MUTATIONS AFFECTING TY-MEDIATED EXPRESSION OF THE *HIS4* GENE OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

We have identified mutations in seven unlinked genes (*SPT* genes) that affect the phenotypes of Ty and δ insertion mutations in the 5′ noncoding region of the *HIS4* gene of *S. cerevisiae*. *Spt* mutants were selected for suppression of *his4-912δ*, a solo δ derivative of Ty912. Other Ty and δ insertions at *HIS4* are suppressed by mutations in some but not all of the *SPT* genes. Only *spt4* suppresses a non-Ty insertion at *HIS4*. In addition to their effects on Ty and δ insertions, mutations in several *SPT* genes show defects in general cellular functions—mating, DNA repair and growth.

INSERTION of a transposable element in or adjacent to a structural gene can result in dramatically altered expression of that gene. The phenotype of the insertion mutation depends upon the particular transposable element, the position (with respect to the gene) at which it has inserted and the genetic background of the strain. Insertion of an element within the coding region of a gene will usually destroy the function of that gene (SHAPIRO 1969; JORDAN, SAEDLER and STARLINGER 1968; MALAMY 1970). Insertion into the regulatory regions outside of a gene can affect the amplitude of gene expression like other *cis*-acting mutations in regulatory regions. These effects range from turning gene expression off to turning it on at a high constitutive level (for reviews see KLECKNER 1981; ROEDER and FINK 1983; VARMUS 1982).

The phenotype of an insertion mutation can be affected by *trans*-acting genes unlinked to the insertion. In maize, unlinked genetic loci have a variety of effects on insertion mutations, including suppression and destabilization (for reviews see MCCLINTOCK 1965; FINCHAM and SASTRY 1974; PETERSON 1977; FEDEROFF 1983). In *Drosophila melanogaster* insertion mutations by the transposable element gypsy can be suppressed by suppressor of Hairy wing (MODELELL, BENDER and MESELSON 1983). In *E. coli* and Salmonella, mutations in the gene coding for the transcription termination factor *rho* can suppress the

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polarity caused by insertions in an operon (DAS, COURT and ADHYA 1976; CIAMPI, SCHMID and ROTH 1982).

In the yeast *Saccharomyces cerevisiae*, Ty insertions have one of two distinct effects when inserted in the 5′ regulatory regions of genes: they cause either constitutive gene expression (ERREDE *et al.* 1980; WILLIAMSON, YOUNG and CIRIACY 1981) or inhibition of gene expression (ROEDER *et al.* 1980). Both of these phenotypes are manifest at the transcriptional level, but the mechanisms by which the regulatory effects are exerted are not yet understood. An additional unexplained complexity is that the phenotype of Ty insertions which cause constitutive gene expression is often dependent upon the mating type of the cell (ERREDE *et al.* 1980; LEMOINE, DUBOIS and WIAME 1978; ROEDER and FINK 1982).

Ty insertion mutations at two different positions in the 5′ noncoding region of the *HIS4* gene have been isolated (ROEDER *et al.* 1980). These insertions result in inhibition of *HIS4* transcription (S. J. SILVERMAN and G. R. FINK, unpublished results). His^+ revertants of the insertion mutations can occur by several mechanisms. These include chromosomal rearrangements (CHALEFF and FINK 1980; ROEDER and FINK 1980), gene conversion in which the Ty at *HIS4* has been replaced by a Ty from somewhere else in the genome (ROEDER and FINK 1982) and mutations in unlinked suppressor genes (CHALEFF 1980; ROEDER *et al.* 1980).

In this paper we present data on the isolation and analysis of a large number of suppressors of Ty and δ insertions. The results presented show that mutations in any one of seven genes can lead to suppression of Ty or δ insertions at *HIS4*. These suppressor mutations have little or no effect on the stability of Ty elements themselves. In addition to their effects on Ty and δ insertions at *HIS4*, different classes of suppressor mutations display a variety of other phenotypes which affect mating, DNA repair and growth.

MATERIALS AND METHODS

Yeast strains: The designations for all yeast strains are standard (SHERMAN, FINK and LAWRENCE 1978). We have changed the designation *SPM* (ROEDER *et al.* 1980) to *SPT* (suppressor of Ty's) to make the symbol correspond more closely to the phenotype (see the section on δ-δ recombination). The yeast strains used in this study are listed in Table 1, except for some of the *spt* mutants referred to in Table 2. All *spt* mutants were spontaneous isolates derived from strains 8874-4B, 8875-28C, 7748(7)-8B, DC152 and 5748-19C. Strains listed without a source or reference were constructed in the course of this study. The *spo11* tester strains were clonally isolated before use in the mapping experiments. The designation *his4-912* refers to a strain carrying Ty912 inserted at position −161 from the start of translation of the *HIS4* gene, and *his4-917* refers to a strain carrying Ty917 inserted at position −71 from the start of translation. The designation *his4-912(URA3)* refers to a strain carrying Ty912 with the *URA3* gene inserted at a *Hin*dIII site within the element (ROEDER and FINK 1982). The *a* and *b* designations for this allele refer to the two orientations of *URA3*: *his4-912(URA3a)* has *URA3* in the same transcriptional orientation as Ty912 and *his4-912(URA3b)* has the opposite orientation.

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).

Media: All media were made as described by SHERMAN, FINK and LAWRENCE (1978). These include rich media (YPD), minimal media (SD) and sporulation media. SC-his, SC-ura and SC-arg

are complete synthetic media (SHERMAN, FINK and LAWRENCE 1978) lacking histidine, uracil and arginine, respectively. Solid media contained 2% agar. Medium for selection of *ura3* mutants was SD with uracil and histidine plus 5-fluoroorotic acid (5FOA) at a concentration of 500 μg/ml.

Isolation of mutants: Nearly all of the mutations described in this paper were isolated in strains 8874-4B and 8875-28C (Table 1). The remaining mutations were isolated from strains 7748(7)-8B, DC152 and 5748-9D. All of these strains have a His^- phenotype due to an insertion mutation in the *HIS4* region. All of the *spt* mutants were isolated by selecting for His^+ revertants on SC-his plates. Most of the mutant isolations were performed in the following way: Single colonies of 8874-4B and 8875-4B were grown on YPD plates. The colonies were picked and patched with sterile toothpicks onto YPD plates, with ten patches arranged on each plate. The patches were grown for approximately 24 hours at 30° and were then replica plated to SC-his plates which were incubated at 30° for 3–5 days. We found that using SC-his plates instead of SD plates supplemented with all growth requirements except for histidine minimizes background growth at 30°. To ensure the independence of each mutation, a single His^+ colony from each patch was purified by streaking once on SC-his plates and once on YPD plates. In a few other cases *spt* mutants were isolated by essentially the same procedure except that 0.1 ml of an overnight culture was spread on plates lacking histidine.

Complementation analysis: For the initial complementation analysis, all *spt* mutants isolated in strain 8874-4B (*MAT***a**) were grown in parallel stripes on a set of YPD plates, and all *spt* mutants isolated in strain 8875-28C (*MATα*) were grown on another set of YPD plates. After 1 day of growth at 30°, the sets of *MAT***a** and *MATα* stripes were transferred to the same YPD plates by replica plating such that the *MAT***a** stripes were perpendicular to the *MATα* stripes. All possible combinations of *MAT***a** × *MATα* strains were made by a series of such cross replications. The patterns on these cross plates were then replicated to SD plates with all nutritional requirements except leucine and histidine (selection for Leu^+ selects for diploids). In this complementation test, a His^+ phenotype indicates failure of the *spt* mutations to complement, whereas a His^- phenotype indicates complementation of the *spt* mutations. The parental strains were included on each plate to test for the possible dominance of the *spt* mutations.

One representative from each *SPT* complementation group was used to construct heterozygous diploids in all possible combinations. In most cases the diploids were isolated by prototrophic selection. In cases in which selection against both parents was not possible, diploids were isolated by the micromanipulation of zygotes after the parental strains had mated for 5 hours on a YPD plate at 30°. The ability of each diploid to grow on plates without histidine was then determined.

Mapping the SPT2 *gene*: *SPT2* was localized to a chromosome by the *spo11* method of KLAPHOLZ and ESPOSITO (1982). For mapping *SPT2* we used strain 8966-19C which has a *URA3* gene inserted at the *SPT2* region by integrative transformation and the allele *ura3-52* at the *URA3* locus. We scored the Ura phenotype as a marker for the location of *SPT2* in these initial experiments. To construct the *spo11/spo11* diploids, we crossed 8966-19C with the three *spo11* tester strains, K381-9D, K393-35C and K396-22B (Table 1). The diploids were sporulated and plated on SC-arg with canavanine (60 μg/ml) and YPD with cycloheximide (2 μg/ml). These media select for viable meiotic products which are haploid for at least the chromosome carrying the recessive drug resistance allele. These drug-resistant strains were then scored for the relevant markers. The desired result in these experiments is that the only surviving meiotic progeny are parental; no recombinants of linked markers should be observed. We monitored the frequency of recombination in *spo11/spo11* diploids by several pairs of markers, including *LEU2-MAT*, *HOM3-CAN1*, *CYH2-ADE6* and *CYH2-LEU1*. Several *spo11/spo11* combinations in which one parent was a member of the *spo11* tester set gave different results—some showed up to 10% recombination between markers tested. We assume that there are modifiers of *spo11* in some of our strains.

The initial localization of *SPT2* to a chromosome was confirmed by mitotic recombination analysis. We then further localized *SPT2* by tetrad analysis, scoring *SPT2* directly by suppression of *his4-912δ*, which was homozygous in the cross.

Mapping the SPT3 gene: The *SPT3* gene was localized to a chromosome using the 2μ-mapping technique (FALCO and BOTSTEIN 1983). This method relies on the observation that, when a segment of the yeast plasmid 2μ circle is inserted into the yeast chromosome, markers on that chromosome become unstable during normal mitotic growth (FALCO *et al.* 1982). For this proce-

TABLE 1

Yeast strains

Strain	Genotype	Source
8874-4B	*MAT***a** *his4-912δ leu2-3 can1-100 ade2-1 lys2-1 SUP4-o*	
8875-28C	*MATα his4-912δ leu1 can1-100 ade2-1 trp5 SUP4-o*	
7748(7)-8B	*MAT***a** *his4-912δ ura3-52*	
DC152	*MAT***a** *his4-917 ino1 ino4*	
5748-9D	*MAT***a** *his4-912 HOL1-1 ino4-8*	
7748(8)-10A	*MATα ura3-52 his4-912*	
8595-6A	*MAT***a** *his4-912δ spt1-1 ura3-52 ino cry1*	
7656-1C	*MATα his4-912δ spt1-1 leu2-1*	
FW236	*MAT***a** *his4-912δ spt2-150 lys1-1 ura3-52 leu2-3*	
FW232	*MATα his4-912δ spt2-150 ade2-1 ura3-52*	
8930-2C	*MAT***a** *his4-912δ spt3-2 ura3-52*	
8930-7A	*MATα his4-912δ spt3-2 leu2-101*	
FW221	*MAT***a** *his4-912δ spt4-3 ura3-52 ade2-1 lys1-1 can1*	
FW251	*MATα his4-912δ spt4-3 leu2-3 lys1-1*	
FW224	*MAT***a** *his4-912δ spt5-194 ade2-1*	
FW226	*MATα his4-912δ spt5-194 ura3-52*	
FW247	*MAT***a** *his4-912δ spt6-140 leu2-3*	
FW141	*MATα his4-912δ spt6-140 ura3-52 ade2-1 cry1*	
FW229	*MAT***a** *his4-912δ spt7-159 leu2-3 lys1-1 can1*	
FW259	*MATα his4-912δ spt7-159 leu2-3 ura3-52*	
8966-19C	*MAT***a** *spo11 SPT2{URA3 spt2-1} ura3-52 trp1-289 his7 hom3 can1 cyh2*	
7519-9C	*MAT***a** *his4-912δ spt2-1 lys2 cry1*	
9244-8C	*MATα his4-912δ rad4-4 ura3-52*	
7770-3D	*MAT***a** *his4-912δ spt3-1 leu2-1*	
FW333	*MATα his4-912δ lys4 ura3-52 trp1*	
FW417	*MAT***a** *his4-912δ trp4 ade8*	
FW418	*MATα his4-912δ spt3-1 lys4*	
FW454	*MATα his4-912δ spt3-1 leu2-3 ade8*	
FW458	*MAT***a** *his4-912δ leu2-3 SUF3*	
K382-23A	*MAT***a** *spo11 ura3 can1 cyh2 ade2 his7 hom3*	KLAPHOLZ and ESPOSITO (1982)
K382-19D	*MATα spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1*	KLAPHOLZ and ESPOSITO (1982)
K381-9D	*MATα spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	KLAPHOLZ and ESPOSITO (1982)
K393-35C	*MATα spo11 ura3 his2 leu1 lys1 met4 pet8*	KLAPHOLZ and ESPOSITO (1982)
K396-22B	*MATα spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	KLAPHOLZ and ESPOSITO (1982)
5856-11D	*MAT***a** *his4-912 leu2-3*	
7655-7B	*MAT***a** *his4-912 spt1-1 lys2 cry1*	
9526-6B	*MATα his4-912 spt2-150 ura3-52 lys*	
9527-1C	*MAT***a** *his4-912 spt3-101 lys2*	
FW369	*MATα his4-912 spt4-3 leu2-3 ade2-1 lys1-1*	
FW370	*MATα his4-912 spt5-194 ade2-1*	
FW371	*MATα his4-912 spt6-140*	
FW258	*MATα his4-912 spt7-159 ura3-52*	
7767-2C	*MAT***a** *spt1-1 his4-917*	
7596-7A	*MATα spt2-1 his4-917*	

TABLE 1

continued

Strain	Genotype	Source
7555-5A	*MAT***a** *spt3-1 his4-917*	
FW362	*MATα spt4-3 his4-917*	
FW364	*MAT***a** *spt5-194 his4-917 ura3-52*	
FW366	*MAT***a** *spt6-140 his4-917 ura3-52 leu2-1*	
FW367	*MATα spt7-159 his4-917 ura3-52*	
FW128	*MAT***a** *his4-917δ spt3-1 {SPT3 URA3} ura3-52 ade2-1 ino1 ino4*	
8581-8C	*MATα his4-917δ spt1-1 ura3-52*	
8574-9B	*MAT***a** *his4-917δ spt2-1 ura3-52*	
8582-10B	*MAT***a** *his4-917δ spt3-1*	
FW419	*MAT***a** *his4-917δ spt6-140 leu2-3*	
S456	*MAT***a** *his4-917(456) ura3-52 ade2-1 ino1-13 ino4-8*	ROEDER and FINK (1983)
S466	*MAT***a** *his4-917(466) ura3-52 ade2-1 ino1-13 ino4-8*	ROEDER and FINK (1983)
S469	*MAT***a** *his4-917(469) ura3-52 ade2-1 ino1-13 ino4-8*	ROEDER and FINK (1982)
8588-2C	*MATα his4-917(456) spt2-1*	
8590-1D	*MATα his4-917(456) spt3-1*	
8591-2B	*MATα his4-917(466) spt2-1 ura3-52 ino*	
8593-2A	*MATα his4-917(466) spt3-1 ura3-52 ino*	
8594-3B	*MATα his4-917(469) spt2-1 ino*	
8596-1D	*MATα his4-917(469) spt3-1 ino*	
9504-7A	*MAT***a** *his4-TAX ura3-52 lys2-52*	
L1463	*MAT***a** *his4-TAX spt2-150 ura3-52*	
L1462	*MAT***a** *his4-TAX spt3-101 ura3-52*	
9604-2B	*MAT***a** *his4-TAX spt4-3 ura3-52*	
9626-3A	*MAT***a** *his4-TAX spt5-194 ura3-52 ade2-1*	
9531-5A	*MAT***a** *his4-TAX spt6-140 ura3-52*	
9532-1A	*MAT***a** *his4-TAX spt7-159 ura3-52*	
9161-2B	*MAT***a** *his4-912(URA3a) ura3-52*	
9162-10B	*MATα his4-912(URA3b) ura3-52*	
9260-1B	MAT**a** *his4-912(URA3a) ura3-52 spt2-1*	
9262-4D	*MATα his4-912(URA3b) ura3-52 spt2-1*	
9259-2B	*MAT***a** *his4-912(URA3a) ura3-52 spt3-1*	
9261-7A	*MAT***a** *his4-912(URA3b) ura3-52 spt3-1*	
FW585	*MAT***a** *his4-912(URA3a) ura3-52 rad52-1*	
FW586	*MAT***a** *his4-912(URA3a) ura3-52*	
FW587	*MATα his4-912(URA3b) ura3-52 rad52-1*	
FW588	*MATα his4-912(URA3b) ura3-52*	

dure we used a plasmid derivative of YEp24 (BOTSTEIN *et al.* 1979) containing a Sau3A partial restriction fragment of yeast DNA containing the *SPT3* gene (F. WINSTON and G. R. FINK, unpublished results). This plasmid was isolated from a recombinant DNA bank containing random Sau3A partial restriction fragments of yeast DNA (CARLSON and BOTSTEIN 1982). Stable transformants using this plasmid as the source of the DNA were shown to be at the *SPT3* locus by genetic and biochemical criteria. These transformants were isolated and mated by the strains K382-9D, K393-35C and K396-22B (Table 1), and diploids were isolated by micromanipulation. The prototrophic diploids were plated on YPD plates for single colonies, which were then replica plated

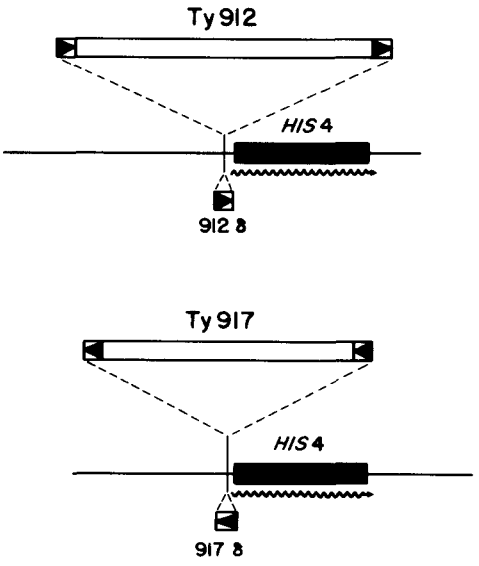

FIGURE 1.—Structure of insertion mutations in the *HIS4* region. *His4-912* and *his4-917* result from insertions of Ty912 and Ty917, respectively, into different sites in the *HIS4* 5′ noncoding region. Both of these mutations cause a His$^-$ phenotype. *His4-912δ* is a derivative of *his4-912* with a solo δ sequence at the same position as Ty912 is in *his4-912*. *His4-912δ* causes a cold-sensitive His$^+$ phenotype. *His4-917δ* is a solo δ derivative of *his4-917* and has a nonconditional His$^-$ phenotype. The wavy line represents the direction of *HIS4* transcription.

to SC-ura plates and to SD plates supplemented with uracil. In this way we were able to identify segregants that had become Ura$^-$ and segregants that had gained some other auxotrophy. Linkage of *SPT3* to markers on chromosome IV was demonstrated by tetrad analysis. In these crosses *spt3-1* was directly scored by suppression of *his4-912δ* which was homozygous.

Measurement of δ-δ recombination frequencies: To measure the frequency of δ-δ recombination for Ty912 in an unbiased manner (independent of selection for a His$^+$ phenotype) we used a construction containing the *URA3* gene in Ty912 (ROEDER and FINK 1982). This allele, *his4-912(URA3)*, is identical with *his4-912* with the addition of the *URA3* marker. Medium containing the compound 5FOA (P-L Biochemicals, Inc.) selects for *ura3* mutants (F. Lacroute, personal communication). Therefore, we were able to select for δ-δ recombination in strains with *his4-912(URA3)* and *ura3-52* at the *URA3* locus by selection for Ura$^-$.

For each strain to be tested, overnight cultures from ten single colonies were grown in YPD. The cultures were washed two times in sterile water and plated on YPD, SC-his and 5FOA plates. The 5FOA^R (Ura$^-$) colonies were of two classes: δ-δ recombinants and presumed gene convertants (in which Ty912 is replaced by a recombination event with another Ty element.) To measure the relative frequency of each event, we scored colonies on the 5FOA plates for the Ura and His phenotypes. Strains representing each of the different genotypes were also analyzed by SOUTHERN (1975) hybridization analysis to determine the DNA structure at *HIS4* (ROEDER and FINK 1980).

RESULTS

Isolation of spt *mutants*: We selected strains carrying spontaneous *spt* mutations as His$^+$ revertants of insertion mutations at the *HIS4* locus. In the initial studies, three different insertion mutations at *HIS4* were used: *his4-912*, *his4-912δ* and *his4-917* (Figure 1). *His4-912* and *his4-917* are the result of two different Ty elements, Ty912 and Ty917, inserted at different sites in the *HIS4* 5′

regulatory region (FARABAUGH and FINK 1980; ROEDER *et al.* 1980). These two Ty elements are inserted in opposite orientation with respect to each other, with Ty912 in the same transcriptional orientation as *HIS4* and Ty917 in the opposite orientation as *HIS4*. In general, Ty elements are transcribed across their entire length with transcription beginning in one δ and terminating in the other (ELDER, LOH and DAVIS 1983). The *his4-912δ* mutation is a derivative of *his4-912* that results from recombination between the direct δ repeats (δ-δ recombination) which flank the element (FARABAUGH and FINK 1980; ROEDER and FINK 1980). Strains carrying *his4-912δ* have a weak His^+ phenotype at 37°, a very weak His^+ phenotype at 30° and a His^- phenotype at 23°.

To avoid isolation of His^+ revertants such as chromosomal rearrangements and gene convertants, which can arise as revertants of the intact Ty in *his4-912* (CHALEFF and FINK 1980; ROEDER and FINK 1982), we selected most *spt* mutants as revertants of *his4-912δ* at 30°. Although we concentrated on these revertants, we were also able to isolate some *spt* alleles among revertants of *his4-912* and *his4-917*. In fact, *spt1-1* was isolated as a revertant of *his4-912*, several *spt2* alleles arose as revertants of *his4-912* (which had conveniently undergone δ-δ recombination, CHALEFF 1980) and *spt3-1* was isolated as a suppressor of *his4-917*.

To isolate His^+ revertants of a strain carrying an insertion, we patched single colonies on permissive media and after 24 hours of growth at 30° the patches were replica printed to SC-his plates as described in MATERIALS AND METHODS. The plates were incubated at 30° and after 3–5 days, 20–50 His^+ colonies grew in each patch. One colony from each patch was picked and purified selectively on SC-his plates and then permissively on YPD plates.

Complementation and dominance tests: The initial assignment of each mutation to a complementation group was done by the replica-plating test described in MATERIALS AND METHODS. Since half of the mutations were isolated in a *MAT***a** strain and the other half in a *MATα* strain, we first tested the ability of all mutants of one mating type to complement with all mutants of the opposite mating type. Some of the *spt2* and *spt3* mutations were identified by direct complementation with strains carrying *spt2* and *spt3* mutations identified previously. The complementation analysis (Table 2) identified seven *SPT* complementation groups. Each group except *spt1* is represented by at least two independently isolated alleles.

More rigorous complementation tests were performed with a strain carrying a representative allele of each complementation group by studying the growth of diploids made by crossing the *spt* strains with each other in all possible combinations. The purified diploids were tested for suppression of *his4-912δ* at 23°. The growth of these diploids was compared with that of diploids homozygous and heterozygous for each of the *spt* mutations. All diploids heterozygous for pairs of *spt* mutations from different groups are His^-, indicating complementation. Furthermore, every diploid that is SPT^+/spt^- for only one *spt* mutation is also His^-, verifying the conclusion that each mutation used in these tests is recessive. Every diploid homozygous for any *spt* mutation has a His^+ phenotype. From the results of the complementation test we conclude that these mutations represent seven *SPT* complementation groups.

TABLE 2

SPT *complementation groups*

SPT gene	No. of mutants	Dominance	Growth on MMS	Growth at 37°
1	1	r	+	+
2	169	d,r	+	+
3	3	r	+	+
4	2	r	−	+
5	3	r	+	+
6	17	r	+	−
7	4	r	+	+/−

spt1-1 and *spt2-1* were isolated as revertants of *his4-912. spt3-1* was isolated as a revertant of *his4-917.* All other *spt* mutants were isolated as revertants of *his4-912δ.* Six of the 17 *spt6* mutants have a temperature-sensitive phenotype. One of the two *spt4* mutants has an MMS-sensitive phenotype. All *spt* mutations are recessive (r) except 79 of the *spt2* mutations, which are dominant (d).

Dominant *SPT* mutations were uncovered only in the *spt2* complementation group. Seventy-nine (47%) of the mutations in the *spt2* group show at least some degree of dominance for suppression of the *his4-912δ* phenotype. That is, for these alleles the *spt*$^-$/*SPT*$^+$ *his4-912δ/his4-912δ* diploid grows significantly better on SC-his than the *SPT*$^+$/*SPT*$^+$ *his4-912δ/his4-912δ* diploid. These alleles show a large range in effect, varying from a barely detectable influence on the phenotype of the diploid (weak His$^+$) to a fully dominant phenotype (strong His$^+$).

Since dominance compromises the complementation test, the dominant *SPT* mutations have been classified in the *spt2* category by several criteria. First, some alleles of *spt2* which had been isolated previously show a partially dominant phenotype (CHALEFF 1980), so we surmised that the newly isolated dominants might also be *spt2* mutants. Second, most of these mutations are not fully dominant (approximately 80%) and in combination with a recessive *spt2* allele give a more extreme phenotype (better growth on SC-his) than when in combination with *SPT*$^+$. Third, one fully dominant *SPT* mutant strain, when crossed by a strain containing a representative *spt2* allele, *spt2-1*, yields no *SPT*$^+$ recombinants in 13 tetrads, showing that this dominant *SPT* mutation is very tightly linked to and probably in the same gene as *spt2-1.* Based on these results, we have tentatively classified our dominant mutations as *spt2* mutations.

Deletion mutations of SPT2 *and* SPT3: Deletion mutations of *SPT2* and *SPT3* have been identified. In both cases, the deletion mutation confers the suppressor phenotype and is recessive to wild type. The *spt2* deletion (*spt2-150*) was found among the spontaneously isolated *spt2* mutations as one that was also radiation sensitive, indicating that the mutation also affected the adjacent *RAD4* gene (see the section on mapping *SPT2*). This mutation was confirmed to be a deletion by Southern hybridization analysis (F. WINSTON and G. R. FINK, unpublished results). Both *spt3* deletion (*spt3-201*) and frameshift (*spt3-101*) mutations were constructed *in vitro* and transplaced into the genome, replacing the wild-type *SPT3* gene (F. WINSTON and G. R. FINK, unpublished

TABLE 3

spt × spt *pairwise crosses*

Parents	Parental alleles	His⁺:His⁻ segregation 4:0	3:1	2:2	Viability of double mutant
8595-6A × FW232	*spt1-1, spt2-150*	2	5	1	+
8595-6A × 8930-7A	*spt1-1, spt3-2*	0	7	2	+
8595-6A × FW251	*spt1-1, spt4-3*	4	5	4	+
FW224 × 7656-1C	*spt1-1, spt5-194*	4	2	4	+
8595-6A × FW141	*spt1-1, spt6-140*	0	7	1	+
8595-6A × FW259	*spt1-1, spt7-159*	2	5	1	+
FW236 × 8930-7A	*spt2-150, spt3-2*	0	6	2	+
FW236 × FW251	*spt2-150, spt4-3*	1	14	1	+
FW236 × FW226	*spt2-150, spt5-194*	7	7	1	+
FW247 × FW232	*spt2-150, spt6-140*	1	3	1	+
FW229 × FW232	*spt2-150, spt7-159*	1	5	0	+
8930-2C × FW251	*spt3-2, spt4-3*	5	13	3	+
FW224 × 8930-7A	*spt3-2, spt5-194*	1	3	5	+
8930-2C × FW141	*spt3-2, spt6-140*	0	6	2	+
8930-2C × FW259	*spt3-2, spt7-159*	0	4	3	+
FW224 × FW251	*spt4-3, spt5-194*	2	6	2	−
FW247 × FW251	*spt4-3, spt6-140*	4	5	0	−
FW221 × FW259	*spt4-3, spt7-159*	2	6	0	+
FW247 × FW226	*spt5-194, spt6-140*	0	7	2	−
FW229 × FW226	*spt5-194, spt7-159*	1	5	0	+
FW229 × FW141	*spt6-140, spt7-159*	1	6	1	+

The His⁺ segregation in tetrads is shown. All strains contain the *his4-912δ* mutation. For those crosses in which the *spt* double mutant is lethal, we have classified the inviable spores in the His⁺ category.

results). For these two *SPT* genes, we have used these null mutations for many of the characterizations of the respective mutants. The recessive nature of these mutations demonstrates that suppression results from loss of function.

Allelism tests between spt *mutations*: We performed pairwise crosses between representatives of each of the *SPT* complementation groups in order to determine whether the seven complementation groups represent seven different genes. Two *spt* mutations in the same gene should give a very low frequency of *SPT* meiotic recombinant spores (His⁻ phenotype); two *spt* mutations in different genes should give a large number of *SPT* spores in meiotic tetrads. The results of these crosses, summarized in Table 3, show that, in every case, crosses between *spt* mutants in different complementation groups yield a high percentage of *SPT* recombinants. These results confirm that the seven different *SPT* complementation groups represent seven different *SPT* genes. The recombination analyses also showed that three combinations of *spt* double mutants are inviable.

Mapping SPT2: The *SPT2* gene was localized to a chromosome by the *spo11* method of KLAPHOLZ and ESPOSITO (1982). The rationale for this method is that *spo11/spo11* diploids undergo meiosis in the absence of recombination

(KLAPHOLZ and ESPOSITO 1982). Therefore, genetic markers on any one chromosome will cosegregate. In the transformed parent the *URA3* gene is integrated at the *SPT2* locus and this parent is canavanine resistant (*can1*). When we plated the sporulated diploids on plates selecting for *can1* segregants (see MATERIALS AND METHODS), all of the *can1* colonies were Ura$^+$. The association of Ura$^+$ with can^R indicates that *SPT2* is on the same chromosome as *CAN1*, chromosome *V*. Mitotic recombination analysis confirmed this result (data not shown). Further meiotic crosses localized *SPT2* to within 0.25 cM of the *RAD4* gene on the right arm of chromosome *V* (Figure 2). In a cross of strains 7519-9C and 9244-8C, there was only one reciprocal recombination event between *SPT2* and *RAD4* found in 201 tetrads. There were also three gene conversion events, two *SPT2* to *spt2-1* and one *RAD4* to *rad4-1*. Although these two genes are extremely close, the isolation of recombinants displaying both mutant phenotypes suggests that they are not the same gene.

Further evidence for close linkage between *spt2* and *rad4* comes from the observation that strains carrying the *spt2* deletion mutation are also radiation sensitive and fail to complement *rad4*. The radiation sensitivity of the deletion is probably a result of deletion of both the *RAD4* and *SPT2* genes rather than deletion of a single gene responsible for both functions. None of the other strains carrying the 168 *spt2* alleles show radiation sensitivity. Moreover, none of the strains carrying other *rad4* alleles show the *spt* phenotype. Finally, a DNA segment that contains the *SPT2* gene fails to complement the *rad4-3* allele (G. R. FINK and G. S. ROEDER, unpublished results).

Mapping SPT3: The *SPT3* gene was first localized to a chromosome by the 2μ-mapping method (FALCO *et al.* 1982; FALCO and BOTSTEIN 1983). In one of the diploids in which the 2μ plasmid containing *URA3* and *SPT3* is integrated at the *SPT3* locus, we found co-loss of the Ura$^+$ phenotype with the Trp$^+$ phenotype at low frequency (five Trp$^-$ colonies among 193 Ura$^-$ segregants). Since the multiply marked parent (K381-9D) used to construct this diploid with the trasformed parent has a *trp1* marker, the co-loss of Trp$^+$ with Ura$^+$ localizes the *SPT3* gene to chromosome *IV*. We mapped the *SPT3* gene more exactly by tetrad analysis (Table 4). These results show that *SPT3* is tightly linked to *SUF3* between *TRP4* and *ADE8* on the right arm of chromosome IV (Figure 2). The order *SUF3—SPT3—ADE8* is based on the one tetratype tetrad listed in Table 4.

Temperature sensitivity: The *spt* mutants were screened for their ability to grow at high temperature on complete medium (YPD). The mutants were subjected to a preliminary screen by growing each in a small patch on YPD plates at 30° and then replica printing them to both SC-his plates and YPD plates at 23°, 30° and 37°.

Five of 17 *spt6* mutants exhibit a temperature-sensitive lethal phenotype which cosegregates with the *spt* phenotype. These mutations suppress the His$^-$ phenotype of *his4-912δ* weakly at 23° and well at 30° but fail to grow at all, even on rich medium at 37°. This result suggests that the *SPT6* gene product is essential for growth. In addition, at least one of the *spt7* mutants shows significantly poorer growth at 37°.

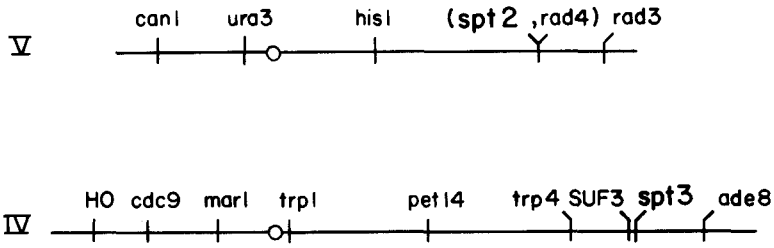

FIGURE 2.—Map positions of *spt2* and *spt3*. A partial genetic map of chromosomes *4* and *5* show the map positions of *spt2*, tightly linked to *rad4* on the right arm of chromosome *V*, and of *spt3*, tightly linked to *SUF3* on the right arm of chromosome *IV*.

Sensitivity to methyl methanesulfonate (MMS): The *spt* mutants were also screened for their sensitivity to the compound MMS. MMS sensitivity is a phenotype frequently associated with mutations affecting recombination and/or DNA metabolism in yeast (PRAKASH and PRAKASH 1977). Representative *spt* mutants were screened by spotting liquid suspensions of cells (in water) onto YPD plates with 0.02% MMS and without MMS. One of the two *spt4* mutants tested, *spt4-3*, showed MMS sensitivity.

Suppression of his4-912 *and* his4-917: Although most *spt* mutations were isolated as suppressors of *his4-912δ*, many suppress other Ty insertions at *HIS4*. We examined the effect of representative mutations in each of the seven *SPT* genes on *his4-912*, *his4-912δ*, and *his4-917* by constructing the appropriate double mutants. The results of this analysis are presented in Table 5. As already described, the representative allele for each *SPT* gene suppresses the His^- phenotype of *his4-912δ*. Suppression of *his4-912* is seen only by *spt1-1*. For *his4-917*, *spt3-1* is a strong suppressor, as judged by the strong His^+ phenotype of the *his4-917 spt3-1* double mutant. Strains with the *spt1-1*, *spt2-1* and *spt6-140* mutations also suppress *his4-917*, although not as well. Strains with *spt4-3*, *spt5-194* or *spt7-159* do not show any suppression of *his4-917* when compared to the SPT^+ control. Some *spt* mutants have been tested for suppression of *his4-917δ* which has a nonconditional His^- phenotype. *Spt3* mutations suppress *his4-917δ* (tested for *spt3-1* and *spt3-101*); *spt1-1*, *spt2-1* and *spt6-140* do not suppress *his4-917δ*. We have not yet tested other alleles of *spt2-spt7* for suppression.

Gene conversion of a derivative of *his4-917*, *his4-917(URA3)*, yields strains with many different Ty elements at the same position in the 5′ regulatory region of *HIS4* (ROEDER and FINK 1982). Those gene convertants that have a His^- phenotype were tested for suppression by *spt2-1* and *spt3-1*. The results (Table 6) show that the His^- phenotype of these Ty insertions is suppressed by the *spt2* and *spt3* mutations.

Suppression of a non-Ty promoter mutation: We examined the ability of mutations in each of the *SPT* genes to suppress a non-Ty insertion mutation caused by insertion of a plasmid into the 5′ noncoding region of *HIS4*. The insertion was created by transformation of a $HIS4^+$ yeast strain with YIp5 (STRUHL *et al.* 1979) containing a 274-base pair restriction fragment (*Taq*I-*Xho*I = TAX) which originates from the 5′ end of the *HIS4* gene (T. F. DONAHUE and G.

TABLE 4

Mapping SPT3 *by tetrad analysis*

segregating markers	PD	NPD	TT	cM
spt3, SUF3	49	0	1	1
spt3, trp4	14	0	9	20
spt3, ade8	53	0	37	21
trp4, ade8	7	0	18	36

The tetrads scored come from crosses of FW417 × FW418 and FW458 × FW454. PD = parental ditype, NPD = nonparental ditype, TT = tetratype. *spt3-1* was scored by its ability to suppress *his4-912δ* which was homozygous.

TABLE 5

Suppression of insertions at HIS4 *by* spt *mutations*

spt allele	*his4-912*	*his4-912δ*	*his4-917*	*his4-917δ*
spt1-1	+/−	+	+/−	−
spt2-1	−	+	+/−	−
spt3-1	−	+	+	+
spt4-3	−	+	−	ND
spt5-194	−	+	−	ND
spt6-140	−	+	+/−	−
spt7-159	−	+	−	ND

Symbols are as follows: + = His^+, +/− = intermediate His^+, − = His^-, ND = not done.

TABLE 6

Suppression of Ty gene convertants by spt2 *and* spt3 *mutations*

	Gene convertant[a]		
Relevant genotype	S456	S466	S469
SPT$^+$	−	−	−
spt2-1	+/−	+/−	+
spt3-1	+	+	+

[a] These gene convertants are new Ty elements at position −8 from the start of transcription in the *HIS4* 5′ noncoding region. They were obtained as spontaneous gene convertants of *his4-917(URA3)* (ROEDER and FINK 1982; G. S. ROEDER and G. R. FINK, unpublished results). The data indicate the ability of strains with these insertion mutations and either *SPT*$^+$, *spt2-1* or *spt3-1* to grow in the absence of histidine.

R. FINK, unpublished results). The *Taq*I site is in the 5′ noncoding region 174 base pairs from the start of translation, and the *Xho*I site is within the gene, 100 base pairs from the start of translation. Integration of the plasmid at the *HIS4* locus by a single crossover creates a duplication that has a size, structure and position within *HIS4* similar to that of Ty912 (Figure 3). *His4-TAX* transformants contain 5600 base pairs of YIp5 flanked by direct repeats (274 base pairs) of the TAX fragment. Strains containing the insertion of this extra DNA

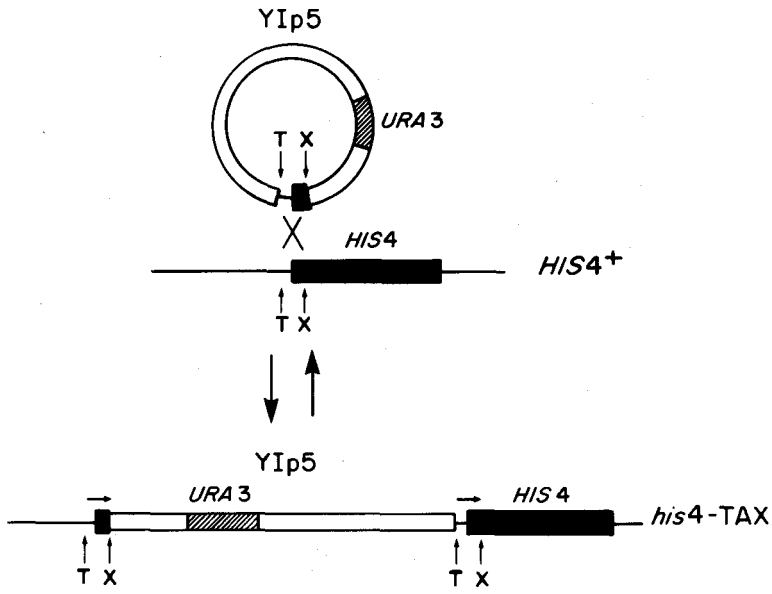

FIGURE 3.—Integration of the YIp5 derivative which contains a small piece of the *HIS4* gene, by a single crossover at the *HIS4* locus, results in integration of the plasmid at *HIS4*, creating *his4-TAX*. The integrated plasmid is flanked by direct repeats consisting of 274 base pairs of the *HIS4* 5′ region. T = *Taq* and X = *Xho*, denoting the restriction sites at the ends of this fragment in *HIS4*. The horizontal arrows indicate the direct repeats in the *his4-TAX* mutation.

in the 5′ noncoding region have a His^- phenotype, presumably as a result of disruption of the promoter sequence.

Meiotic segregants containing both an *spt* mutation and the *his4-TAX* mutation were tested for growth on SD at 23°, 30° and 37°. Strains containing *spt4* grow weakly on this medium at 37°, suggesting that *spt4* suppresses the *his4-TAX* mutation. None of the other *spt* mutations suppress *his4-TAX*.

We also examined the ability of *spt3-101* to affect the phenotype of two deletion mutations in the *HIS4* 5′ noncoding region. The His^- phenotypes of the two deletions we tested, *his4-416* and *his4-413* [referred to as Δ-136 and Δ-138, respectively, in DONAHUE *et al.* (1983)] were unaffected by *spt3-101*.

δ-δ recombination in SPT^+ *and* spt^- *strains*: The frequency of δ-δ recombination in *SPT*$^+$, *spt2-1* and *spt3-1* strains are listed in Table 7. These frequencies were determined by selection for Ura^- derivatives of strains with *his4-912(URA3)* and the *ura3-52* mutation at the *URA3* locus. Selection in this way avoids selection for His^+ and, hence, any bias due to the difference in the His^+ phenotype of *his4-912δ* in *SPT*$^+$ (weak His^+) and *spt*$^-$ (strong His^+) strains. Measurement of δ-δ recombination by this method demonstrates that neither *spt2* nor *spt3* have a significant effect on the frequency of δ-δ recombination for Ty912. When δ-δ recombination is measured by selection for His^+ a different result is obtained, due to the bias imposed by the different His^+ phenotypes in *SPT*$^+$ and *spt*$^-$ strains. When *his4-912* is plated at 37°, His^+ colonies arise at a 50-fold greater frequency in an *spt* background than in an *SPT*$^+$ background. This type of result led to the earlier suggestion that *spt* mutations

TABLE 7

Frequency of δ-δ recombination for Ty912

Strain	*SPT* allele	Recombination frequency	His^+ frequency
9161-2B	SPT^+	0.9 ± 0.5	0.05 ± 0.02
9162-10B	SPT^+	0.8 ± 0.3	$<5.4 \times 10^{-4}$
9260-1B	*spt2-1*	1.6 ± 0.8	3.9 ± 1.6
9262-4D	*spt2-1*	2.0 ± 1.4	5.8 ± 2.9
9259-2B	*spt3-1*	2.6 ± 3.0	3.0 ± 2.3
9261-7A	*spt3-1*	4.4 ± 7.6	4.6 ± 6.8

The numbers are the numbers of recombinants per 10^5 total cells. Each value represents measurement of the frequency and the standard deviation in ten independent cultures of each strain.

TABLE 8

Effect of rad52-1 *on δ-δ recombination*

Strain	*RAD52* allele	Recombination frequency
FW586	$RAD52^+$	2.0 ± 0.4
FW588	$RAD52^+$	3.5 ± 2.1
FW585	*rad52-1*	0.08 ± 0.06
FW587	*rad52-1*	0.15 ± 0.08

The numbers are the numbers of recombinants per 10^5 total cells. Each value represents measurement of the frequency and the standard deviation in ten independent cultures of each strain.

enhance δ-δ recombination (ROEDER *et al.* 1980). The measurements of δ-δ recombination by $5FOA^R$ selection demonstrates, however, that at least *spt2* and *spt3* mutations have no significant effect on Ty912 δ-δ recombination.

By the same procedure we also measured the effect of a *rad52-1* mutation on δ-δ recombination. The results (Table 8) demonstrate that *rad52-1* lowers δ-δ recombination in SPT^+ strains by approximately 25-fold. We found the same to be true in an *spt2-1* background (data not shown).

To verify that measurement of δ-δ recombination using *his4-912(URA3)* and selection for $5FOA^R$ reflects the real frequency of recombination we have done several tests. First, to demonstrate that 5FOA itself does not affect recombination, we performed a fluctuation test (LURIA and DELBRUCK 1943). For strain 9259-2B we measured the frequency of $5FOA^R$ in ten independent cultures, using the same plating procedure we used for testing δ-δ recombination in all of our experiments. We then combined the 10 cultures and measured the frequency of $5FOA^R$ of the combined culture ten times. The expectation is that if $5FOA^R$ arises in the individual cultures, as opposed to happening in response to exposure to 5FOA, then the fluctation of the measurements between the independent cultures will be significantly greater than the fluctation of the different platings of the combined culture. For the ten independent cultures the value for the number of $5FOA^R$ colonies per plate was 308 ±

364. The value for the ten platings of the combined culture was 354 ± 35. Therefore, the fluctation was significantly greater in the independent cultures than for multiple platings of the combined culture. From this we conclude that 5FOA itself has no significant effect on the number of $5FOA^R$ colonies we measure.

Second, we showed that the presence of the *URA3* gene in Ty912 does not affect the δ-δ recombination frequency. We compared the frequency of δ-δ recombination for *his4-912* and *his4-912(URA3)* in experiments by selection for His^+. In these cases we obtained the same results: the frequency of His^+ colonies was 20- to 50-fold greater in *spt* mutants than in SPT^+ strains. Therefore, the bias in selection for His^+ is seen in both cases, and the presence of *URA3* in the Ty element does not influence the outcome of the reversion experiment.

Third, the number of $5FOA^R$ colonies we detect is an accurate measure of the number of *ura3* cells plated. We did a reconstruction experiment, plating known amounts of *ura3* cells among 10^7 $URA3^+$ cells. In this experiment, the expected number of *ura3* colonies were found in each case (data not shown). In summary, mutations in *SPT* genes suppress Ty insertion mutations (hence, *SPT*) and do not increase δ-δ recombination.

DISCUSSION

Our studies identify seven genes (*SPT* genes) that affect the phenotype of Ty insertion mutations at the *HIS4* locus in *S. cerevisiae*. Mutations in each of these genes share the phenotype of suppression of the His^- phenotype of *his4-912δ*. Many *spt* mutations also suppress other Ty and δ insertions at *HIS4*. The suppression of *his4-912δ* by *spt1*, *spt2* and *spt3* mutations is known to occur at the transcriptional level (S. J. SILVERMAN and G. R. FINK, unpublished results).

In one sense, these Ty and δ insertions are promoter mutations and our selection could yield promoter mutation suppressors. These suppressors would include mutations in genes that are specific to Ty as well as those that are less specific and affect general cellular functions, such as transcription initiation or termination. Another possibility is a mutation analogous to the *supX* mutations in Salmonella which suppress the *leu-500* promoter mutation (DUBNAU and MARGOLIN 1972). Some facts argue in favor of the specificity of the *spt* mutations for Ty or δ elements. Only mutations in *SPT4* suppress a non-Ty promoter insertion at *HIS4*. In addition, strains containing *spt2* or *spt3* not only suppress *his4-912δ*, *his4-917* and *his4-917δ* but they also suppress several different Ty elements which were created by gene conversion of *his4-917(URA3)* (Table 6). Furthermore, recent experiments have shown that *spt2* and *spt3* mutations can suppress some Ty and δ insertions at the *LYS2* locus (G. SIMCHEN, F. WINSTON, C. STYLES and G. R. FINK, unpublished results).

The phenotypes of strains with mutations in the *SPT3*, *SPT4*, *SPT5*, *SPT6* and *SPT7* genes suggest that these genes may also have a role in general cellular functions. Several *spt6* alleles are temperature-sensitive lethal mutations, and *spt7-159* causes extremely poor growth at elevated temperature. Strains containing *spt4-3* are MMS sensitive, suggesting that *SPT4* may be required for repair of DNA damage. The double mutants *spt4 spt5*, *spt4 spt6*

and *spt5 spt6* are inviable. Strains carrying mutations in *spt3*, including a frameshift and a deletion, show an unusual mating defect—they mate well with SPT^+ strains but fail to produce zygotes with *spt3* strains. Zygotes between *spt3* and *SPT3* strains are formed efficiently when the *spt3* strain is either *MAT***a** or *MAT*α (F. WINSTON and G. R. FINK, unpublished results). The *SPT3* gene is distinct in map position and phenotype from all known *SIR* and *STE* genes which have been mapped (MORTIMER and SCHILD 1981).

Strains carrying mutations in the *SPT1* and *SPT2* genes give no indication that these genes participate in general cellular functions. However, since there is only one allele of *SPT1*, we may not have an adequate representation of the extent of possible phenotypic diversity at this locus. The major class of mutations we found are *spt2* mutations. This bias could have several explanations. For example, mutants containing *spt2* could have a growth advantage over other types of *spt* mutants under our selective conditions.

Other mutations that affect Ty-mediated gene expression in yeast have been identified. These include *tye* mutations (CIRIACY and WILLIAMSON 1981) and *roc* mutations (DUBOIS, JACOBS and JAUNIAUX 1982). Similar to some *tye* mutants, *spt3* mutants appear to have some conjugation defects. Since none of the *tye* mutations have been mapped we do not known whether they fell into the same set as our *SPT* genes.

Suppressors of insertion mutations in other organisms, including Drosophila, maize and bacteria have also been identified. In *D. melanogaster*, suppressors of two types of insertion mutations have been identified. These are *su*(w^a), which suppresses the w^a mutation, an insertion of a *copia* element at the white locus (BINGHAM and JUDD 1981; BINGHAM, LEVIS and RUBIN 1981; Z. ZACHAR and P. BINGHAM, unpublished results) and *su(Hw)* which suppresses several insertion mutations of the mobile element gypsy (MODOLELL, BENDER and MESELSON 1983).

In maize, several two-element controlling systems have been identified (MCCLINTOCK 1965; FINCHAM and SASTRY 1974; PETERSON 1977; FEDEROFF 1983). These two-element systems consist of a transposable receptor element which acts in *cis* on a gene near where it has inserted and in addition a regulatory element which can act in *trans* to alter the stability of the receptor element or expression of a gene adjacent to the receptor element. The two-element controlling systems have highly specific interactions in that the effects of the *trans*-acting regulatory element are limited to a particular receptor element.

In bacteria there is some indication of the molecular basis for suppression of insertion mutations. In both *E. coli* and Salmonella, mutations in the gene coding for the transcription termination factor *rho* suppress the polarity of insertion mutations in operons (DAS, COURT and ADHYA 1976; CIAMPI, SCHMID and ROTH 1982). It seems likely that the basis for suppression is that the *rho* mutation suppresses the transcription termination caused by the insertion element. In addition, the phage λ *N* gene product can suppress the polarity of insertion mutations (BRACHET, EISIN and RAMBACH 1970). Again, the basis of *N* suppression appears to be suppression of transcription termination.

The basis of *spt* suppression of Ty and δ insertions is likely to be different from that described for bacterial insertions. Since *spt* mutations can suppress Ty and δ insertions in either orientation, we think it is unlikely that suppression affects termination of a transcript that initiates in a Ty or δ element. It is still possible that some *spt* mutations affect Ty transcription. If so, they would affect the normal Ty transcript, which lies entirely within the Ty element (ELDER, LOH and DAVIS 1983). Some *spt* mutations could affect other fundamental cellular functions, such as transcription initiation specificity or promoter recognition by RNA polymerase.

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