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The *Saccharomyces cerevisiae SPT13/GAL11* Gene Has Both Positive and Negative Regulatory Roles in Transcription

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To understand the function of *SPT13*, a gene encoding a *trans*-acting factor involved in regulation of Ty-mediated gene expression, we have cloned and sequenced the gene. Our analysis revealed that *SPT13* is the same gene as *GAL11*, a gene in which mutations cause reduced expression of some *GAL4*-regulated genes. Further analysis of *spt13/gal11* mutants suggested that the *SPT13* gene also affects transcription of genes involved in mating-type specialization. We show here that *SPT13* has both positive and negative regulatory roles in transcription.

Ty insertion mutations have provided a convenient selection for the identification of genes encoding *trans*-acting factors involved in transcription. Ty elements are retrotransposons found in the yeast *Saccharomyces cerevisiae* that consist of 5.3 kilobase pairs (kb) of unique sequence flanked by 330-base-pair direct repeats called δ sequences. In addition to approximately 35 Ty elements, there are roughly 100 solo δ elements per haploid genome (7, 15). Insertion mutations caused by full Ty elements and by solo δ sequences in the 5′ noncoding regions of yeast genes can interfere with normal transcription of those genes (13, 17, 50, 60, 66). Ty element insertion mutations often reduce adjacent-gene transcription, whereas solo δ insertion mutations often alter adjacent-gene transcription qualitatively by providing an alternate and preferred site for transcription initiation (29, 59, 69). In both cases, the change in the pattern of transcription of the adjacent gene can lead to a mutant phenotype. For example, Ty and δ insertion mutations at the *HIS4* and *LYS2* genes frequently cause His^- and Lys^- phenotypes, respectively (50, 59, 60, 67). By selection for unlinked suppressors of Ty and δ insertion mutations, we have identified 15 different *SPT* (suppressor of Ty insertion mutation) genes, each of which is likely to encode a protein involved in the regulation of Ty-mediated transcription and possibly in the transcription of other yeast genes (19, 67, 68).

The upstream activation site, TATA, and initiation site sequences for Ty transcription are located in the δ sequences (14, 40). However, control of Ty and adjacent-gene transcription is also mediated by sequences located within the Ty transcription unit (18, 51). At the nucleotide sequence level, the internal regulatory sequences in Ty elements show similarity to the simian virus 40 enhancer core sequence (18, 51). Functional studies also suggest an enhancerlike activity, since these sequences, located downstream of the Ty promoter, contribute to activation of Ty transcription (12, 18, 24, 40, 51). Mutations in genes encoding factors regulating or interacting with these internal signals rather than with the upstream activation site, TATA, or initiation site present in the δ were predicted to suppress Ty but not δ insertion mutations. Mutations in the *SPT13* gene were identified in a search for this specific suppression pattern (19).

In this paper, we present further molecular and genetic analysis of *SPT13*. Our results demonstrate that *SPT13* is the same as the previously identified *GAL11* gene (45, 64), that *spt13* mutations cause pleiotropic effects, and that *SPT13* has both positive and negative regulatory roles in transcription.

MATERIALS AND METHODS

Yeast strains. All yeast strains are shown in Table 1. All strains are from our strain collection or were constructed for this study unless otherwise indicated. The *spt13* mutations used were a spontaneous mutation, *spt13-1*, and two different Tn*10-LUK* insertion mutations, *spt13-100* and *spt13-101*.

Media. The media used were as described by Sherman et al. (55) and included minimal medium (SD) with amino acids added, synthetic complete medium lacking a specific amino acid, and rich medium (YPD). Unless otherwise specified, all yeast strains were grown at 30°C.

Genetic methods. Procedures for yeast crosses, sporulation, and tetrad analysis were as described by Mortimer and Hawthorne (42) and Sherman et al. (55). Yeast transformation using either intact plasmids or DNA fragments was done using the method of Ito et al. (32). For genetic mapping of the *SPT13* gene, 95 tetrads from a cross of strains JF781 (*spt13-100 his4-519 trp1Δ1 leu2-3 ura3-52*) and LM882-13d (*his4-519 SUF17 leu2-3 ura3-52 arg1*) were dissected and analyzed. The *spt13-100* allele was scored by the Ura^+ phenotype conferred by the presence of the Tn*10-LUK* insertion in the *SPT13* gene, and *SUF17* was scored by suppression of the *his4-519* mutation. Linkage was calculated using the formula centimorgans = 50[TT+6(NPD)]/(PD+NPD+TT), where TT is tetratype, NPD is nonparental ditype, and PD is parental ditype.

Plasmids. Plasmids used as probes in Northern (RNA) hybridization analysis were as follows: FB72, a YIp5 derivative carrying an *Eco*RI-*Cla*I fragment from the *LYS2* gene, from which an internal *Bgl*II-*Xho*I fragment was isolated; pMC1871, a pBR322 derivative carrying most of the *Escherichia coli lacZ* coding region (9); pSM39, a pUC18 derivative carrying an internal *Eco*RI-*Xba*I fragment from the *MFA1* gene (41); pHK2, a pBR328 derivative carrying an internal fragment from the *MFα1* gene (38); and CSH238, a pBR322 derivative carrying an *Eco*RI-*Hin*dIII fragment from the *MAT***a** strain provided by J. Thorner. This probe hybridizes to all *MAT* transcripts as well as to transcripts from the *HML* and *HMR* loci. The amount of RNA in Northern

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TABLE 1. Yeast strains

Name	Genotype	Source or reference
JF781	*MAT***a** *spt13-100 his4-519 trp1Δ1 leu2-3 ura3-52*	
LM882-13d	*MAT*α *his4-519 SUF17 leu2-3 ura3-52 arg1*	M. Culbertson
RC634	*MAT***a** *sst1-3 ade2 his6 met1 ura1 rme1*	R. Chan
JF15	*MAT*α *lys2-128δ his4-917 ura3-52 leu2*	
JF15T1	*MAT*α *lys2-128δ his4-917 ura3-52 leu2 spt13-101*	
JF28	*MAT***a** *lys2-128δ his4-917 trp1Δ1 leu2*	
JF576	*MAT*α *spt13-101 lys2-Δ201 leu2 trp1Δ1 his4-917*	
JF577	*MAT***a** *spt13-101 lys2-Δ201 leu2 trp1Δ1 his4-917*	
JF94	*MAT*α *spt3-101 lys2-128δ ura3-52 leu2*	
MCY1345	*MAT*α *ssn6-Δ6::URA3 his4-539 lys2-801 ura3-52 SUC2*	8
JF393	*MAT*α *spt13-1 lys2-Δ201 ura3-52 trp1Δ1 leu2*	
FW979	*MAT***a** *lys2-Δ201 ura3-52*	
JF822	*MAT*α *spt13-101 his4-917 lys2 leu2 ura3-52 GAL2*	
JF906	*MAT*α *spt13-101 his4-917 lys2 leu2 ura3-52 GAL2 gal4::LEU2*	

hybridization analysis was standardized by hybridization to plasmid pFR2, which carries the yeast *PYK1* gene.

The plasmid used for generating deletions of the *SPT13* clone for sequencing was pAA3.7× (1, 2). The *GAL4* disruption plasmid used in constructing the *gal4 spt13* derivative was provided by C. Hardy and D. Shore. It consists of a 3.3-kb *Bam*HI-*Hin*dIII fragment from the *GAL4* gene, disrupted by an insertion of a 2.2-kb *Xho*I-*Sal*I fragment from the *LEU2* gene into the *GAL4 Sal*I and *Sph*I sites.

YCp*lys2-61*-Z was constructed from pGS5 (60), a YIp vector carrying the *lys2-61* allele, as follows. A 2-kb *Eco*RI-*Bam*HI fragment carrying *ARS1* and *CEN3* was isolated from pYE*CEN3*-41 (10), treated with Klenow fragment to fill in the ends, and ligated into the unique *Sma*I site of pGS5 to construct the autonomously replicating derivative, YCp*lys2-61*. After partial digestion with *Cla*I, which cuts once in Ty sequences and once in *LYS2*, linearized full-length YCp*lys2-61* molecules were gel purified, treated with calf intestinal phosphatase, and ligated with a *Taq*I digest of a *Pst*I fragment carrying the *E. coli lacZ* gene from plasmid pMC1871 (9). *lacZ*-containing derivatives were identified by colony hybridization, using the *Pst*I *lacZ* fragment as a hybridization probe, and characterized by restriction analysis. Several different *Taq*I inserts were isolated. Two different isolates, YCp*lys2-61*-Z1 and YCp*lys2-61*-Z2, were used in the experiment shown in Fig. 1.

Sequence analysis. Nested 5′ and 3′ deletions of an 8-kb *Sph*I fragment containing *SPT13* were generated in vivo by using the deletion strategy described by Ahmed (1, 2). Sequencing was accomplished by using the chain termination method (53), [^{35}S]dATP (Amersham Corp.), and the Sequenase sequencing kit (U.S. Biochemical Corp.). All sequencing was performed by using double-stranded templates and a primer, 5′-d(GCCACTGGAGCACCTC) (prepared by Paul Tempst with an Applied Biosystems 380B DNA synthesizer), complementary to IS*1* sequences 44 nucleotides before the deletion junction.

Mating-factor and barrier assays. The mating-factor assay was performed as described by Julius et al. (34), with minor modifications. Approximately 6×10^7 tester cells (RC634) were spread onto a YPD plate that was immediately replica plated to a second YPD plate. Strains to be tested were patched onto the replica plate. Plates containing the patches and the RC634 lawn were incubated at 30°C for 3 days. The production of barrier was also assayed on replica-plated lawns of RC634 by using a modification of the method of Sprague and Herskowitz (62). The strains to be assayed were streaked immediately adjacent to circular patches of strain JF15 (*MAT*α) cells. The plate was incubated at 30°C for 3 days. The production of barrier by the strains being tested was assayed by whether they allowed the lawn to grow in the presence of the α-factor produced by JF15.

RNA isolation and Northern hybridization analysis. Cells were grown to a concentration of 1×10^7 to 2×10^7/ml in YPD or in SD medium supplemented with amino acids. RNA was prepared by the method of Carlson and Botstein (8). Electrophoresis, blotting, and hybridization were performed as previously described (19). ^{32}P-labeled probes were prepared by nick translation (49) or by using random primers according to the method of Feinberg and Vogelstein (20, 21).

RESULTS

Molecular and genetic analyses of the *SPT13* gene. To further investigate the role of *SPT13* in transcription, we cloned, genetically mapped, and began to sequence the *SPT13* gene. To clone the *SPT13* gene, an *spt13 his4-917* mutant strain (JF393; His$^+$ phenotype) was transformed with a yeast genomic library in the vector YCp50 (52). Ura$^+$ transformants were screened for those that had acquired a His$^-$ phenotype. One candidate was obtained out of the 11,000 transformants screened. The identity of the cloned gene was confirmed by showing that it directs integration of a plasmid to the *SPT13* locus. Localization and disruption of the plasmid-born gene were accomplished by Tn*10-LUK* transposon mutagenesis (31). The null phenotype conferred by the disrupted gene (Spt$^-$) confirmed that the *spt13*-mediated suppression pattern was due to loss of *SPT13* function (19). Five different Tn*10-LUK* insertions that disrupted *SPT13* function (failed to rescue the His$^+$ phenotype of an *spt13* mutant) and two that partially disrupted function (resulted in a His$^{+/-}$ phenotype) were obtained. On the basis of the map positions of these insertion mutations, the gene was estimated to be a maximum of 3.8 kb in size.

To determine its genetic map position, the *SPT13* gene was first physically mapped to chromosome XV by hybridization of an *SPT13* probe to a blot of separated yeast chromosomes generated by field inversion gel electrophoresis of total yeast DNA. Tetrad analysis from a three-factor cross placed the gene 3 centimorgans (cM) centromere proximal to *ARG1* on the left arm of chromosome XV (Table 2). The distance calculated for the *SUF17*-to-*ARG1* interval (2 cM) is in reasonable agreement with the previously reported value of 3 cM (25).

***SPT13* is the same gene as *GAL11*.** Our sequence analysis revealed that *SPT13* is identical to the previously identified *GAL11* gene, mutations in which cause a moderate decrease in expression of some but not all *GAL4*-regulated genes (45, 64). Although our *SPT13* sequence was incomplete at the time of the publication of the *GAL11* sequence, the *SPT13* sequence we obtained matches bases −834 through −660,

TABLE 2. Determination of the *SPT13* map position by tetrad analysis

Test markers	Tetrad analysis			Map distance (cM)
	PD	NPD	TT	
spt13, *SUF17*	93	0	2	1.1
spt13, *arg1*	89	0	6	3.2
SUF17, *arg1*	91	0	4	2.1

−31 through 993, 1180 through 1550, and 1730 through 2630 (coordinates of Suzuki et al. [64]) in addition to 1,400 bases of 5′- and 3′-flanking sequence. In addition, the null mutation, *spt13-101*, confers the same weak Gal$^-$ phenotype described for *gal11* mutants (45, 64).

Effect of *spt13* mutations on Ty transcription. Although the precise role of *SPT13* in expression of *GAL4*-regulated genes is not known, this additional *spt13*/*gal11* phenotype suggests that *SPT13* is a positive regulator of gene expression. In contrast, previous Northern hybridization analysis of Ty insertion alleles demonstrated that suppression of Ty insertion mutations in *spt13* mutants is due to elevated levels of adjacent gene transcription, suggesting that, in that case, *SPT13* is a negative regulator of gene expression (19). Given these apparently opposite effects on gene expression, we considered the possibility that the *spt13* effect on Ty-mediated gene expression is an indirect result of a reduction in Ty transcription. By this model, a decrease in Ty transcription in *spt13* mutants would allow the increase in adjacent-gene transcription. Although previous work had demonstrated that *spt13* mutations had no significant effect on total Ty transcription, transcription of the particular Ty element in the insertion mutation in question was not specifically examined. Therefore, an effect on that specific Ty element could not be ruled out.

To establish conclusively that *spt13*-mediated activation of adjacent-gene transcription is not accompanied by reduction in Ty transcription, we examined the effect of an *spt13* mutation on a single Ty element at the *LYS2* locus rather than on total Ty transcription. The *lys2-61* Ty insertion, which is located between the *LYS2* transcription initiation site and the start of translation (23, 60; J. S. Fassler and F. Winston, unpublished results), is suppressed by *spt13* mutations. To analyze transcription of this particular Ty element, fragments from the *E. coli lacZ* gene were inserted into the *Cla*I site at position 3582 in the Ty element of *lys2-61* on plasmid YCp*lys2-61* (Fig. 1A; see Materials and Methods). The Lys$^-$ phenotype of *SPT13*$^+$ *lys2Δ201*(YCp*lys2-61-Z*) and the Lys$^+$ phenotype of *spt13 lys2Δ201*(YCp*lys2-61-Z*) strains showed that the *lacZ* inserts did not interfere with the phenotype of the *lys2-61* mutation or with the ability of *spt13* mutations to suppress the *lys2-61* insertion allele.

Northern hybridization analysis using *LYS2* and *lacZ* probes showed that *spt13*-mediated activation of adjacent-gene transcription was accompanied by a high level of transcription of the adjacent Ty element (Fig. 1B). In fact, transcription of the Ty element was also somewhat elevated in the *spt13* mutant, although much less so than transcription of *LYS2*. This result is consistent with the data of Coney and Roeder (12) showing that the Ty activator region has a strong effect on adjacent-gene expression and a modest effect on Ty expression. This transcription pattern suggests that *SPT13* is a negative regulator of both Ty and Ty-mediated transcription.

On the basis of the model of Suzuki et al. (64), which suggests that *SPT13*/*GAL11* may interact with *GAL4* to activate transcription of the *GAL* genes, we considered the unlikely possibility that *spt13*-mediated suppression of Ty insertion mutations also required *GAL4*. However, we found that *gal4 spt13* (JF906) and *GAL4 spt13* (JF822) strains were indistinguishable in suppression of the Ty insertion mutation, *his4-917* (data not shown). This result strongly suggests that *SPT13* regulates or interacts with other factors in addition to *GAL4*.

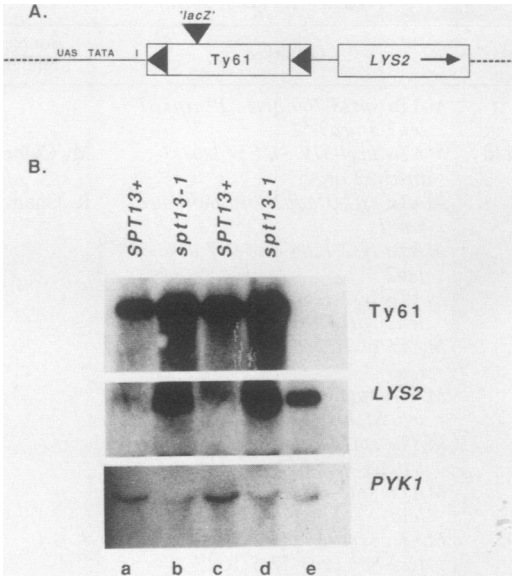

FIG. 1. Effect of an *spt13* mutation on Ty and adjacent-gene transcription. (A) Structure of the modified *lys2-61* allele in plasmids YCp*lys2-61*-Z1 and YCp*lys2-61*-Z2. UAS, Upstream activation sequence. (B) Hybridization analysis of wild-type (FW979) and *spt13* mutant (JF393) strains deleted for the genomic *LYS2* gene (*lys2Δ201*) and carrying either YCp*lys2-61*-Z1 (lanes a and b) or YCp*lys2-61*-Z2 (lanes c and d) (see Materials and Methods). RNA from an *SPT13*$^+$*LYS2*$^+$ control strain carrying no plasmid was probed in lane e. Cells were grown at 20°C in minimal medium lacking uracil to select for the continued presence of the plasmid. The 20°C growth temperature was used because *lys2-61* is a cold-sensitive mutation; in an *SPT13*$^+$ background, the phenotype was Lys$^+$ at 37°C, Lys$^{+/-}$ at 30°C, and Lys$^-$ at 20°C. The filter was hybridized individually with gel-isolated ^{32}P-labeled *LYS2*, *lacZ*, and *PYK1* (pyruvate kinase) probes. The *lacZ* probe hybridizes to Ty*61* transcripts; the *LYS2* probe hybridizes to the *LYS2* transcript; and *PYK1* hybridization was used to normalize amounts of RNA loaded in each lane. Probes were removed from the filter between hybridizations. Exposure times differed for each analysis.

Regulation of mating-type gene expression. The pleiotropic phenotype of *spt13* mutants suggests that *SPT13* may also be involved in regulation of additional genes. In addition to suppression of Ty insertion mutations and reduced *GAL4*-regulated gene expression, *spt13* mutant phenotypes include mating and sporulation defects (19). We therefore tested whether the *spt13*-associated mating defect reflects a role for *SPT13* in transcriptional regulation of mating-type genes.

In mating-type tests, *MAT*α *spt13* but not *MAT***a** *spt13* strains mated poorly. To determine the reason for this apparent α-specific mating defect, we first examined *SPT13* transcription in *MAT***a** and *MAT*α cells. Although suppression of Ty insertion mutations is not specific to *MAT*α *spt13* mutants, we considered the possibility that *MAT*α cells require higher concentrations of SPT13 product to successfully execute an *SPT13*-mediated mating function. In North-

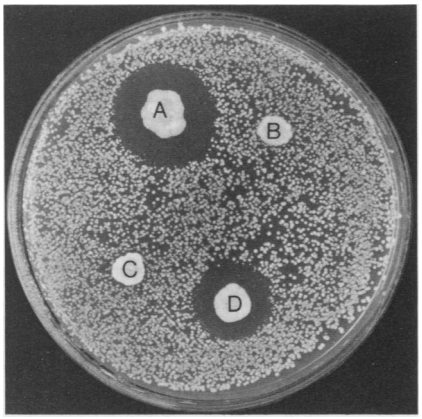

FIG. 2. Effect of an *spt13* mutation on α-factor production. (A) Wild type (JF15); (B) *spt13-101* (JF15T1); (C) *ssn6-Δ6::URA3* (MCY1345) (54); (D) *spt3-101* (JF94). All strains are *MAT*α and were spotted onto a lawn of *MAT***a** cells that are supersensitive to growth inhibition by the opposite mating factors because of a mutation in the *SST1* gene. The zone of growth inhibition around the spotted strains is a measure of the amount of mating factor produced. The tester lawn is RC634 (*MAT***a** *sst1-3*) (34).

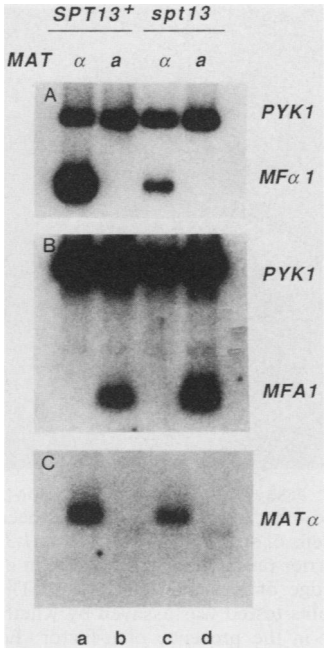

FIG. 3. Effect of an *spt13* mutation on transcription of genes involved in mating-type specification. Transcription of *MFA1*, *MFα1*, and *MAT*α genes is shown in *SPT13*+ and *spt13* strains of both mating types. Approximately 10 μg of total RNA was loaded in each lane. RNA levels were normalized by using hybridization to the *PYK1* message. The filter shown in panel A was stripped and rehybridized with the *MAT*α probe for panel C. Electrophoresis, blotting, and hybridization conditions were as described previously (19) except that the gels were 1.5% agarose. The probes were as follows: (A) *MFα1*, pHK2 (38); (B) *MFA1*, pSM39 (41); and (C) *MAT***a**, pCSH283. Since the *MAT***a** and *MAT*α loci share extensive homology (36), the *MAT***a** probe hybridized to *MAT*α transcripts (lanes a and c) as well as to *MAT***a** transcripts (seen faintly in lanes b and d). In this experiment, the *MATα1* and *MATα2* transcripts comigrated; therefore, the transcript designated *MAT*α is actually a composite transcript consisting of both *MATα1* and *MATα2* transcripts. Lanes: a, *MAT*α *SPT13*+ (JF15); b, *MAT***a** *SPT13*+ (JF28); c, *MAT*α *spt13-101* (JF576); d, *MAT***a** *spt13-101* (JF577).

ern hybridization experiments comparing RNAs from *MAT***a** and *MAT*α *SPT13*+ strains, we detected no difference in the levels of *SPT13* transcripts (data not shown).

Levels of the mating pheromone α-factor were examined in *MAT*α *SPT13*+ and *spt13* strains (Fig. 2). Since mating pheromones arrest growth of cells of the opposite mating type, it is possible to assess relative pheromone levels in different mutants by comparing the extent to which cells of one mating type inhibit growth of cells of the other mating type. In this halo (inhibition of growth) assay, the *spt13* mutant, like the α-specific sterile mutant *ssn6* (54), produced no α-factor halo, in contrast to a 5- to 6-mm halo formed by the *SPT13*+ strain. Like *spt13* mutants, *spt3* mutants are also mating defective and produce less α-factor (28); however, the *spt3* α-factor defect appears to be leakier than that of *spt13* mutants. Northern hybridization analysis showed that transcription of *MFα1*, the gene encoding the majority of the α-factor in the cell (5, 38, 61), was substantially reduced in *spt13* mutants (Fig. 3A, lanes a and c). Therefore, the reduction in α-factor in *spt13* mutants was at least partially caused by a reduction in *MFα1* transcript levels.

Since *MFα1* transcription is positively regulated by *MATα1* (16, 63), *MATα1* transcription was also examined. The *MATα1* and *MATα2* genes are transcribed divergently from a common promoter (27, 36, 43, 44, 58) and are likely to be coordinately regulated in haploids. *spt13* mutants produced reduced levels of *MATα1* or *MATα2* RNA (or both) (Fig. 3C). (Since we were unable to separate the *MATα1* and *MATα2* mRNAs, we could not definitively conclude whether one or both mRNA levels were reduced.) Therefore, we propose that *SPT13* has a positive regulatory role in expression of genes at the *MAT*α locus. The effect of *spt13* on *MFα1* transcription, therefore, may be partially indirect, via the effect on *MATα1* expression. We think it unlikely that the reduction in *MATα1* transcription accounts for the entire *spt13* effect on *MFα1* expression.

Since *MATα2* is responsible for repression of *MAT***a**-specific genes in *MAT*α cells (33), the observation that *MATα2* RNA levels may be reduced in *spt13* mutants suggests that *MAT*α *spt13* mutants might exhibit some **a**-specific gene expression. To test this prediction, we examined transcription of the **a**-specific gene, *MFA1*, one of the two genes that encode **a**-factor (4). Transcription of the *MFA1* gene was not detectable in *MAT*α *spt13* mutants (Fig. 3B, lane c). However, the *spt13* mutation caused a small increase in transcript levels of *MFA1* in *MAT***a** cells. The significance of the small increase in expression of this gene in the *MAT***a** *spt13* strain is not clear.

We also examined expression of the **a**-specific gene *BAR1*, a gene that, like *MFA1*, is expressed specifically in *MAT***a** cells (37). *BAR1* is involved in the extracellular degradation of α-factor in *MAT***a** cells (37). In a standard plate assay for *BAR1* activity, *MAT*α *spt13* mutants were compared with *MAT*α *SPT13*+, *spt3*, and *ssn6* mutants. *MAT*α *spt13* mutants could be clearly shown to express barrier activity (Fig. 4), just as has been shown previously for *ssn6* mutants (54). The effect of the *spt13* mutation on *BAR1* expression in *MAT*α cells is consistent with a reduction in *MATα2* RNA levels in *spt13* mutants. In addition, the aberrant expression of *BAR1* must contribute to the reduction in the α-factor halo size seen in *spt13* mutants.

DISCUSSION

In this work, we show that the *S. cerevisiae SPT13* gene participates in the regulation of a diverse set of genes

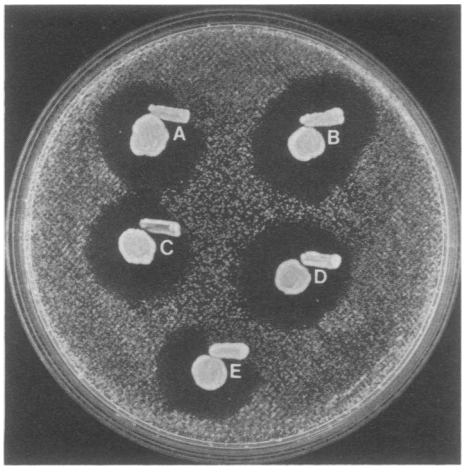

FIG. 4. Barrier assay. Spots of the α-factor-producing strain JF15 were patched onto a YPD plate that had been freshly spread with roughly 10^6 cells of strain RC634 (*MAT***a** *sst1-3*). Each strain to be assayed for barrier production was gridded in a small streak by the upper right edge of a round JF15 patch. The production of barrier by the strains tested was assayed by whether they allowed the lawn to grow in the presence of α-factor. For example, the barrier-producing control strain in streak E allowed growth of the lawn near the streak; the barrier-nonproducing control strain in streak B had a large clear zone around the streak. Strains tested were as follows: (A) *MAT*α *spt13-101* (JF15T1); (B) *MAT*α *SPT13*$^+$ (JF15); (C) *MAT*α *ssn6-Δ6::URA3* (MCY1345); (D) *MAT*α *spt3-101* (JF94); and (E) *MAT***a** *SPT13*$^+$ (JF28).

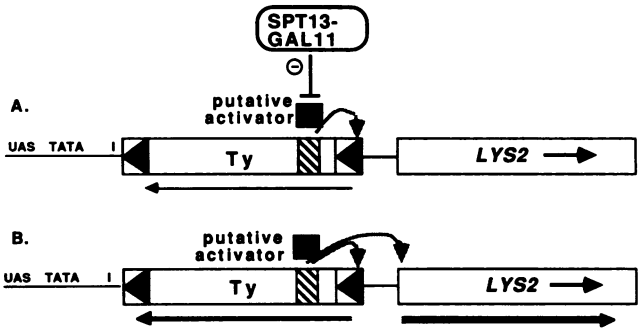

FIG. 5. Model for *SPT13*-mediated regulation of Ty and adjacent-gene transcription. Activation of Ty and adjacent-gene transcription is mediated by a positive regulatory factor of unknown identity whose activity is negatively regulated by *SPT13*. (A) *SPT13*$^+$ represses activation of adjacent-gene transcription while permitting activation of Ty transcription; (B) in an *spt13* mutant, both Ty transcription and adjacent-gene transcription are increased. UAS, Upstream activation sequence.

(summarized in Table 3). Analysis of the mating-defective phenotype of *spt13* mutants shows that in addition to regulation of Ty, Ty-mediated, and *GAL* gene expression, *SPT13* also has a role in regulation of genes involved in specifying mating type. The major effect of *spt13* mutations on mating-type gene expression that we observed is the reduction in the level of *MF*α*1* transcripts. A smaller effect on *MAT*α*1* and *MAT*α*2* gene transcripts was also observed. Although we were unable to distinguish the individual *MAT*α transcripts, we found that the activities of both *MAT*α*1*- and *MAT*α*2*-regulated genes were affected in a way that suggests a reduction in expression of both genes (α-factor production was lowered and barrier production was not fully repressed in *MAT*α cells). The conclusion that *MAT*α*2* expression is decreased in *spt13* mutants, although supported by the presence of BAR1 activity in *MAT*α cells, is not supported by the absence of detectable *MFA1* expression in *MAT*α cells. Possibly, *BAR1* expression and *MFA1* expression are differentially sensitive to the reduced *MAT*α*2* levels.

From the analysis of *spt13* mutant phenotypes, we conclude that *SPT13* can participate in both positive and negative regulation. The transcriptional role of *SPT13* appears to vary with the gene being regulated. The data do not allow discrimination among at least three distinct possibilities: (i) SPT13 is a DNA-binding protein with both positive and negative regulatory activities, (ii) SPT13 regulates the expression of other transcription factors, or (iii) SPT13 interacts with other regulatory factors to achieve positive or negative regulation.

TABLE 3. Summary of *SPT13*-regulated genes

Affected gene(s)	Role of *SPT13*	Reference(s)
Ty	Negative regulator	19; this work
Ty adjacent	Negative regulator	19; this work
GAL4 regulated	Positive regulator	45, 64
*MF*α*1*	Positive regulator	This work
*MAT*α*1* and *MAT*α*2*	Positive regulator	This work

Although the *SPT13* sequence includes a weak helix-turn-helix domain (64), there is currently no experimental support for the *SPT13* gene product binding directly to DNA. Computer-assisted searches for sequence similarity between Ty1 and the *GAL1-GAL10* promoter reveal no significant matches. Furthermore, in gel retardation experiments involving Ty enhancer fragments, the absence of SPT13 from the protein extract had no effect on the mobility of the shifted bands (J. Fassler, unpublished results).

We therefore favor a model in which SPT13 interacts with additional proteins to carry out its effects. By this model, the nature of the effect at a given gene would depend on the other proteins involved. For example, SPT13 might interact with GAL4 to help activate transcription at some GAL4-regulated genes. With respect to Ty transcription and Ty-mediated gene expression, SPT13 could interact with proteins that bind to the Ty enhancerlike region, resulting in inhibition of adjacent-gene expression (Fig. 5). Several Ty DNA-binding activities that are potential targets for SPT13 action have been identified (11, 26). We cannot, of course, rule out the possibility that the negative effect on Ty-mediated gene expression by *SPT13* is indirect and occurs by positive regulation of a negative regulator.

The observation that the SPT13 gene product is involved in both positive and negative regulation is reminiscent of the dual regulatory activities of the regulatory factor RAP1 (6, 30, 56, 57). RAP1 was identified as a DNA-binding protein likely to be involved in repressing expression of mating-type genes at the silent mating-type loci (57). In contrast to its presumed role as a repressor at the silent mating-type locus, RAP1 is believed to activate transcription at a variety of other loci, including genes for ribosomal protein, translation elongation factors, *TEF1* and *TEF2*, and the *MAT*α locus (6, 30, 57). The RAP1 protein has been shown to bind to the *MAT*α upstream activation sequence (56).

Although *RAP1* is an essential gene and *SPT13* is not, we examined the possibility that *SPT13* regulates expression of *RAP1*. In preliminary experiments, we detected no change in levels of *RAP1* transcripts in *MAT*α *SPT13*$^+$ and *spt13*

strains. The *MATα* genes are therefore apparently under the control of both *RAP1* and *SPT13*. The possibility that SPT13 interacts with RAP1 or regulates its activity is currently under investigation.

A strong parallel between the activities of SPT13 and the transcription factor PRTF (3, 35, 46) can also be made. PRTF, also called GRM (35) and *MCM1* (46), though present in all cell types, interacts with cell-type-specific factors, turning on α-specific gene expression in α cells (3) and **a**-specific gene expression in **a** cells (3) and turning off **a**-specific gene expression in α cells (35). Thus, PRTF is both a positive and a negative regulator that functions at the level of protein-protein rather than DNA-protein interaction. This is similar to the role we envision for SPT13.

One noteworthy feature of the predicted SPT13 protein is the prevalence of glutamine: 170 of 965 amino acid residues are glutamine (64). As noted by Suzuki et al. (64), many of the glutamine residues occur within two tracts of poly (glutamine) and one of poly(glutamine-alanine). Although the significance of poly(glutamine) tracts is unknown, this sequence feature has been noted in several other *S. cerevisiae* genes of diverse function, including *SSN6* (54), *HAP2* (47), and *MCM1* (46), as well as several *Drosophila* homeotic genes such as *notch* (65), *engrailed* (22, 48), and *antennapedia* (39).

In addition to this and other similarities between *SSN6* and *GAL11/SPT13* noted by Suzuki et al. (64), we show that *spt13* mutants, like *ssn6* mutants, have an α-specific sterile phenotype. In both mutants, the **a**-specific gene, *BAR1*, is aberrantly expressed in *MATα* cells. However, the *spt13* mating defect differs from that of *ssn6* in that *spt13* mutants show a substantial reduction in *MFα1* transcription, whereas the *ssn6* mutation has no effect on *MFα1* expression. Interestingly, mutations in the *MCM1* gene, which encodes the poly(glutamine)-containing transcription factor PRTF, also result in an α-specific sterile phenotype (46).

In conclusion, *SPT13* is both a positive and a negative regulatory factor that affects transcription of a diverse set of genes. The mechanism of *SPT13* participation in these regulatory systems and the identification of other roles for SPT13 will hopefully be elucidated by further genetic and molecular analyses.

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