Isolation and Analysis of a Novel Class of Suppressor of Ty Insertion Mutations in *Saccharomyces cerevisiae*

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ABSTRACT

Using a new scheme for the isolation of suppressor of Ty insertion mutations (*spt* mutations) in yeast, we have identified six new *SPT* genes. Mutations in two of these genes, *SPT13* and *SPT14*, exhibit a novel suppression pattern: suppression of complete Ty insertion mutations, but not of solo δ insertion mutations. Transcriptional analysis shows that *spt13*- and *spt14*-mediated suppression of Ty insertion mutations is the result of an elevation in the levels of adjacent gene transcription. In spite of the failure of these mutations to suppress solo δ insertion mutations, they do cause changes in transcription of at least one solo δ insertion mutation. In addition, *spt13* and *spt14* mutations are epistatic to mutations in certain other *SPT* genes that do suppress solo δ insertion mutations. These results suggest that the *SPT13* and *SPT14* gene products may act via sequences in both the δ and ϵ regions of Ty elements. Finally, mutations in *SPT13* cause sporulation and mating defects and *SPT14* is essential for growth, suggesting that these two genes have important roles in general cellular functions.

THE Ty elements of *Saccharomyces cerevisiae* are a set of transposable genetic elements, approximately 5.9 kb long and flanked by long terminal repeats, called δ sequences. Ty elements provide an excellent model system for study of the mechanism of eukaryotic transcription. Ty insertion mutations in the 5′ noncoding region of a gene often interfere with adjacent gene expression because of the presence of Ty-encoded transcription signals (ROEDER and FINK 1982; SILVERMAN and FINK 1984; WINSTON, DURBIN and FINK 1984). Insertion mutations that result in the inhibition of adjacent gene expression can be used to select for suppressors; these suppressor mutations may identify genes encoding yeast transcription factors that recognize Ty transcription signals. Analysis of such suppressor mutations has the potential to provide insight into the eukaryotic transcription apparatus.

There are approximately 35 Ty elements per haploid yeast genome and at least some of these are abundantly transcribed (CAMERON, LOH and DAVIS 1979; ELDER *et al.* 1981). Ty transcription initiates in the 5′ δ sequence and terminates in the 3′ δ sequence (ELDER, LOH and DAVIS 1983). Therefore, Ty transcription signals for both transcription initiation and termination must exist in δ sequences. Other signals presumably exist that distinguish the 5′ and 3′ δ sequences with respect to initiation and termination. In addition, ϵ sequences near the 5′ δ border appear to modulate expression of the adjacent gene (ERREDE *et al.* 1985; ROEDER, ROSE and PEARLMAN 1985). This region shows homology to the SV40 enhancer core sequence (ERREDE *et al.* 1985; ROEDER, ROSE and PEARLMAN 1985).

Both complete Ty and solo δ insertion mutations have been isolated in the 5′ noncoding regions of yeast genes. Such insertion mutations alter the expression of the adjacent gene, either by inhibiting the expression of a gene that is normally on (ROEDER *et al.* 1980; SIMCHEN *et al.* 1984; EIBEL and PHILIPPSEN 1984), or by allowing expression of a gene that is normally off (ERREDE *et al.* 1980; WILLIAMSON, YOUNG and CIRIACY 1981). These changes occur at the transcriptional level (DUBOIS, JACOBS and JAUNIAUX 1982; SILVERMAN and FINK 1984; WILLIAMSON *et al.* 1983; WINSTON, DURBIN and FINK 1984).

Trans-acting mutations that alter Ty- and δ-mediated gene expression have been selected as suppressors of two solo δ insertions mutations that inhibit adjacent gene expression. These mutations identify a large set of genes [*SPT* genes (*SPT* = *Sup*pressor of *T*y insertion mutations)] (WINSTON *et al.* 1984, 1987) that can be divided into two classes based on suppression patterns conferred by *spt* mutations. The first class of mutations (in *SPT3, SPT7* and *SPT8*) are strong suppressors of both Ty insertion mutations and δ insertion mutations (WINSTON *et al.* 1984, 1987). The second class of mutations (in *SPT4, SPT5* and *SPT6*), while strong suppressors of solo δ insertion mutations, generally suppress Ty insertion mutations poorly (WINSTON *et al.* 1984; CLARK-ADAMS and WINSTON 1987; J. FASSLER and F. WINSTON, unpublished results).

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The distinction between these two classes is reinforced by their different effects on the pattern of Ty transcription. Mutations in *SPT3*, *SPT7*, and *SPT8* abolish normal Ty δ-δ transcription and result instead in a low level of a new Ty transcript, that initiates approximately 800 bp farther downstream (WINSTON, DURBIN and FINK 1984; WINSTON *et al.* 1987). In contrast, mutations in *SPT4*, *SPT5* and *SPT6* do not affect the level or the length of Ty transcripts (CLARK-ADAMS and WINSTON 1987; J. FASSLER and F. WINSTON, unpublished results). The differences between the suppression and transcription patterns in class 1 and class 2 *spt* mutants suggest that they act by different mechanisms. These differences may reflect differences in the transcriptional control elements recognized by the *trans*-acting factors encoded by the *SPT* genes.

In the present study, we have isolated and characterized additional *spt* mutations. To maximize the probability of isolating mutations that identify new classes of *SPT* genes, perhaps encoding factors that recognize different transcription elements, we selected for suppressors of two insertion mutations, one a complete Ty element, the other a solo δ sequence, which had not been used in previous isolation schemes.

From this study, we have identified six new *SPT* genes. Among the new *SPT* genes, mutations in two of them, *SPT13* and *SPT14*, confer a novel suppression pattern (suppression of Ty insertion mutations but not of solo δ insertion mutations) and comprise a third phenotypic class of *spt* mutants.

MATERIALS AND METHODS

Yeast strains: The yeast strains used in this study are listed in Table 1. All strains, with the exception of A120 from Anne Happel, were from our laboratory collection or were constructed for these studies and are derivatives of strain S288C (*MAT*α *ga12*). The Ty and δ insertion mutations have been described previously and are illustrated in Figure 1. The *his4-917* insertion mutation consists of a Ty element inserted at position −7 relative to the start site of *HIS4* transcription (ROEDER *et al.* 1980), and the *lys2-128*δ insertion mutation (SIMCHEN *et al.* 1984) consists of a solo δ sequence inserted at +158 relative to the *LYS2* translation initiation site (F. WINSTON, unpublished results).

A set of *spt* tester strains was constructed for the complementation and linkage studies. These strains contain a representative mutant allele for the *SPT1-SPT6* linkage groups plus the two insertion mutations used in the selection for new *spt* mutants, *his4-917* and *lys2-128*δ. Two strains were constructed for each *spt* mutant: a *MAT***a** strain carrying *trp1*Δ*1* and a *MAT*α strain carrying *ura3-52*. The auxotrophic markers were included to allow the prototrophic selection for diploids in both complementation and linkage tests. Complementation tests against *spt7* and *spt8* mutants were done using strains FW1116, FW1117, FW1136 and FW1137.

Genetic methods: The methods used for yeast crosses and sporulation of diploids were as described by MORTIMER and HAWTHORNE (1969) and SHERMAN, FINK and LAWRENCE (1978). Diploids were isolated by selection for complementation of recessive auxotrophies in each parent or by micromanipulation of zygotes. In some cases, where zygotes were difficult to identify, the entire mating patch was transferred to sporulation plates. Diploids were then sporulated on plates (SHERMAN, FINK and LAWRENCE 1978). At least ten tetrads were dissected for each cross. In most

TABLE 1

Strains

Strain	Genotype
A) Parental Strains:	
JF28	*MAT***a** *his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF15	*MAT*α *his4-917 lys2-128*δ *ura3-52 leu2*
B) *spt* Tester Strains	
JF110	*MAT***a** *spt1-1 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF108	*MAT*α *spt1-1 his4-917 lys2-128*δ *ura3-52*
JF96	*MAT***a** *spt2-150 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF97	*MAT*α *spt2-150 his4-917 lys2-128*δ *ura3-52 leu2*
JF95	*MAT***a** *spt3-101 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF94	*MAT*α *spt3-101 his4-917 lys2-128*δ *ura3-52 leu2*
JF112	*MAT***a** *spt4-3 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF113	*MAT*α *spt4-3 his4-917 lys2-128*δ *ura3-52*
JF118	*MAT***a** *spt5-194 his4-917 lys2-128*δ *trp1*Δ*1*
JF117	*MAT*α *spt5-194 his4-917 lys2-128*δ *ura3-52 leu2*
JF93	*MAT***a** *spt6-140 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF88	*MAT*α *spt6-140 his4-917 lys2-128*δ *ura3-52*
FW1243	*MAT***a** *spt7-217 lys2-128*δ *his4-912*δ or *his4-917*δ *trp1*Δ*1*
FW1185	*MAT*α *spt7-217 lys2-128*δ *his4-912*δ or *his4-917*δ
FW1116	*MAT***a** *spt7-217 his4-917 lys2-173R2 trp1*Δ*1 leu2-1*
FW367	*MAT*α *spt7-159 his4-917 ura3-52*
FW1117	*MAT*α *spt7-217 his4-917 lys2-173R2 ura3-52 leu2-1*
FW1146	*MAT***a** *spt8-113 lys2-128*δ *ura3-52 trp1*Δ*1 his4-912*δ or *his4-917*δ
FW1118	*MAT*α *spt8-113 lys2-128*δ *his4-917*δ *trp1*Δ*1 ura3-52*
FW1136	*MAT***a** *spt8-113 his4-917 lys2-173R2 ura3-52 leu2-1*
FW1137	*MAT*α *spt8-113 his4-917 lys2-173R2 ura3-52 leu2-1*
C) Other strains	
A120	*mat***a***1 his4-917*δ *trp1*Δ*1* (A. HAPPEL)
JF10	*MAT***a** *lys2-128*δ *leu2-3,112*
JF14	*MAT*α *lys2-128*δ *leu2-3,112 trp1*Δ*1*
JF16	*MAT***a** *his4-917 lys2-128*δ *ura3-52 leu2*
JF296	*MAT***a** *his4-912*δ *spt14-1 leu2*
JF305	*MAT*α *spt13-1 his4-912*δ *lys2-128*δ
JF372	*MAT***a** *spt13-1 lys2-128*δ
JF397	*MAT*α *spt13-1 his4-917 ura3-52 leu2*
JF398	*MAT***a** *spt13-1 his4-917 lys2-201 trp1*Δ*1 leu2*
JF441	*MAT***a** *spt14-1 his4-917 ura3-52 leu2 lys2-128*δ
JF444	*MAT*α *spt14-1 his4-917 lys2-128*δ *trp1*Δ*1 leu2*
JF564	*MAT***a**/*MAT*α *SPT13/spt13-1 his4-917/his4-917 ura3-52/ura3-52 TRP1/trp1*Δ*1 leu2/leu2*
JF577	*MAT***a** *spt13-101*::Tn*10-LUK his4-917 lys2-201 leu2 trp1*Δ*1*
JF580	*MAT***a** *spt13-100*::Tn*10-LUK his4-917 lys2-128*δ *leu2 trp1*Δ*1*
JF582	*MAT***a**/*MAT*α *SPT14/spt14-1 his4-917/his4-917 ura3-52/ura3-52 leu2/leu2 TRP1/trp1*Δ*1*
JF590	*MAT***a**/*MAT*α *SPT14/spt14-200*::Tn*10-LUK his4-917/his4-917 ura3-52/ura3-52 leu2/leu2 TRP1/trp1*Δ*1*
JF593	*MAT***a**/*MAT*α *spt14-1/spt14-200*::Tn*10-LUK his4-917/his4-917 ura3-52/ura3-52 leu2/leu2 TRP1/trp1*Δ*1*
JF605	*MAT***a** *spt13-100*::Tn*10-LUK his4-912*δ *lys2-128*δ *ura3-52 trp1*Δ*1*
FW605	*MAT*α *his4-917(480) ura3-52 cry1*^{R}
FW1238	*MAT*α *his4-912*δ *lys2-128*δ *ura3-52*

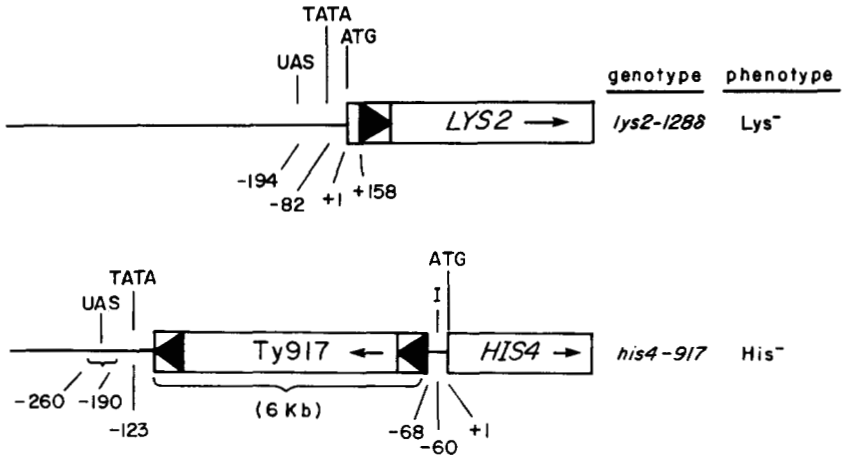

FIGURE 1.—Structure of the *lys2-128δ* and *his4-917* insertion alleles. Positions of the insertions relative to specific transcription signals are shown. UAS, upstream activation sequence; I, transcription initiation site; →, direction of transcription.

cases, spores were germinated on YPD plates at 30°. However, in the case of some very sick mutants, germination was improved at 23°. Yeast transformation was performed as described by ITO *et al.* (1983).

Media: The media used were as described by SHERMAN, FINK and LAWRENCE (1978), and included minimal media with amino acids added (for example, SD + his), synthetic complete media, lacking a specific amino acid (for example, SC-his), and rich media (YPD). Unless otherwise specified, all yeast strains were grown at 30°.

Isolation of mutants: All of the mutants described in this paper were spontaneous isolates from strains JF15 and JF28 which are isogenic except at *MAT*, *URA3* and *TRP1* (Table 1). These strains have a His^- Lys^- phenotype due to the insertion mutations, *his4-917* and *lys2-128δ*. Single colonies of JF15 or JF28 were picked and patched onto YPD plates and incubated overnight at 30°. The patches were replica plated to SC-his, SC-lys or SC-his, lys plates which were then incubated approximately 5–7 days. Several revertants from each patch were restreaked on selective media (Lys^+ revertants isolated on SC-lys plates were purified on SC-lys plates, etc.), and replica plated to SC-lys, SC-his and YPD at 20°, 30° and 37°. This test provided a preliminary characterization of the range of mutant suppression phenotypes. One isolate was saved from each patch, unless different isolates from a single patch showed distinct phenotypes. All isolates are considered to be independent. After this initial screen, mutants were again single colony purified on the appropriate selective media (*e.g.*, SC-lys), and then on permissive media (YPD). The phenotype of each purified strain was rechecked and all strains were frozen as 15% glycerol stocks. All further characterization was performed on cells derived from the frozen stocks.

Complementation analysis and dominance/recessiveness tests: Lys^+ His^+ and Lys^+ His^- mutants were tested for complementation and dominance based on suppression of *lys2-128δ*. Mutants with a Lys^- His^+ phenotype were analyzed separately, based on suppression of *his4-917*.

Complementation tests were done as described by FINK (1966). The Lys^+ mutants were grouped according to mating type and grown as sets of stripes on YPD plates. Stripes of mutants isolated in the *MAT***a** background were replica plated perpendicularly onto YPD replicas of stripes of *MAT*α mutants. The grid of *MAT***a** and *MAT*α mutants was then replica plated to SD plates lacking uracil, tryptophan and lysine. The absence of uracil and tryptophan selects for diploids, and the absence of lysine tests the Lys phenotype in the heterozygous diploids. In this test, a Lys^+ intersection indicates that the *spt* mutations failed to complement. The SPT^+ parental strains were included to test for dominance of the *spt* mutations. Any ambiguity in the results of these tests was resolved by examining the His and Lys phenotypes of diploids purified from the intersections.

The His^+ Lys^- mutants were tested for complementation based on suppression of *his4-917*. Since the His phenotype was very difficult to assess in unpurified intersections, all heterozygous diploids were first purified on selective media, patched to YPD plates, and then replica plated to SC-his at 30° to test for complementation and dominance.

Test of mating type control: To evaluate the *MAT*α His^+ Lys^- mutants for mating type regulation of *his4-917* expression, each of the *MAT*α mutants was mated by a *mat***a***1* SPT^+ strain (A120). Diploids were purified and tested for their His phenotype on SC-his plates.

RNA isolation and Northern hybridization analysis: Cells for the preparation of RNA were grown in supplemented SD to $1–2 \times 10^7$ per ml. Yeast RNA was prepared according to the method of CARLSON and BOTSTEIN (1982). For each sample, 5 μg of total RNA were loaded onto a 1% formaldehyde-agarose gel. Electrophoresis was for a total of 500 V-hr. Blotting and hybridization were performed using the dextran sulfate method described in the GeneScreen (New England Nuclear Corp., Boston, MA) manual, except that prior to hybridization, the RNA was UV-cross-linked to the filter (1200μW/cm², 2 min; CHURCH and GILBERT 1984). The amount of RNA per lane was standardized by hybridization to plasmid pFR2 which carries the yeast *PYK1* gene. ^{32}P-labeled DNA probes were prepared by nick translation (RIGBY *et al.* 1977). Plasmids used as probes were pBR322 derivatives, each containing an internal fragment from a specific yeast gene as follows: pFW45, an internal *Bgl*II-*Sal*I restriction fragment from the *HIS4* gene; B161, an internal *Bgl*II restriction fragment from Ty1 (R. SUROSKY, B.-K. TYE, and G. R. FINK, unpublished data); and pFR2, the *PYK1* gene (kindly provided by P. SINHA).

Construction of null alleles of *SPT13* and *SPT14*: Null insertion alleles of *SPT13* and *SPT14* were isolated in plasmids that contain the cloned *SPT13* and *SPT14* genes (J. FASSLER, and F. WINSTON, unpublished results) using the Tn*10-lacZ-kan*^{R}*-URA3* (Tn*10*-LUK) gene fusion transposon as described (HUISMAN *et al.* 1987). For analysis of the null phenotype, a restriction fragment carrying the null allele was isolated and used to transform diploid strains homozygous for *ura3-52* and *his4-917*, and heterozygous for *spt13-1* (JF564) or *spt14-1* (JF582). In the Ura^+ transformants either the wild or mutant *spt* allele of the diploid was replaced by the null allele through homologous recombination (ROTHSTEIN 1983). *spt* null/*spt* diploids were His^+,

while *spt* null/*SPT*$^+$ diploids were His$^-$. The null phenotype in haploids was examined following sporulation of each diploid strain and dissection of tetrads. Two different *spt13*::Tn*10*-LUK insertions 1.4 kb apart, and three different *spt14*::Tn10-LUK insertions, spanning 1.0 kb, were analyzed.

RESULTS

Isolation of *spt* mutants: To optimize isolation of new classes of *spt* mutations, we constructed strains containing two insertion mutations that had not been used in previous *spt* selections. Furthermore, the insertion mutations chosen differ from each other in several important respects (Figure 1). One insertion mutation, *his4-917*, is a complete Ty inserted into the 5′ noncoding region of the *HIS4* gene between the TATA box and the site of transcription initiation in the opposite transcriptional orientation with respect to the *HIS4* gene (ROEDER *et al.* 1980). The second insertion mutation, *lys2-128δ* is a solo δ within the early coding region of the *LYS2* gene (F. WINSTON, unpublished data) in the same transcriptional orientation as *LYS2*. These mutations cause His$^-$ Lys$^-$ phenotypes respectively, by inhibiting transcription of the adjacent gene. Suppression of both *his4-917* and *lys2-128δ* in various *spt* mutants occurs at the transcriptional level (WINSTON, DURBIN and FINK 1984; CLARK-ADAMS and WINSTON 1987).

Using strains JF15 and JF28, which carry both of these insertion mutations, we have performed three separate selections: for His$^+$, for Lys$^+$ or simultaneously for His$^+$, Lys$^+$ revertants. Mutations conferring all three possible suppression patterns (Lys$^+$ His$^+$, Lys$^+$ His$^-$ and Lys$^-$ His$^+$) were isolated. Representatives of the first two classes, Lys$^+$ His$^+$ and Lys$^+$ His$^-$ were expected on the basis of the phenotypes of the known *spt* mutants. In addition, we isolated mutations that conferred a novel suppression pattern. These Lys$^-$ His$^+$ mutants appear to suppress complete Ty insertion mutations, but not solo δ insertion mutations, suggesting that they cause a transcriptional alteration distinct from those in previously characterized *spt* mutants.

Complementation analysis of Lys$^+$ His$^+$ and Lys$^+$ His$^-$ revertants: Each *MAT***a** Lys$^+$ mutant and the *MAT***a** parent, JF28, was crossed by each *MAT*α Lys$^+$ mutant and the *MAT*α parent, JF15, to test for complementation and dominance as described in MATERIALS AND METHODS. Twelve of the 115 Lys$^+$ mutations tested were dominant and are possibly *spt2* mutations. This possibility is supported by the fact that one of the dominant mutations was demonstrated to be alleleic to a known *spt2* mutation and the prior observation that a large percentage of *spt2* mutations are dominant (CHALEFF 1980; WINSTON *et al.* 1984). Seventy-five of the remaining 103 recessive mutations fell into ten complementation groups, six of which (*SPT3-SPT8*) had been identified previously (Tables 2 and 3). The remaining 28 mutants complemented all other *spt* mutants and were not studied further.

TABLE 2

Isolation of new alleles of *SPT1-SPT8*

	Selection				Phenotype	
Gene	L$^+$	H$^+$	H$^+$L$^+$	Total	H$^+$L$^+$	H$^-$L$^+$
SPT1	0	0	0	0	NA	NA
SPT2	12	0	0	12	ND	ND
SPT3	4	2	3	9	9	0
SPT4	3	0	0	3	3	0
SPT5	2	0	0	2	1	1
SPT6	4	0	0	4	3	1
SPT7	2	0	1	3	2	1
SPT8	3	0	1	4	3	1

Selections were performed on SC-lys, SC-his or SC-his and lys plates. Lys$^+$ (L$^+$), His$^+$ (H$^+$) and His$^+$ Lys$^+$ (H$^+$L$^+$) revertants were isolated and characterized as described in MATERIALS AND METHODS. The mutant phenotype has been designated His$^+$ or Lys$^+$ if growth of the mutant on SC-his or SC-lys plates is improved at all relative to the *SPT*$^+$ parent. A "$^+$" designation therefore includes a wide range of phenotypes. NA, not applicable; ND, not determined.

TABLE 3

Distribution of new *spt* mutations into complementation groups

Complementation group	Selection				Phenotype		
	L$^+$	H$^+$	H$^+$L$^+$	Total	H$^+$L$^+$	H$^-$L$^+$	H$^+$L$^-$
9	4	7	7	18	15	2	1
10	5	3	10	18	18	0	0
11	2	0	0	2	2	0	0
12	0	2	10	12	12	0	0
13	0	5	0	5	0	0	5
14	0	3	0	3	0	0	3
15 (*HIS4*)	0	8	0	8	0	0	8

Selections were performed on SC-lys, SC-his or SC-his and lys plates. Lys$^+$ (L$^+$), His$^+$ (H$^+$) and His$^+$ Lys$^+$ (H$^+$L$^+$) revertants were isolated and characterized as described in MATERIALS AND METHODS. The mutant phenotype has been designated His$^+$ or Lys$^+$ if growth on SC-his or SC-lys plates is improved at all relative to the *SPT*$^+$ parent. A "$^+$" designation therefore includes a wide range of phenotypes.

Complementation analysis of His$^+$ Lys$^-$ revertants: Complementation analysis of the His$^+$ Lys$^-$ mutants was less straightforward as we first needed to distinguish *cis*-acting alterations at *HIS4* from recessive *spt* mutants. Since only the His phenotype was altered in these mutants, it was possible that the phenotype was the result of a rearrangement or gene conversion event at the Ty that activated *HIS4* expression (CHALEFF and FINK 1980; ROEDER and FINK 1982). Since mating type can affect expression of genes adjacent to Ty insertion mutations that activate gene expression (ELDER *et al.* 1981; ERREDE *et al.* 1980; WILLIAMSON, YOUNG and CIRIACY 1981), a Ty gene convertant with a His$^+$ phenotype may appear recessive (His$^-$) because *HIS4* expression has been reduced by *MAT***a***1*/*MAT*α*2* control.

Therefore, to distinguish between *MAT***a***1*/*MATα2* control and true recessiveness of the His$^+$ Lys$^-$ revertants, each *MATα* His$^+$ mutant was mated by a *mat***a***1 SPT*$^+$ strain. Although *mat***a***1* mutants retain the capacity to mate with *MATα* cells, the *mat***a***1*/*MATα* diploid is defective in *MAT***a***1*/*MATα2* regulation, and is phenotypically an α cell (KASSIR and SIMCHEN 1976). Therefore, *MAT***a***1*/*MATα2* control of Ty mediated gene expression is not observed in *mat***a***1*/*MATα* diploids. Using this test, two out of 22 of the *MATα* His$^+$ Lys$^-$ mutants gave a weak His$^+$ phenotype, similar to that observed for a *mat***a***1*/*MATα* diploid containing a known Ty917 gene convertant, *his4-917(480)* (ROEDER and FINK 1982). These two mutants are therefore likely to be gene convertants or other types of rearrangements of Ty917, and were not studied further. The other 20 mutants were His$^-$ in the *mat***a***1*/*MATα* diploid, and were assumed to be recessive *spt* mutants.

To determine the number of additional complementation groups specified by the His$^+$ Lys$^-$ mutants, each recessive *MATα spt* mutant was crossed by each of the *MAT***a** *spt* mutants to test for complementation of the His phenotype. Three complementation groups (CG13, CG14 AND CG15) were identified, accounting for 16 out of the 47 His$^+$ Lys$^-$ mutants (Table 3). The remaining 31 mutants complemented all mutants of the opposite mating type and were not studied further.

Although the dominance/recessiveness tests demonstrated that most of the *MATα* His$^+$ Lys$^-$ mutations were recessive and therefore unlikely to be rearrangements of Ty917, we used linkage tests to confirm this hypothesis. One representative from each of the three complementation groups was crossed to the *HIS4*$^+$ *SPT*$^+$ strains, JF10 (*MAT***a**) and JF14 (*MATα*). These crosses demonstrated that one of the three complementation groups, CG15, contained mutations tightly linked to *HIS4*. In this cross, all 10 tetrads showed 4:0 segregation for His$^+$:His$^-$. The finding of recessive *HIS4*-linked mutants suggests that some Ty917 gene convertants (probably those with weak His$^+$ phenotypes) will appear His$^-$ in a diploid even in the absence of mating type regulation. However, the possibility of an *SPT* gene that is tightly linked to *HIS4* has not been definitively ruled out. Mutations in complementation group 15 were not studied further.

Linkage analysis: To examine linkage between mutations in the different *SPT* complementation groups, representatives from each of the six new *spt* complementation groups were crossed both by each other and by representative mutants for each of the previously identified *SPT* genes. Although at least 10 tetrads were dissected for each cross, in many crosses spore viability was poor, yielding few tetrads with four viable spores. In these crosses allelism was evaluated based on the presence or absence of wild type recombinant spores, rather than on the frequency of PD, NPD and TT tetrads. In general, the results of these crosses verified that the new complementation groups represent unlinked genes. Interestingly, the complementation groups CG11 and CG12 are tightly linked. We have tentatively designated these as two genes, *SPT11* and *SPT12* (Table 3). Mutants in these groups are being studied further.

Double mutant analysis: Previous work showed that for particular alleles of *SPT4, SPT5* and *SPT6*, double mutants are inviable (WINSTON *et al.* 1984) suggesting possible interactions between these genes. Although germination was poor in many crosses, we found no further double mutant inviability for combinations of new *spt* mutations with *spt4, spt5* and *spt6* mutations. However, in pairwise crosses between representative *spt9-spt14* strains, a viability pattern suggestive of double mutant inviability was seen in crosses of *spt11* and *spt12* mutants by *spt10* mutants. Crosses of two different *spt10* alleles with one *spt11* and one *spt12* allele resulted in a 4:0, 3:1 and 2:2 viability pattern diagnostic of double mutant lethality. The suppression phenotypes of the surviving spores was consistent with inviability of the *spt10 spt11* and *spt10 spt12* double mutant strains.

Additional *spt* mutant phenotypes: Among the original *spt* mutants, several additional mutant phenotypes not obviously related to suppression of Ty and δ insertion mutations were observed (WINSTON *et al.* 1984). For example, *spt3* mutants are mating and sporulation deficient, some *spt4* mutants are sensitive to methylmethane sulfonate and some *spt6* mutants are temperature sensitive for viability. To determine the extent of some of these phenotypes among the new *spt* mutants, each mutant was tested for temperature sensitivity and representatives of each new complementation group were tested for sporulation defects.

Two of the newly isolated mutations are temperature sensitive for growth. One *spt9* allele confers temperature sensitive growth on YPD and one *spt14* allele confers a leaky temperature sensitive phenotype. In all cases the temperature sensitivity and suppression phenotypes cosegregate in crosses.

In addition, mutations in *SPT13* were found to confer a tight sporulation defect. After 8 days of incubation on sporulation media no tetrads were present among several hundred cells examined and the cells were predominantly unbudded.

Efficiency of diploid formation was also reduced in *spt13* mutants. In two separate experiments in which *spt13-100*::Tn*10-LUK* strains were mated to each other, 60% (19 of 31) of the zygotes isolated after six hours of mating were inviable and 33% (4 of 12) of the apparent zygotes that formed colonies

TABLE 4

Suppression of δ and Ty insertion mutations in *spt13* and *spt14* mutants

Insertion mutation	Orientation	Wild type	*spt13*[a]	*spt14*
δ				
lys2-128δ	→[b]	−	−	−
his4-917δ	←	−	−	−
lys2-61δ	←	−	−	−
his4-912δ	→	−	−	−
Ty				
his4-917	←	−	+	+
lys2-61	←	−	+	+
his4-912	→	−	−	−

[a] Suppression of each insertion was tested in both *spt13-1* and *spt13-100*::Tn*10-LUK* (null) backgrounds.

[b] The transcriptional orientation of the δ or Ty is indicated by the arrow. ←, the transcriptional orientation of the inserted element is opposite that of the adjacent gene; →, the transcriptional orientation of the element is the same as the adjacent gene.

+, suppression; −, no suppression.

were not diploids: two of the four tested as *MAT*α and the other two tested as *MAT***a.**

Suppression patterns in *spt13* and *spt14* mutants: Based on their initial characterization, it seemed possible that mutations in *spt13* and *spt14* might, in general, suppress complete Ty insertion mutations and fail to suppress solo δ insertion mutations. To test this possibility, we constructed a series of strains containing *spt13* or *spt14* mutations and various complete Ty and δ insertion mutations. Three additional solo δ insertion mutations were crossed into the *spt13* and *spt14* backgrounds, including one, *his4-917*δ, that is located in the same position as *his4-917*. The *his4-917*δ insertion, unlike the *lys2-128*δ insertion, has a transcriptional orientation opposite that of the adjacent gene. None of the solo δ insertion mutations tested were suppressed by the *spt13* or *spt14* mutations (Table 4).

Two other Ty insertion mutations, *his4-912* and *lys2-61*, were also crossed into *spt13* and *spt14* backgrounds. Of the two, only *lys2-61* was suppressed. This insertion mutation is located in the 5′ noncoding region of the *LYS2* gene and resembles the *his4-917* insertion mutation (which is also suppressed by *spt13* and *spt14*) in that the Ty element is oriented such that the direction of Ty transcription is opposite that of the adjacent gene. The second insertion mutation, *his4-912*, also located in the 5′ region of the *HIS4* gene, was not suppressed. Ty912 is transcribed in the same direction as *HIS4*. These results suggest that it is the presence of unique (ε) Ty sequences that allows suppression by *spt13* and *spt14* mutations and that a complete Ty is suppressed only when its transcriptional orientation is opposite that of the adjacent gene.

***spt13* and *spt14*-mediated suppression occurs at the transcriptional level:** To determine whether

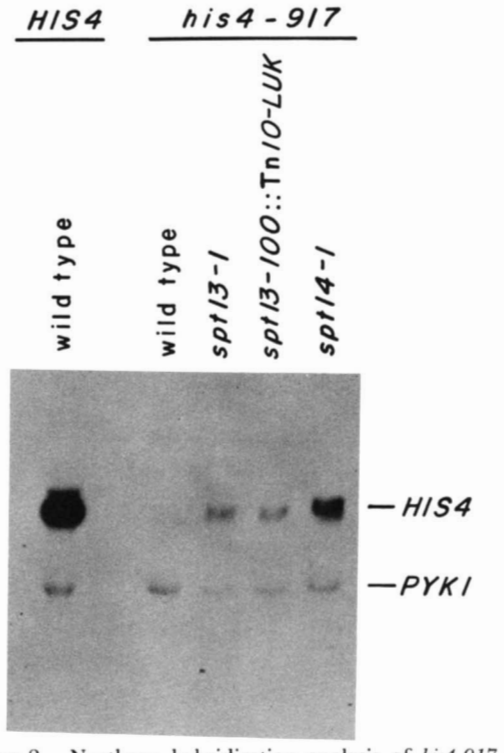

FIGURE 2.—Northern hybridization analysis of *his4-917* transcription. Total RNA was prepared from strains S288C (*SPT*$^+$, HIS4$^+$), JF15 (SPT$^+$, *his4-917*), JF397 (*spt13-1, his4-917*), JF580 (*spt13-100*::Tn*10-LUK*), *his4-917*), and JF444 (*spt14-1, his4-917*). Hybridization was to the *HIS4* probe, pFW45, and the *PYK1* probe, pFR2.

suppression of *his4-917* occurs at the transcriptional level, we examined *HIS4* transcription in *spt13* and *spt14* mutants by Northern hybridization analysis. The effect of *his4-917* on *HIS4* transcription in an *SPT*$^+$ strain is to virtually abolish *HIS4* transcription (WINSTON, DURBIN and FINK 1984). In the *spt13* or *spt14* mutants, however, *HIS4* transcription is partially restored (Figure 2). The same effect is seen for the Ty insertion mutation, *lys2-61* (data not shown). In an *SPT*$^+$ background, no detectable *LYS2* transcript is seen. In *spt13* strains, the *LYS2* transcript level is greatly increased.

To test the possibility that the *spt13* and *spt14* mutations might affect transcription of the wildtype *HIS4* and *LYS2* loci we analyzed *HIS4* and *LYS2* transcription in *SPT*$^+$, *spt13* and *spt14* strains. The wild-type *HIS4* (Figure 3) and *LYS2* (data not shown) transcript levels are only slightly reduced by the *spt13* or *spt14* mutations. This confirms that the increase in *HIS4* and *LYS2* transcript levels observed for Ty insertion mutations at these loci caused by *spt13* and *spt14* mutations is the result of a specific effect on expression of the insertion mutation alleles.

Effects of *spt13* and *spt14* mutations on δ insertion mutations: While neither *spt13* nor *spt14* suppress solo δ insertion mutations, two results demonstrate that the *spt13* and *spt14* mutations affect δ-mediated gene expression. First, the *his4-912*δ insertion muta-

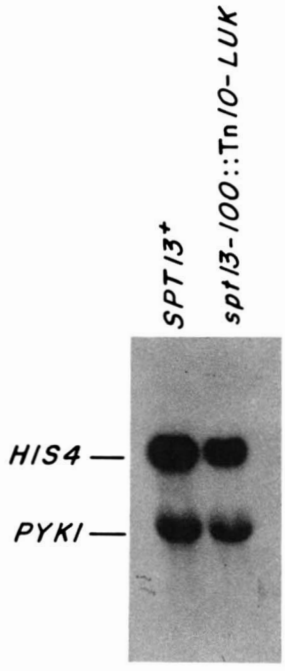

FIGURE 3.—Northern hybridization analysis of *HIS4* transcription. Total RNA was prepared from strains S288C (SPT$^+$) and JF372 (spt13-1). Hybridization was to the *HIS4* probe, pFW45 and the *PYK1* probe, pFR2.

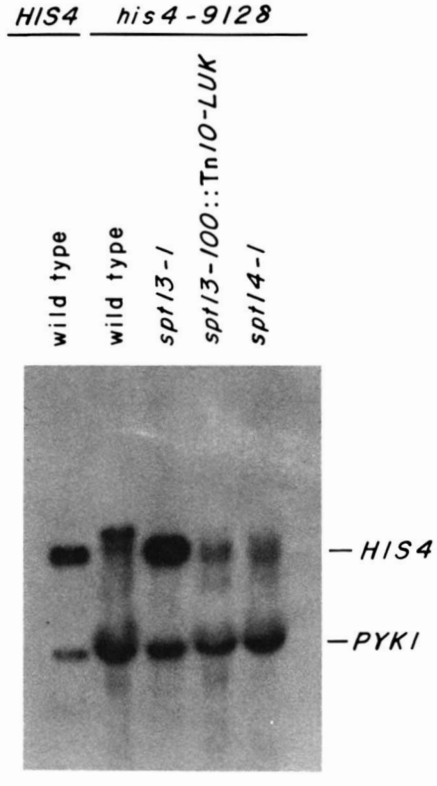

FIGURE 4.—Northern hybridization analysis of *his4-912δ* transcription. Total RNA was prepared from strains S288C (*SPT$^+$ HIS4$^+$*), FW1238 (*SPT$^+$*, *his4-912δ*), JF305 (*spt13-1*, *his4-912δ*), JF605 (*spt13-100*::Tn*10-LUK his4-912δ*) and JF296 (*spt14-1*, *his4-912δ*). Hybridization was to the *HIS4* probe, pFW45, and the *PYK1* probe, pFR2.

tion which is normally cold-sensitive (His$^+$ at 37°, weak His$^+$ at 30°, and His$^-$ at 20°) is His$^-$ at all temperatures in *spt13* and *spt14* backgrounds. To further analyze the effect of *spt13* and *spt14* on *his4-912δ*, we examined the pattern of *his4-912δ* transcription by Northern hybridization analysis. In *SPT$^+$* strains grown at 30°C, the *his4-912δ* transcripts initiate in the δ and at the wild type *HIS4* position (Figure 4, lane 2; SILVERMAN and FINK 1984). Interestingly, the only *his4-912δ* transcript in *spt13-1*, *spt13-100*::Tn*10-LUK* and *spt14-1* strains migrates near the position of the wildtype *HIS4* transcript (Figure 4, lanes 3 and 4). Further analysis is in progress to determine whether the His$^-$ phenotype of these strains can be explained by this transcriptional change. The *spt13-1* mutant produces a much greater amount of this transcript than *spt13-100*::Tn*10-LUK* or *spt14-1*.

A second indication that *spt13* mutations affect δ-mediated gene expression was seen in studies of *spt* double mutants with respect to suppression of solo δ insertion mutations. We found that *spt13* is epistatic to some other *spt* mutations for nonsuppression of δ insertion mutations. The epistasis of *spt13-1* mutation with respect to representative alleles in all other identified *SPT* genes was examined with respect to suppression of *lys2-128δ* (Table 5). This analysis showed that *spt13-1* is epistatic to the *spt1-1*, *spt3-101*, *spt7-217* and *spt8-113* mutations and weakly epistatic to *spt6-140*. Mutations in *spt14*, like those in *spt13*, fail to suppress δ insertion mutations; however, with the exception of *spt1-1*, *spt14-1* is not epistatic to other *spt* mutations for suppression of *lys2-128δ*.

Ty transcription in *spt13* and *spt14* mutants: Although *spt13*- and *spt14*-mediated suppression of Ty insertion mutations is phenotypically indistinguishable from suppression of Ty insertion mutations by *spt3*, *spt7* and *spt8* mutations, the two groups of mutants differ in their pattern of Ty transcription. While *spt3*, *spt7* and *spt8* mutations greatly reduce the level of full length Ty transcripts, *spt13* and *spt14* mutations cause no significant reduction in the level of full length Ty transcripts. A low level of a shorter Ty transcript that comigrates with the short transcript made in *spt3* mutants is observed (Figure 5, lane 3). The *spt14-1* mutation does not have any apparent effect on Ty transcription (Figure 5, lane 2).

Null phenotypes for *SPT13* and *SPT14*: To examine if the *spt13* and *spt14* suppression phenotype is due to loss of function rather than to alteration or gain of function, null mutations in each gene were isolated. The cloned *SPT13* and *SPT14* genes (J. FASSLER, and F. WINSTON, unpublished results) were disrupted as described in MATERIALS AND METHODS. In each case, the disrupted gene was substituted for the genomic allele by one-step gene replacement (ROTHSTEIN 1983). In the case of *spt13*, the null

TABLE 5

Epistasis tests of *spt13* and *spt14* with *spt1-12* for suppression of *lys2-128δ*

	Lys phenotype	
	spt13	*spt14*
spt1	−	−, −/+
spt2	+	+
spt3	−, −/+	+
spt4	+	+
spt5	+	+
spt6	+/−	+
spt7	−, −/+	+, +/−
spt8	−	NT
spt9	+	+
spt10	+	+
spt11	+	+
spt12	NT	NT

The suppresssion phenotype of each *spt* double mutant was scored in crosses of *spt13* and *spt14* with all of the other spt mutants. Some variability in the phenotype of *spt1 spt14* recombinant spores, as well as in *spt7 spt13*, and *spt3 spt13* double mutants spores was observed. The range in phenotypes is given. −, no suppression; −/+, weak suppression; +/−, moderately strong suppression, +, strong suppression. NT, not tested. For the single mutants *spt2-spt12*, suppression of *lys2-128δ* was observed. *spt1-1* is a very weak suppressor of *lys2-128δ*.

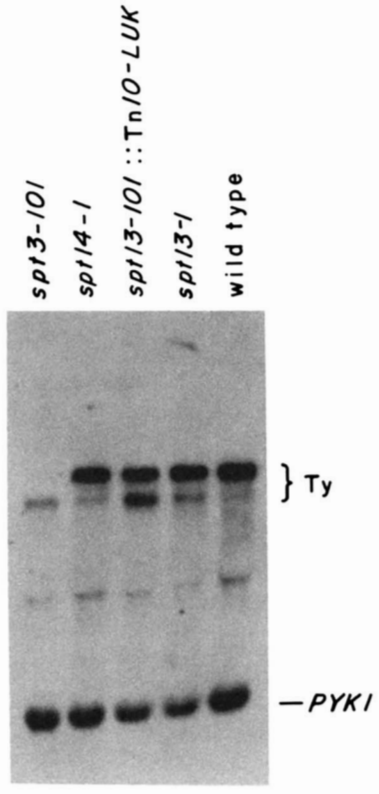

FIGURE 5.—Northern hybridization analysis of Ty transcription. Total RNA was prepared from strains JF94 (*spt3-101*), JF441 (*spt14-1*), JF577 (*spt13-101*::Tn*10-LUK*), JF398 (*spt13-1*), and JF16 (*SPT*$^+$). Hybridization was to the Ty probe, B161.

mutant exhibited the same suppression and transcription phenotypes as *spt13-1*, a spontaneously isolated mutant. All of the insertion mutations tested shown in Table 2 were also crossed into the *spt13* null (*spt13-100*::Tn*10-LUK*) background (JF580). As in the *spt13-1* mutant, no δ insertion mutations were suppressed. In addition, a diploid homozygous for the *spt13-100*::Tn*10-LUK* mutation failed to sporulate. We therefore conclude that for *SPT13*, suppression is due to loss of function.

Null mutations in *SPT14* resulted in a lethal phenotype in haploid strains. Tetrads dissected from *spt14-1/spt14-200*::Tn*10-LUK* (JF590) and *SPT14/spt14-200*::Tn*10-LUK* (JF593) diploids segregated 2:2 for viability. This is consistent with the temperature sensitivity of *spt14-1* mutants and demonstrates that *SPT14* is essential for growth. However, we cannot yet conclude for *SPT14* that suppression is due to loss (or reduction) of normal *SPT14* function.

DISCUSSION

By selection for *trans*-acting suppressors of a complete Ty insertion mutation at *HIS4* and a solo δ insertion mutation at *LYS2*, we have isolated mutations in 13 *SPT* genes. These genes include seven out of eight of the previously identified *SPT* genes (WINSTON *et al.* 1984, 1987) and six previously unidentified *SPT* genes. Among the newly identified genes, we have found two, *SPT13* and *SPT14*, in which mutations cause a novel suppression pattern: suppression of complete Ty insertion mutations but not of solo δ insertion mutations.

A total of 162 mutants were examined, 37 of which contained mutations allelic to mutations in seven of the eight previously identified *SPT* genes. Fifty-eight additional mutations identified six new *SPT* genes. The remaining mutations complemented representative mutations from all of the complementation groups. Since only 67% of the new mutants examined were able to be categorized into complementation groups consisting of more than one member, additional genes that can mutate to give an Spt$^-$ phenotype are likely to exist.

Selection for suppressors of Ty and solo δ insertion mutations is predicted to yield mutations in genes encoding transcription factors. M. CIRIACY (personal communication) has found that *S. cerevisiae* strains that lack Ty elements (but which still contain solo δ sequences) are unaffected in their growth. Hence, the fact that many *spt* mutants are slow growing and that several *SPT* genes are essential for growth, suggests that an alteration in Ty-mediated gene regulation is merely a symptom of more general defects. The nature of these defects may be directly related to cellular transcription. However, the possibility remains that for at least some *spt* mutants, alterations

in transcription may be an indirect consequence of some other defect.

Mutations in *SPT13* and *SPT14* suppress complete Ty insertion mutations by elevation of the level of adjacent gene transcription. Mutations in *SPT3*, *SPT7* and *SPT8* also suppress complete Ty insertion mutations by elevating adjacent gene transcription. However, *spt3*, *spt7* and *spt8* mutations greatly reduce full length Ty transcription, whereas *spt13* and *spt14* mutations have little or no effect on full length Ty transcription. This suggests that the levels of Ty transcription and adjacent gene transcription are not necessarily related. Similarly, PARKHURST and CORCES (1986a,b) have reported that, while both su(Hw) and su(f) mutations suppress gypsy insertion mutations in *Drosophila melanogaster*, a su(Hw) mutation decreases gypsy transcript levels and a su(f) mutation increases gypsy transcript levels.

Although *spt13* and *spt14* mutants have related patterns of suppression and transcription, two lines of evidence suggest that the two genes are functionally distinct. First, *SPT14* is essential, whereas *SPT13* is not. And second, *spt13*, but not *spt14* mutations are epistatic to certain other *spt* mutations with respect to suppression of solo δ insertion mutations. In the case of *SPT13* and *SPT14*, therefore, similarity in suppression phenotype is not consistent with the genes acting at a common step in transcription.

The suppression phenotype and the transcription patterns in *spt13* and *spt14* mutants suggest that *SPT13* and *SPT14* identify factors that may interact at transcription signals located in the internal (ϵ) region of the Ty. However, our finding that δ-mediated suppression and transcription can be affected by *spt13* and *spt14* mutations suggests that, despite the failure of *spt13* and *spt14* mutations to suppress δ insertion mutations, *SPT13* and *SPT14* may recognize sequences in the δ as well as in the ϵ region of the Ty.

A region required for activation of adjacent gene transcription by Ty elements of adjacent genes has been localized to a 1.5-kb region at the 5′ end of Ty (ERREDE *et al.* 1984, 1985; ROEDER, ROSE and PEARLMAN 1985; ERREDE, COMPANY and HUTCHISON 1987). Within this region there is repeated sequence homology to both the SV40 enhancer core sequence and to the *MATa1/MATα2* control site which itself is partially homologous to SV40 transcriptional control sequences. In light of the finding that *spt13* and *spt14* mutations restore expression to genes only when the Ty is inserted with the 5′ end proximal to the adjacent gene, these 5′ sequences are possible candidates for sites of action for the *SPT13* and *SPT14* products. Work currently in progress will address the possibility that the *SPT13* or *SPT14* genes encode factors which regulate transcription through the Ty enhancer.

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