed for the **a**/α strain (Fig. 3A), but again this may be due to experimental variation. It has been shown previously that repression in **a**/α cells does not depend on the *MAT*α*1* gene (8), which is itself turned off in **a**/α cells (9, 12).

We thus demonstrate that *STE12* function is under a kind of negative feedback because synthesis of its transcript is repressed by products present only in the diploid state. Repression of *STE12* expression in the **a**/α cell requires the combined activity of both the *MAT***a***1* and *MAT*α*2* products, as mutation of either gene restored the haploid level of expression. However, the reduction in the **a**/α cell relative to the haploid was only 5- to 10-fold. In this regard, *STE12* differs from genes like *HO* (8) or *MAT*α*1* (9, 12), whose transcripts are totally absent in the **a**/α cell, a repression greater than 100-fold. The partial repression of *STE12* is similar to that observed for the *MAT*α*2* gene, whose transcript is reduced 5-fold in the **a**/α cell (12), and for the *RME1* gene, whose transcript is reduced 10- to 20-fold in the **a**/α cell (11). This level of repression of the *RME1* gene is sufficient to allow **a**/α but not **a** or α cells to undergo sporulation (11).

Regulation of cell-type-specific expression is summarized in Fig. 4. In α cells, α-specific gene expression requires the *MAT*α*1* and *STE12* products, while **a**-specific gene expression is repressed by the *MAT*α*2* product. In **a** cells, **a**-specific

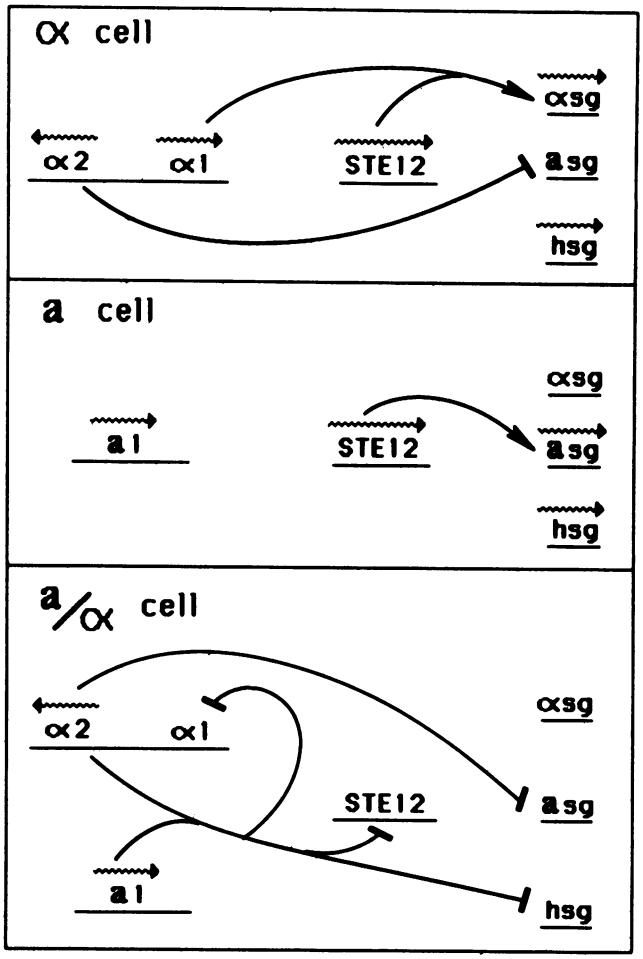

FIG. 4. Control of cell type-specific expression in α, **a**, and **a**/α cells. For each panel, the expression of the mating-type locus (*MAT*) alleles is shown on the left, *STE12* in the center, and unlinked cell type-specific genes on the right. Straight lines terminating with arrowheads represent stimulation of expression, and straight lines terminating with bars represent inhibition of expression. Wavy lines indicate gene expression. Abbreviations: α*sg*, α-specific genes; **a***sg*, **a**-specific genes; *hsg*, haploid-specific genes.

gene expression is activated by the *STE12* product. The set of haploid-specific genes is expressed in both **a** and α cells. In the **a**/α cell, the **a**1-α2 function represses transcription of *MATα1*, *STE12*, and the haploid-specific genes, and the α2 function represses transcription of **a**-specific genes. By repressing *STE12*, the diploid employs a redundant mechanism to repress α- and **a**-specific transcription.

The *STE12* product is also known to be required for RNA production driven by insertion of the transposable element Ty1 into the promoter regions of genes such as *CYC7* (4). In addition, transcription of *CYC7* in these strains is repressed approximately 5- to 10-fold in the **a**/α cell (3), as is transcription of ordinary Ty1 elements themselves (2). There are at least two different ways in which this repression of Ty1 expression might come about: it could be a direct consequence of **a**1-α2 activity, or it could be an indirect consequence of *STE12* repression. It is also possible that both mechanisms may apply, providing a redundant layer of control in the **a**/α cell to repress expression of these genes.

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LITERATURE CITED

1. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
2. **Elder, R. T., T. P. St. John, D. T. Stinchcomb, and R. W. Davis.** 1981. Studies on the transposable element Ty1 of yeast. I. RNA homologous to Ty1. Cold Spring Harbor Symp. Quant. Biol. **45**:581–584.
3. **Errede, B., T. S. Cardillo, F. Sherman, D. Dubois, J. Deschamps, and J.-M. Wiame.** 1980. Mating signals control expression of mutations resulting from insertion of a transposable repetitive element adjacent to diverse yeast genes. Cell **25**:427–436.
4. **Errede, B., T. S. Cardillo, G. Wever, and R. Sherman.** 1981. Studies on transposable elements in yeast. I. ROAM mutations causing increased expression of yeast genes: their activation by signals directed toward conjugation functions and their formation by insertion of Ty1 repetitive elements. Cold Spring Harbor Symp. Quant. Biol. **45**:593–602.
5. **Fields, S., and I. Herskowitz.** 1985. The yeast *STE12* product is required for expression of two sets of cell-type-specific genes. Cell **42**:923–930.
6. **Hartwell, L.** 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. J. Cell Biol. **85**:811–822.
7. **Herskowitz, I., and Y. Oshima.** 1981. Control of cell type in *Saccharomyces cerevisiae*: mating type and mating-type interconversion, p. 181–209. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Jensen, R., G. F. Sprague, Jr., and I. Herskowitz.** 1983. Regulation of yeast mating type interconversion: feedback control of *HO* gene expression by the mating type locus. Proc. Natl. Acad. Sci. USA **80**:3035–3039.
9. **Klar, A. J. S., J. N. Strathern, J. R. Broach, and J. B. Hicks.** 1981. Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature (London) **289**:239–244.
10. **Kuo, C.-L., and J. L. Campbell.** 1983. Cloning of *Saccharomyces cerevisiae* DNA replication genes: isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. Mol. Cell. Biol. **3**:1730–1737.
11. **Mitchell, A. P., and I. Herskowitz.** 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature (London) **319**:738–742.
12. **Nasmyth, K. A., K. Tatchell, B. D. Hall, C. Astell, and M. Smith.** 1981. A position effect in the control of transcription at yeast mating type loci. Nature (London) **289**:244–250.
13. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
14. **Siliciano, P., and K. Tatchell.** 1984. Transcription and regulatory signals at the mating type locus in yeast. Cell **37**:969–978.
15. **Sprague, G. F., Jr., and I. Herskowitz.** 1981. Control of yeast cell type by the mating type locus. I. Identification and control of expression of the **a**-specific gene, *BAR1*. J. Mol. Biol. **153**: 305–321.
16. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**: 1035–1039.
17. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.