Three Genes Are Required for trans-Activation of Ty Transcription in Yeast

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ABSTRACT

Mutations in the SPT3 gene were isolated as one class of suppressors of Ty and solo δ insertion mutations in Saccharomyces cerevisiae. Previous work has shown that null mutations in SPT3 abolish the normal Ty δ-δ transcript; instead, a transcript that initiates 800 bases farther downstream is made, suggesting that SPT3 is required for transcription initiation in δ sequences. We have selected for new spt mutations and have screened for those with the unique suppression pattern of spt mutations with respect to two insertion mutations. Our selection and screen has identified two additional genes, SPT7 and SPT8, that are also required for transcription initiation in δ sequences. We show that mutations in SPT7 or SPT8 result in the same alteration of Ty transcription as do mutations in SPT3. In addition, mutations in all three genes cause a sporulation defect. By assay of a Ty-lacZ fusion we have shown that spt3, spt7 and spt8 mutations reduce transcription from a δ sequence by 10–25-fold. Finally, we show that SPT3 mRNA levels are unaffected in either spt7 or spt8 mutants, suggesting that these two genes do not regulate transcription of SPT3.

The Ty elements of the yeast Saccharomyces cerevisiae are a dispersed set of repetitive transposable genetic elements. They are 5.9 kb long, flanked by direct terminal repeats called δ sequences (Cameron, Lo and Davis 1979). Ty elements are a member of a group of eukaryotic transposable elements termed retrotransposons (Boeke et al. 1985). This group also includes copia-like elements of Drosophila and retroviral proviruses of mammals. Ty elements share several structural characteristics with these other elements. They contain two open reading frames, analogous to the gag and pol open reading frames of retroviruses (Clare and Farabaugh 1985; Warmington et al. 1985; Hauber, Nelbock-Hochstetter and Feldmann 1985); Ty transcription occurs via an RNA intermediate (Boeke et al. 1985); and Ty elements encode a reverse transcriptase (Garfinkel, Boeke and Fink 1985; Mellor et al. 1985).

Insertion of Ty elements or their solo δ derivatives in the 5' noncoding region of genes can inhibit or otherwise alter adjacent gene expression [for a review, see Roeder and Fink (1983)]. Ty transcription initiates in the 5' δ (LTR) and proceeds across the element, terminating in the 3' δ (Elder, Lo and Davis 1983). Ty transcription and the effect of Ty insertion mutations on expression of adjacent genes can be affected by the state of several different yeast genes, including those that affect mating (MAT and some STE genes; Errede et al. 1980) and several genes designated SPT, TYE and ROC (Winston et al. 1984; Ciriacy and Williamson 1981; Dubois, Jacobs and Jauniaux 1982).

We have previously shown that the yeast SPT3 gene product is necessary for transcription initiation in δ sequences of Ty elements (Winston, Durbin and Fink 1984). In the absence of SPT3, Ty transcription initiates 800 bp farther downstream, in the internal region of Ty elements. A similar transcriptional effect on solo δ insertions in the 5' noncoding region of genes also has been observed in spt3 mutants. In wild-type strains, transcription initiates in the solo δ; in spt3 mutants transcription initiates farther downstream. The virtual abolition of normal length Ty transcripts in an spt3 mutant results in elimination of Ty transcription, since Ty RNA is an essential intermediate in the transposition process (Boeke, Styles and Fink 1986).

Mutations in SPT3 cause several other striking mutant phenotypes, including suppression of Ty and δ insertion mutations and defects in mating and sporulation (Winston et al. 1984; Winston, Durbin and Fink 1984). This variety of phenotypes suggests that SPT3 is important for normal transcription of cellular sequences in addition to Ty elements or that Ty transcription is required for these functions.

In this paper, we describe the identification of two additional yeast genes, SPT7 and SPT8, that are also required for transcription from δ sequences. Our results show that spt7 and spt8 mutants display many of the same phenotypes as spt3 mutants. These include suppression of insertion mutations and a sporulation...
defect. Furthermore, we show that SPT7 and SPT8 do not regulate transcription of SPT3. These results indicate that these three genes act together in regulation of Ty transcription.

MATERIALS AND METHODS

**Yeast strains:** The designations for all yeast strains are standard (SHERMAN 1981). The yeast strains used in this study are listed in Table 1. Genotypes listed in brackets indicate integrated plasmids.

**General genetic methods:** Standard yeast genetic procedures of crossing, sporulation and tetrad analysis were followed as described by Mortimer and Hawthorne (1969) and Sherman, Fink and Lawrence (1978). For measurement of sporulation frequency, cultures were sporulated for 1 day at 23° and 2 days at 30° on solid sporulation medium. Sporulated cultures were examined by light microscopy in a hemacytometer. Sporulation frequency is the number of tetrads divided by the sum of tetrads and unsporulated cells.

**Media:** All media were made as described by Sherman, Fink and Lawrence (1978). These include rich media (YPD), minimal media (SD) and sporulation media. SD complete refers to SD media containing the amino acid requirements of the particular strain being grown. SC-his and SC-lys are complete synthetic media (Sherman, Fink and Lawrence 1978) lacking histidine and lysine, respectively. Solid media contained 2% agar.

**Isolation of mutants:** All of the mutations described in this paper were isolated in strains FW667 and L37 (Table 1). Both of these strains have a His* Lys* phenotype due to the insertions his4-9126 and lys2-173R2. The spt mutants were isolated essentially as described by making patches from single colonies on YPD plates and then replica-plating them to SC-his plates (Winston et al. 1984). For mutants isolated from strain FW667, we picked a single His* colony from each patch, purified it on SC-his plates and then scored the His and Lys phenotypes. For mutants isolated from strain L37, we replica-plated the SC-his selection plates directly onto SC-lys and SC-his plates. Then, we picked one His* Lys* candidate from each patch for colony purification and further analysis.

**Complementation and dominance analysis:** Analysis of His* mutants to determine if they contained spt3 mutations was done by a replica plating test. For mutants isolated from strain FW667, we replica-plated patches of the candidates to YPD plates. We then replica-plated confluent lawns of strains L6 and L37 to the same YPD plates and incubated the plates overnight. The following day, the YPD plates were replica-plated to SD plates containing histidine and lysine to score for diploid formation and to SD plates containing only lysine to score for dominance (in the tests with strain L37) or complementation of the spt3 mutation (in the tests with strain L6). For mutants isolated from strains L37 we followed the same procedure, using strains FW508 and FW667 as the testers for complementation and dominance, respectively.

Complementation tests between the new mutants were initially done by cross replica plating stripes of the different candidates as previously described (Winston et al. 1984). We then constructed heterozygous diploids using representative alleles from each group and the ability of each purified diploid to grow on plates without histidine was determined.

**Northern hybridization analysis:** Analysis of transcription by Northern hybridization experiments was done as previously described (Winston, Durbin and Fink 1984). In some cases, instead of baking the filter at 80° for 2 hr, RNA was cross-linked to Genescreen (New England Nuclear) by irradiation of the filter for 2 min with 1200 μW/cm² of UV light (Church and Gilbert 1984). Ty mRNA was measured with the probe B161, an internal Ty BglIII restriction fragment in pBR322 (R. Surosky, B. Tye and G. R. Fink, unpublished data). SPT3 mRNA was detected with the probe pFW42, an EcoRI-XhoI SPT3 fragment in pBR322. This probe also faintly detects a second RNA slightly larger than SPT3 mRNA (Winston and Hart 1986). The intensities of bands on the autoradiograms were normalized to those for pyruvate kinase mRNA (Burke, Tekamp-Olson and Najarian 1983), detected with the plasmid pFR2, provided by Dr. P. Sinha.

**S1 nuclease protection analysis:** S1 protection experiments were done as previously described (Winston, Durbin and Fink 1984). The probe was B163, a Ty BglI restriction fragment in pBR322 (R. Surosky, B. Tye and G. R. Fink, unpublished data).

**Construction of Ty-lacZ fusions:** To analyze a Ty-lacZ fusion, we constructed strains that contain a single copy of the Ty912Δ44-lacZ fusion integrated into the yeast genome at the URA3 locus. To construct such an integrant, we began with plasmid pJCA44, a 2-μm circle containing plasmid that contains 338 bp of Ty912 fused to the Escherichia coli lacZ gene, encoding a hybrid haβ-galactosidase protein (J. Clare and P. Farabaugh, unpublished data). From this, we constructed plasmid pFW82, which lacks the yeast 2-μm circle EcoRI fragment. To integrate a single copy of pFW82 into the genome, it was digested with the restriction enzyme SmaI, which cuts the plasmid once, in the URA3 gene. Such a linearized plasmid, upon transformation into yeast, should integrate at the URA3 locus (Orr-Weaver, Szostak, and Rothstein 1981). We transformed the linearized plasmid into strain FW1094 (Table 1) and selected Uras transformants. Transformants were confirmed to contain a single copy of the plasmid integrated at URA3 by two tests. First, we crossed the transformants by a URA3 strain and observed 4:0 segregation for Ura*:Ura- in all tetrads. Second, to ensure that only a single copy of the plasmid had integrated, we did Southern hybridization analysis of BamHI-digested DNA prepared from the transformants. By these criteria, all transformants contained a single copy of the plasmid integrated at URA3. One transformant of strain FW1094, strain FW1154, was used in crosses to generate all strains subsequently used for analysis of the Ty-lacZ fusion. All progeny of these crosses used in the β-galactosidase assays were rechecked by Southern hybridization analysis to verify that they contained a single copy of the Ty-lacZ fusion.

**β-Galactosidase Assays:** β-Galactosidase assays were performed as described by Rose, Casadaban and Botstein (1981). β-Galactosidase levels were normalized to total cellular protein (Bradford 1976). To grow cells for preparation of extracts, overnight cultures were first grown in SD complete media. These cultures were used to inoculate fresh SD complete media and cultures were grown to approximately 2 x 10⁶ cells/ml. For wild type and for each spt mutant, we assayed four different strains. All assays were repeated at least three times.

**RESULTS**

**Isolation and complementation of new spt mutants:** We initially identified seven SPT genes by selection for suppressors of the solo δ insertion mutation, his4-9126 (Winston et al. 1984). When representative mutations in these seven genes were examined for their ability to suppress other Ty and δ insertion mutations, we discovered that spt3 mutations had a
unique suppression pattern among the seven classes of spt mutants. Only spt3 mutations both suppress the His⁻ phenotype of his₄-9176 and change the lys₂-173R2 phenotype from Lys⁺ to Lys⁻ (WINSTON et al. 1984; WINSTON, DURBIN and FINK 1984). Also, by transcriptional analysis, only spt3 mutations appeared to cause a defect in initiation of transcription in δ sequences. If other genes, not found in our first mutant selection (WINSTON et al. 1984), also participate in this function, either directly or by regulation of SPT3, then mutations in those unidentified genes should have the same suppression pattern as spt3 mutations. To attempt to identify all genes involved in this function, then, we selected and screened for new suppressors with the same suppression pattern as spt3.

To select for new suppressors, we began with two strains, FW667 and L37, that contain the insertions his₄-9176 and lys₂-173R2 and, therefore, have His⁻ Lys⁺ phenotypes. His⁺ revertants were selected as described in MATERIALS AND METHODS and were screened for a Lys⁺ phenotype. We isolated 58 spontaneous, independent, recessive His⁺ revertants, 25 of which had also acquired a Lys⁻ phenotype. Of these, 18 were spt3 mutants, as judged by complementation tests. The other seven mutants, however, complemented spt3 mutants, suggesting that these strains contained mutations in genes other than SPT3. The mutant strains were crossed by each other to test for complementation and were shown to fall into two complementation groups, comprised of five and two members.

The earlier group of spt mutants we studied were selected primarily as suppressors of a different insertion mutation, his₄-9126 (WINSTON et al. 1984); therefore, it was possible that some of those were actually alleles of the two newly identified groups of mutations, but failed to have the distinctive spt3 suppression
alleles. Even though spt7-159, an insertion mutation suppressible by all of these spt mutations. By this test we discovered that the one new group that contains five members failed to complement a previously identified mutation in SPT7, spt7-159, indicating that these new mutations were spt7 alleles. The other group complemented all other spt mutations and we designated this gene SPT8.

Linkage tests: Two sets of genetic crosses between spt mutants were performed to examine linkage relationships. First, to verify that the new spt7 mutations segregate as alleles of those previously identified, we crossed a mutant containing one of the spt7 mutations identified in our earlier study, spt7-159 (WINSTON et al. 1984), by strains containing new spt7 isolates. From two crosses, FW1088 (spt7-115) by FW228 (spt7-159) and FW229 (spt7-159) by FW1087 (spt7-217), ten tetrads with four viable spores segregated 0:4 for SPT7:spt7, demonstrating close linkage between these alleles. Even though spt7 mutations confer a sporulation defect, as discussed in a later section, these spt7-159/spt7 diploids sporulated, presumably because spt7-159 is leaky.

Second, to verify that SPT7 and SPT8 are both unlinked to each other and unlinked to SPT3, we did pairwise crosses between strains carrying mutations in these three genes. The results of these crosses (Table 2) demonstrate that mutations in spt3, spt7 and spt8 represent three unlinked genes. Linkage between the new spt7 alleles and between different spt8 alleles could not be determined as spt7/spt7 and spt8/spt8 diploids do not sporulate.

Map position of SPT7: In the course of genetic crosses, we discovered that SPT7 and LYS2 are meiotically linked. The results of tetrad analysis (Table 3) show that they are approximately 13 cM apart on the right arm of chromosome II. Weak linkage to PET9 (47 cM) suggests that SPT7 is on the centromere proximal side of LYS2 (Figure 1) (MORTIMER and SCHILD 1985). The calculated distance between PET9 and LYS2 confirms the map order PET9-CEN2-SPT7-LYS2.

Map position of SPT8: SPT8 was mapped to chromosome XII by a combination of field inversion gels (CARLE, FRANK and OLSON 1986) and tetrad analysis. First, we separated intact yeast chromosomes by field inversion gels, blotted the separated chromosomes to nitrocellulose by the method of SOUTHERN (1975) and probed the blot with a 32P-labeled SPT8 probe. Since we did not detect any hybridization with the SPT8 probe, but did with control probes (HIS4 and URA3), we concluded that SPT8 was probably located on chromosome IV or chromosome XII, neither of which resolved well in our gel. This analysis was followed by tetrad analysis, using spt8 and markers on these two chromosomes. By this analysis, we have shown that SPT8 is linked to ASP5 and to GAL2 on the right arm of chromosome XIII (Table 4). From the map distance and the relative frequencies of recombinants between the three markers in the same crosses, we conclude that the map order is CEN12-ASP5-SPT8-GAL2 (Figure 1).

Transcriptional effects of spt7 and spt8 mutations: Mutations in SPT3 result in a specific transcriptional defect in yeast cells: the inability to initiate transcription in δ sequences (WINSTON, DURBIN and FINK 1984). This defect results in aberrant Ty transcripts that have initiated 800 bases farther downstream, within the ε portion of the Ty element. These transcripts are also made at a reduced level. We have examined Ty transcription to see if it is similarly affected in spt7 and spt8 mutants. Our results (Figure 1)
TABLE 4
Mapping \textit{spt8} by tetrad analysis

<table>
<thead>
<tr>
<th>Segregating markers</th>
<th>PD</th>
<th>NPD</th>
<th>TT</th>
<th>cM</th>
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<tr>
<td>\textit{spt8}, \textit{asp5}</td>
<td>36</td>
<td>0</td>
<td>34</td>
<td>24.3</td>
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<td>\textit{spt8}, \textit{gal2}</td>
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<td>28</td>
<td>31.2</td>
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<td>17</td>
<td>1</td>
<td>46</td>
<td>40.6</td>
</tr>
</tbody>
</table>

The tetrads scored come from crosses of BM25 × FW927 and FW928 × BM24. PD = parental ditype, NPD = nonparental ditype, TT = tetratype. \textit{spt8} mutations were scored by their ability to suppress \textit{his4-9176}, which is present in all strains. Centimorgans were calculated by \textsc{perkins}' (1949) formula.

2) show that Ty transcription is altered in \textit{spt7} and \textit{spt8} mutants and the alteration is similar to that in \textit{spt3} mutants: there is a large reduction in the level of full length Ty transcripts and an increase in the level of a shorter Ty RNA. The shorter RNA seen in the wild-type lane is of unknown origin but may be the Ty RNA with a shorter 5' end reported by \textsc{elder}, \textsc{loh} and \textsc{davis} (1983).

The structure of the Ty RNAs in wild-type and in \textit{spt7} and \textit{spt8} mutants was examined by S1 protection experiments to locate the site of the alteration in the Ty transcript (Figure 3). The largest DNA fragment protected in wild-type strains corresponds to transcripts initiating in the \(\delta\). The lower band may be due to sequence heterogeneity among Ty elements. The 5' end of total Ty RNA from \textit{spt3}, \textit{spt7} and \textit{spt8} mutants maps to a site downstream of the \(\delta\), in the internal region of the Ty. These results demonstrate that the change in the Ty transcripts in \textit{spt7} and \textit{spt8} mutants is identical to that seen in the \textit{spt3} mutant: the smaller Ty transcripts are shorter at the 5' end by approximately 800 bases. Therefore, \textsc{spt7} and \textsc{spt8}, like \textsc{spt3}, appear to be required for initiation of transcription in \(\delta\) sequences.

\textbf{Effect of \textit{spt3}, \textit{spt7} and \textit{spt8} mutations on expression of a Ty-lacZ fusion:} Expression of a Ty912-lacZ fusion in wild type and \textit{spt3}, \textit{spt7} and \textit{spt8} mutants was used to measure the reduction in \(\beta\)-initiated transcription we had seen by Northern hybridization analysis and S1 protection experiments. We used an in-frame protein fusion between the first open reading frame of Ty912 (\textsc{clare} and \textsc{farabaugh} 1985) and the \textit{E. coli} lacZ gene (Figure 4). The fusion contains 388 bp of Ty912 (\textsc{j. clare} and \textsc{p. farabaugh}, unpublished data), and therefore does not contain the downstream Ty transcription initiation site used in \textit{spt3}, \textit{spt7} and \textit{spt8} mutants. Comparison of \(\beta\)-galactosidase levels in wild-type, \textit{spt3}, \textit{spt7} and \textit{spt8} strains, therefore, compares the levels of transcription initiation in the \(\delta\) sequence in these different genetic backgrounds. The results of \(\beta\)-galactosidase assays (Table 5) shows a significant decrease in the level of \(\beta\)-galactosidase in either the \textit{spt3}, \textit{spt7} and \textit{spt8} mutants compared to the wild-type strains, ranging from a 25-fold decrease in the \textit{spt3} mutant to 10–13-fold in the \textit{spt7} and \textit{spt8} mutants. The smaller effect in the \textit{spt7} and \textit{spt8} mutants may be due to the fact that these mutations are not null alleles.
Figure 4.—Ty912-lacZ fusion. This fusion contains 388 base pairs of Ty912 fused to the E. coli lacZ gene, creating an in-frame fusion between Ty912-lacZ. The fusion was integrated at the URA3 locus as described in Materials and Methods. The wavy arrow indicates the hybrid Ty-lacZ mRNA that initiates in the b sequence of the fusion.

Table 5

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>β-Galactosidase units</th>
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<tr>
<td>Wild type</td>
<td>860</td>
</tr>
<tr>
<td>spt3-202</td>
<td>34</td>
</tr>
<tr>
<td>spt7-217</td>
<td>66</td>
</tr>
<tr>
<td>spt8-113</td>
<td>83</td>
</tr>
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</table>

Effect of spt mutations on the expression of Ty912A44-lacZ fusion. The numbers reported represent the average of β-galactosidase activities measured in at least three independent experiments on four different strains carrying the same spt mutation. Strains used for wild type were FW1163-FW1166; for spt3-202, FW1154-FW1162; for spt7-217, FW1197-FW1200; and for spt8-113, FW1229-FW1232. The standard error is less than 17%.

Transcription of SPT3 in spt7 and spt8 mutants:

One model to explain the phenotypes of spt7 and spt8 mutants is that SPT7 and SPT8 regulate expression of SPT3. According to this explanation, the SPT7 and SPT8 gene products would be required for transcription. One model to explain the phenotypes of spt7 and spt8 mutants would be that SPT7 and SPT8 mutants for other spt3 mutant phenotypes. The SPT3 mRNA in wild type, an spt3 deletion mutant and in spt7 and spt8 mutants shows that there is no significant difference in the level of SPT3 mRNA in either spt7 or spt8 mutants compared to wild type (Figure 5 and the densitometric analysis described in Materials and Methods). The most intense band in the wild-type, spt7 and spt8 lanes is the SPT3 mRNA. In the spt3-202 deletion mutant (Winston and Minehart 1986), this band is gone and the shorter mRNA of the deletion can be seen. From this experiment, we conclude that SPT7 and SPT8 do not regulate SPT3 transcription.

spt7, spt8, and multiple mutant phenotypes: We also screened spt7 and spt8 mutants for other spt3 mutant phenotypes. We found that spt7/spt7 and spt8/spt8 double mutants fail to sporulate, as do spt3/spt3 double mutants (Table 6). This defect is recessive, since in SPT7/spt7 and SPT8/spt8 double mutants, sporulation was normal.

We have also examined the suppression pattern of a set of Ty and b insertion mutations by representative spt7 and spt8 alleles. The results (Table 7) show that suppression of a total of five different insertions at HIS4 and LYS2 follows the same pattern for mutations in SPT3, SPT7 and SPT8.

Finally, we have constructed spt3 spt7 (FW1058), spt3 spt8 (FW1060), and spt7 spt8 (FW1063) double mutants. The numbers reported in Table 7 were determined for suppression of the HIS4-9178 and LYS2-128R2 alleles. Symbols indicate growth on media lacking histidine for his4-9178, his4-917 and on media lacking lysine for lys2-173R2 and lys2-128R2. + = strong growth; = intermediate strong growth; ± = weak growth; = = no growth; cs = cold sensitive. The spt7 and spt8 mutations were selected for suppression of his4-9178 and screened for reduced expression of lys2-173R2. Suppression was determined by replica plating onto SD plates supplemented with the appropriate amino acids.
and an spt3 spt7 spt8 triple mutant (FW1225). All of these multiply mutant strains have essentially the same phenotypes as an spt3 single mutant with respect to effects on the insertion mutations his4-917b and lys2-173R2 and with respect to growth rate.

**DISCUSSION**

Three genes, SPT3, SPT7 and SPT8, are required for normal transcription of Ty elements in *S. cerevisiae*. Mutation in any one of these genes results in similar transcriptional defects for Ty elements: failure to initiate transcription in the 5' δ sequence and transcription initiation at a site farther downstream, internal to the Ty element.

The spt3 mutant phenotype was shown to result from loss of function, because null alleles constructed in *vitro* and recombined back into the yeast genome, replacing the wild-type SPT3 gene confer the Ty transcriptional defect (WINSTON, DURBIN and FINK 1984). The spt7 and spt8 transcriptional defects are also likely due to loss of function as every allele we have isolated is fully recessive.

The mutant phenotypes of spt3, spt7 and spt8 appear to be identical. By both Northern hybridization analysis and S1 nuclease protection experiments, mutations in any of these three genes greatly reduce the amount of full length Ty transcript and cause an increase in a species of Ty RNA that initiates some 800 bp 5' of the normal start site. In addition, mutations in any of these three genes result in the same pattern of suppression of Ty and δ insertion mutations. It is likely that suppression is a result of the altered pattern of Ty transcription. We also expect that spt7 and spt8 mutations reduce the frequency of Ty transposition as do spt3 mutations (BOEKE, STYLES and FINK 1986).

Mutations in SPT7 and SPT8, like mutations in SPT3, also result in a sporulation defect in homzygous diploids. This defect in sporulation suggests that either this phenotype is caused by an effect on Ty transcription (or other δ-initiated transcription) or that these three genes are required for transcriptional regulation of other sequences as well as of δ sequences. Mutations in SPT3 also cause a defect in mating, believed to be caused by a reduction in expression of mating pheromone genes (WINSTON, DURBIN and FINK 1984; J. HIRSCHHORN and F. WINSTON, unpublished data); preliminary results indicate that spt7 and spt8 mutations cause a similar defect.

These three SPT functions are analogous to products encoded by some retroviruses that are required for *trans-activation* of transcription of proviral LTR sequences. Most notably, HTLV I, HTLV II and Rous sarcoma virus all encode *trans-acting* functions required for transcription from their LTRs (ROSANI, ROSEN and HASELTINE 1984; FUJISAWA et al. 1985; BROOME and GILBERT 1985). These *trans-acting* transcription factors may affect expression of host as well as viral genes. Since Ty elements are not intercellularly transmitted (GARFINKEL, BOEKE and FINK 1985), they can rely exclusively on host encoded transcriptional factors for their expression. There are a few possible reasons for the requirement of three genes for transcription initiation in δ sequences. First, one of the genes may encode the product that acts directly on transcription initiation and the other two regulate expression of that gene. From results presented in this paper, we can conclude that neither SPT7 nor SPT8 are involved in transcriptional regulation of SPT3. Conceivably, SPT7 or SPT8 are regulated by SPT3, or regulation of SPT3 is at a post-transcriptional level. Second, the three gene products may act independently, but are all required at the same step to activate transcription. Third, the three gene products may interact to form a complex that is directly required for activation of transcription from the δ sequence.

Since SPT3, SPT7 and SPT8 serve to specify transcription initiation, one or all of them may be δ-specific DNA binding proteins that recognize the δ TATA region. Recent work has shown that disruption of TATA regions in yeast results in an alteration of transcription initiation specificity (NAGAWA and FINK 1985; HAHN, HOAR and GUARENTE 1985; McNEIL and SMITH 1986). Whether the SPT3, SPT7 and SPT8 gene products form a complex that acts at a single site or act independently at different sites (not necessarily in the δ) remains to be elucidated. DURBIN (1985) and BOEKE, STYLES and FINK (1986) demonstrated that when the 5' 240 bp (the U3 region) of a δ is replaced by the promoter for the GAL1 gene of yeast, Ty transcription is independent of SPT3. The U3 region of δ sequences, therefore, is a strong candidate to contain the site(s) of action of SPT3, SPT7, or SPT8. We cannot, however, rule out a less direct effect.

Finally, by selection for suppressors of a variety of different Ty and δ insertion mutations, at least 12 different SPT genes have been identified (WINSTON et al. 1984; J. FASSLER and F. WINSTON, unpublished data). Further isolation of mutants by the selection and screen described in this paper has yielded mutations only in SPT3, SPT7 and SPT8, plus one additional gene, mutations in which do not affect Ty transcription (F. WINSTON, unpublished data). Therefore, among the entire set of SPT genes, mutations in only three of them, SPT3, SPT7 and SPT8, alter Ty transcription as described in this paper. However, other classes of spt mutants do suppress insertion mutations by transcriptional alterations (SILVERMAN and FINK 1984; CLARK-ADAMS and WINSTON 1987; J. FASSLER and F. WINSTON, unpublished data). We believe, therefore, that selection for suppressors of different Ty and δ insertion mutations selects for different classes of transcriptional mutants. SPT3, SPT7 and
SPT8 define one class; other classes may encode transcription factors that recognize other transcription signals.

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