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Saccharomyces cerevisiae SPT3 Gene Is Required for Transposition and Transpositional Recombination of Chromosomal Ty Elements

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Mutations in the *Saccharomyces cerevisiae SPT3* gene have dramatic effects on the expression of Ty elements and genes adjacent to the element. The *SPT3* gene is essential for Ty transposition, because transposition of chromosomal Ty elements ceased when the *SPT3* gene was replaced with the frameshift mutation *spt3-101*. Presumably, the elimination of transposition was due to the effect of the *SPT3* gene product on Ty transcription; the transcripts of chromosomal Ty elements were largely abolished in the *spt3-101* strain (F. Winston, K. J. Durbin, and G. R. Fink, Cell 39:675–682, 1984). Ty transcription in an *spt3-101* strain could be reestablished by introduction of the pGTyH3 plasmid, in which transcription of the Ty element TyH3 is under the control of the *GAL1* promoter; these plasmid-derived Ty transcripts were *SPT3*-independent. Ty transposition resumed after galactose induction in *spt3-101* strains containing the pGTyH3 plasmid. In *spt3* mutants nearly all of the resulting transposition events derived from pGTyH3 plasmids and not from chromosomal elements.

Ty elements are a family of retrotransposons found in the yeast *Saccharomyces cerevisiae*. Ty elements are about 6 kilobases (kb) long and carry long terminal direct repeat (LTR) sequences of 335 base pairs (bp) called δ elements. The δ's flank a central region called ε (4). The major transcript of the Ty elements extends from δ to δ (10). The transposition of Ty elements proceeds via an RNA intermediate and resembles the process of retroviral reverse transcription and integration (2, 13). Thus, Ty elements can be viewed as endogenous viruslike parasites of the yeast genome. Ty elements carry an enhancerlike sequence which is capable of activating adjacent genes (12, 28).

There are many chromosomal single-copy yeast genes, designated *SPT*, *ROC*, and *TYE* (5, 8, 37), which interact with Ty elements. It is not known whether any of these chromosomal genes are essential to the transposition process. One of the best-characterized *trans*-acting genes is the *SPT3* gene (37, 38; F. Winston and P. Minehart, submitted for publication; F. Winston and P. L. Minehart, Nucleic Acids Res., in press). Specifically, it has been shown that both the Ty transcript and the transcripts from various solo LTRs (LTRs not associated with a Ty element) are greatly decreased in abundance and that such transcripts are altered in structure. In *spt3* mutants there is little if any full length δ-to-δ transcript. Instead, a small amount of a transcript which is 800 bp shorter at the 5′ end is seen (38; K. Durbin, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1985). One would predict that since Ty RNA is an intermediate in transposition, inactivation of the *SPT3* gene by mutation should abolish Ty transposition.

In this paper we investigate the role of the *SPT3* gene in transposition by studying transposition in isogenic *SPT3* and *spt3-101* strains. This comparison shows that the *SPT3* gene is essential for the transposition of chromosomal Ty elements. (We use the term "chromosomal Ty element" to describe Ty elements which use the native Ty [δ] promoter, as opposed to the plasmid-borne pGTy elements we have constructed, which have a *GAL1* promoter. We do not mean to imply that the *SPT3* gene product affects plasmid-borne and chromosomally located elements differently.) We have previously described plasmid constructs (pGTyH3 and its derivatives) which contain a fusion between the inducible *GAL1* promoter and the Ty element TyH3 on a high-copy-number (2μm plasmid based) vector (2). *GAL1*-promoted Tys are transcribed from these plasmids in galactose-grown *spt3* strains because the *GAL1* promoter is *SPT3*-independent. Replacing the native Ty promoter with the *GAL1* promoter restores Ty transcription, which in turn leads to Ty transposition. Nearly all of the transpositions in the *spt3-101* strain are derived from the *GAL1*-promoted Ty. The transposition events recovered in the *spt3-101* strain differ from those recovered in the *SPT3* strains in two ways. Transposition in *SPT3* strains results in a high rate of sequence changes at certain polymorphic restriction sites within the Ty element (2). We show that such sequence changes during transposition are rare or absent in the *spt3-101* strain, suggesting that the sequence changes which occur in the wild-type strain derive from some form of recombination between Ty transposition intermediates. The most likely form of recombination is at the level of template switching (7) or strand displacement and assimilation during reverse transcription (reviewed in Skalka et al. [32]). Second, the distribution of sites at which Ty insertions are recovered (i.e., positions of insertion) in *spt3-101* strains extends further upstream than in the wild-type strain. Analysis reveals that Ty insertions which lie more than 175 bp 5′ to the *his3Δ4* target gene (2) are His^+ in the *spt3-101* strain but His^- in the *SPT3* strain.

MATERIALS AND METHODS

Strains, plasmids, media, and DNA manipulations. Isogenic *SPT3* and *spt3-101* (38) derivatives of strain JB183 (*MATa ura3-52 his3Δ200 trp1-289 lys2 SPT3 GAL*$^+$) were constructed by transformation of the *spt3-101*-integrating plasmid pFW33 (generously provided by F. Winston) into JB183. *spt3-101* is a frameshift mutation in the *SPT3* gene generated in vitro (38). Isogenic *SPT3* and *spt3-101* strains were selected from an integrant with 5-fluoroorotic acid

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TABLE 1. Temperature effect on Ty transposition

Growth temp (°C)	P(0)[a]	Mutation rate to His$^+$ [b] ± SE	Ty fraction[c]	Estimated transposition rate[d]
15	21/74	$7.5 (\pm 0.23) \times 10^{-8}$	9/14	4.8×10^{-8}
30	32/86	$1.6 (\pm 1.1) \times 10^{-8}$	1/20	8.0×10^{-10}

[a] Number of independent cultures containing no His$^+$ revertants over number tested.

[b] Mean number of His$^+$ revertants [from P(0)] divided by mean titer of Trp$^+$ (pAB100-containing) cells per culture. Standard error calculated by the method of Lea and Coulson (21).

[c] About 20 independent His$^+$ revertants from each experiment were examined by colony hybridization (of derived *E. coli* transformants) to determine whether the pAB100 derivative in question contained Ty sequences.

[d] Product of mutation rate and Ty fraction. The actual transposition rate is probably about twice this number, as >90% of the Ty insertions recovered were in one orientation (see Table 4).

medium (3). Yeast media were those described by Sherman et al. (30). *SPT3* and *spt3-101* strains were identified by a mating test with *SPT3* and *spt3* tester strains (*spt3* × *spt3* crosses give rise to a very low frequency of diploids [38]). The presence of the *spt3-101* mutation was confirmed by Southern blot analysis (the mutation destroys a *Sal*I site in the *SPT3* gene) and by crosses to a strain containing an *spt3*-suppressible *lys2* mutation. All *S. cerevisiae* strains used in this paper are isogenic to strain JB183, except that some carry the *spt3-101* mutation (as indicated throughout the text) or various plasmids. The plasmids used in this paper are described in Boeke et al. (2). The DNA manipulations were essentially as described in Maniatis et al. (23).

Determination of P(0). Independent 1-ml cultures of the strain to be tested were grown for 5 days in SC-Trp medium at 15°C. The cultures (50 to 100 per strain or condition) were harvested by filtration on sterile black nitrocellulose filters (0.8-μm pore size, 47-mm diameter; Micro Filtration Systems, Dublin, Calif., catalog no A080P047A), which were placed on individual 50-mm petri plates containing SC-histidine medium. The average titer of Trp$^+$ cells (i.e., cells carrying the target plasmid) per milliliter was determined by averaging the titers from five cultures. The plates were incubated for 1 week at 30°C to visualize His$^+$ revertants.

Determining the Ty fraction among His$^+$ revertants. His$^+$ revertants from strains containing only the *his3Δ4* target plasmid pAB100 were analyzed by colony hybridization. Ten or more ampicillin-resistant *Escherichia coli* HB101 transformants from each His$^+$ revertant were patched onto LB plates containing ampicillin. The patches were then probed by the Grunstein-Hogness (16) procedure with a Ty-specific probe. In this case, the Ty fraction was defined as the fraction of His$^+$ revertants whose plasmids hybridized to the Ty-specific probe. The colony hybridization procedure cannot be used on revertants from strains containing both pGTyH3 (or its derivatives) and pAB100 because His$^+$ revertants with Ty homology can arise by recombination between the two plasmids. When two plasmids were present, the plasmid responsible for the His$^+$ phenotype was purified by segregating out the His$^-$ plasmid (if necessary), followed by isolation of the His$^+$ plasmid (2) in *E. coli* cells. Rapid plasmid preparations were then analyzed by digestion with *Bgl*II and other enzymes to classify the plasmids. Plasmids bearing Ty insertions give a characteristic band pattern with *Bgl*II.

Mapping of Ty insertion points in pAB100. Several Ty insertions in pAB100 were sequenced to determine the exact point of insertion into the target plasmid (2; unpublished data). The sequenced Ty insertions served as standards for high-resolution restriction mapping of the insertion points of the unsequenced Tys. The high-resolution mapping was performed as follows. Samples (1 μl) of plasmid DNA (approximately 200 ng), prepared by the method of Holmes and Quigley (17), were treated sequentially with T4 DNA ligase (to repair nicks in the DNA), *Nco*I restriction enzyme (to linearize the DNA at a unique site near the point of Ty insertion), DNA polymerase Klenow fragment plus [α-^{32}P]dCTP (to label the 3′ ends generated by *Nco*I), and secondary restriction enzymes to release the radioactive restriction fragments indicative of the site of insertion. The secondary enzymes were *Alu*I and *Mbo*II, both of which cut within the δ sequences. The molecular weights of the novel radioactive fragments were compared with those of standards on a DNA sequencing gel. The position of the insertions is accurate to within 5 bp for insertions which lie within 130 bp of the *Bam*HI site and to within 10 bp for those farther than 130 bp from the *Bam*HI site (see Fig. 3).

RESULTS

Quantitative assay for the transposition of chromosomal Ty elements. We have described a shuttle plasmid, pAB100, which can be used as a target for Ty transposition events (2). This plasmid carries a mutant *S. cerevisiae HIS3* gene, called *his3Δ4*, which is nonfunctional because the *HIS3* promoter has been deleted and replaced with sequences from bacteriophage λ (29, 33). *S. cerevisiae* JB183 contains pAB100 and has a complete chromosomal deletion (*his3Δ200*) of the *HIS3* gene, resulting in a His$^-$ phenotype. His$^+$ revertants of this strain isolated at low temperature often contained a Ty insertion in the λ sequences 5′ to the *HIS3* structural gene (Tables 1 and 2). Other events, mostly partial duplications or deletions of the plasmid with one breakpoint near the 5′ end of the *HIS3* structural gene, can also give rise to a His$^+$ phenotype (29). Ty insertions were distinguished from other plasmid rearrangements by a simple colony hybridization method (see Materials and Methods) in *E. coli*. The fraction of His$^+$ revertants caused by Ty insertion was determined as the number of His$^+$ revertants arising via Ty insertion in a given experiment divided by the total number of revertants examined.

The P(0) method of Luria and Delbrück (22) was used to determine the overall mutation rate to His$^+$. The estimated Ty transposition rate was calculated as the product of the mutation rate to His$^+$ and the Ty fraction.

The Ty transposition frequency is strongly influenced by temperature (27). Our assay reflected this temperature ef-

TABLE 2. Transposition of chromosomal Ty elements in isogenic *SPT3* and *spt3-101* strains[a]

Strain	Relevant genotype	P(0)	Mutation rate to His$^+$ (±SE)	Ty fraction	Estimated transposition rate
JB183	*SPT3*[b]	16/73	$5.8 (\pm 0.84) \times 10^{-8}$	12/20	3.5×10^{-8}
JB234	*SPT3*[c]	8/77	$1.3 (\pm 0.25) \times 10^{-7}$	17/21	1.1×10^{-7}
JB198	*spt3-101*[c]	32/59	$3.1 (\pm 0.46) \times 10^{-8}$	0/21	$<1.5 \times 10^{-9}$

[a] Experiments were performed at 15°C. See Table 1, footnotes *a* through *d*.

[b] Pretransformation.

[c] After removal of the *spt3-101* allele by 5′-fluoroorotic acid treatment (3).

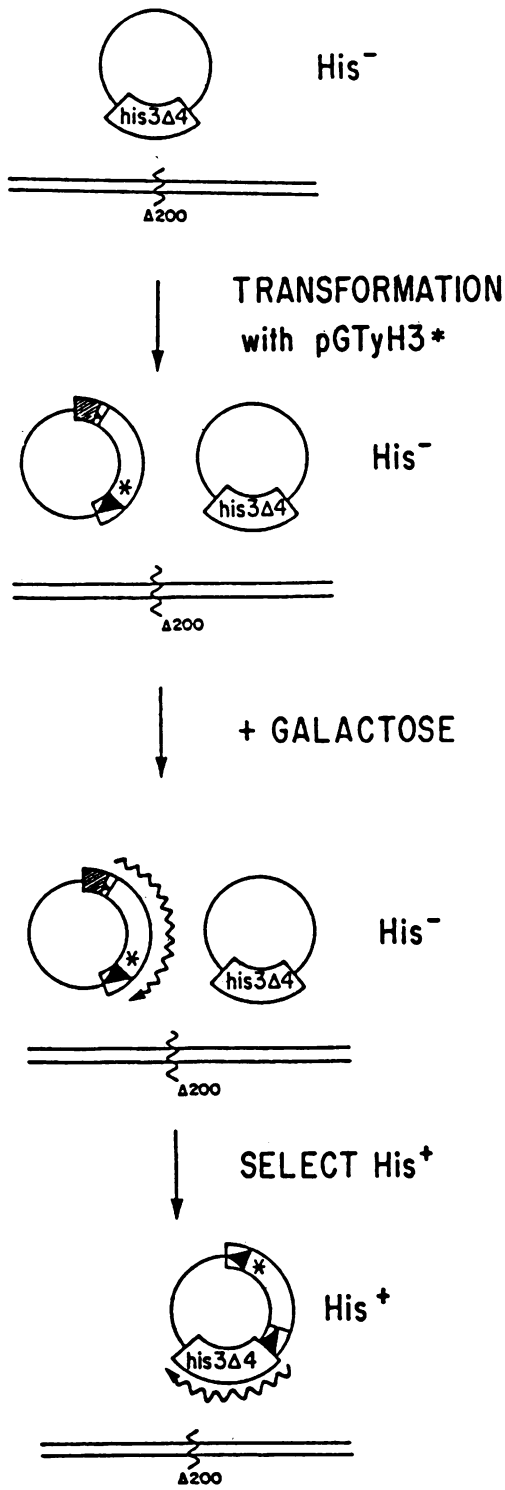

FIG. 1. Transposition of a marked Ty to a target plasmid. *S. cerevisiae* cells which are chromosomally *his3Δ200* (a complete deletion of the *HIS3* gene) and bear the plasmid pAB100, which carries the *his3Δ4* "gene," are His⁻ because the *his3Δ4* gene is not transcribed. A marked pGTyH3 plasmid (hatched box, *GAL1* promoter sequence; solid triangles, δ sequence; wary arrows, transcripts; *, marker [usually a synthetic *lacO* sequence]) is introduced by transformation. The addition of galactose results in transcription and subsequent transposition of the marked Ty (see text and Boeke et al. [2] for further details). Finally, transpositions of the marked element into the target plasmid are recovered as His⁺ revertants.

TABLE 3. His⁺ revertants from *SPT3* and *spt3-101* strains containing marked pGTyH3 plasmids

Parent strain	Relevant genotype	No. of revertants analyzed	Ty fraction[a] (%)	No. of Tys containing marker/no. tested (%)
JB396	*SPT3*	266	247/266 (93)	40/247 (16)
JB451	*spt3-101*	77	42/77 (55)	39/42 (93)

[a] Calculated as described in Materials and Methods (includes marked and unmarked Tys).

fect; transposition of chromosomal Ty elements at low temperature (15°C) occurred at a 10- to 50-fold-higher rate than at 30°C (Table 1).

***SPT3* gene required for transposition of chromosomal Ty elements.** Isogenic *SPT3* (JB234) and *spt3-101* (JB198) strains were constructed by allele replacement as described in Materials and Methods and by Boeke et al. (3). The transposition rate of chromosomal Ty elements in the *spt3-101* strain was at least 10-fold lower than in *SPT3* strains (Table 2). To ensure that Ty insertion into the *his3Δ4* plasmid resulted in a His⁺ phenotype in the *spt3-101* background, we transformed 45 Ty-mediated revertant plasmids originally obtained in the *SPT3* strain into the *spt3-101* strain by using the *TRP1* selectable marker in the plasmid for selection of transformants. All 45 plasmids conferred a full His⁺ phenotype in both *SPT3* and *spt3-101* strains. Moreover, under special conditions (see below), it was possible to isolate Ty-mediated revertants in the *spt3-101* strain. In view of these results, the low frequency of His⁺ revertants in *spt3* mutants is not due to a block in expression of *his3Δ4* plasmids bearing Ty insertions.

***GAL1*-promoted Ty transcription leads to transposition in *spt3* mutants.** The implication that the *spt3-101* mutation interferes with Ty transposition at the level of Ty transcription can be tested directly by studying a Ty whose transcription is independent of *SPT3*. In pGTyH3 and its marked derivatives, Ty transcription is driven by the *GAL1* promoter, which replaces the normal Ty promoter. pGTyH3 expressed TyH3 RNA abundantly in both *SPT3* and *spt3-101* strains when the *GAL1* promoter was induced (a condition referred to as transposition-induction). This plasmid produces a Ty RNA in both *SPT3* and *spt3-101* strains that is structurally indistinguishable from normal Ty RNA (A. Bystrom and J. Boeke, unpublished results). We examined Ty transposition in the *SPT3* and *spt3-101* strains by constructing strains containing both the pGTyH3-*lacO* plasmid and the *his3Δ4* target plasmid pAB100. Transposition was induced by growth on galactose (Fig. 1). Numerous His⁺ revertants from both strains were analyzed (Table 3). A large fraction of the revertants selected in both *SPT3* and *spt3-101* backgrounds resulted from Ty insertions into the target plasmid. Thus, unlike chromosomal Ty transposition, pGTyH3-promoted transposition appears to be *SPT3*-independent.

Transposons recovered in *SPT3* and *spt3-101* strains. The Ty elements obtained in these transposition experiments were examined to determine how frequently a marker (a synthetic *lacO* fragment) placed within the Ty portion of the pGTyH3 plasmid was found in the transposed Ty elements. The structure of the recovered Ty elements was studied by digestion with the restriction enzymes *Bgl*II, *Hha*I, *Hin*dIII, and *Xho*I. The last three enzymes are useful because they reveal differences in structure between different members of the Ty1 family of elements. In the *SPT3* strain, only 16% of

TABLE 4. Heterogeneity and orientation of marked Ty elements which transposed in *SPT3* and *spt3-101* strains

Genotype[a]	No. of marked Tys examined	No. of restriction site polymorphisms in transposed Tys[b]			Orientation of Ty[c]		Position of Ty[d]	
		0	1	>1	A	B	5′	3′
SPT3	40	31	5	4	36	4	0	40
spt3-101	38	38	0	0	38	0	12	27

[a] Genotype of strain in which transposition event occurred.
[b] All marked Tys were examined with the enzymes *Bgl*II, *Hha*I, *Hin*dIII, and *Xho*I.
[c] Ty is transcribed away from (A) or towards (B) *HIS3* (see Fig. 1).
[d] With respect to the *Bam*HI site (see Fig. 2 and 3).

the Tys recovered carried the *lacO* marker (similar results were obtained with other markers). We believe that the Tys recovered without the *lacO* marker originated from chromosomal Tys rather than from TyH3 because the unmarked Ty elements, as a group, showed greater heterogeneity at restriction sites known to be polymorphic among Ty elements (J. Boeke and D. Garfinkel, unpublished data).

Transposition was dramatically different in the *spt3-101* strain: 93% of the transposons recovered were marked with *lacO* (similar results were obtained when other markers were inserted in the Ty element). Only three unmarked elements were recovered from the *spt3-101* strain; at least two of these were different at polymorphic restriction sites. About 80 pAB100 derivatives (about 40 each from *SPT3* and *spt3-101*) carrying a marked Ty element were examined in detail by restriction mapping. Whereas more than 20% of the marked Tys recovered in the *SPT3* strain showed one or more polymorphisms with respect to the expected pattern of sites, none of the 38 marked Tys recovered from the *spt3-101* strain showed any evidence of polymorphisms with the enzymes used (Table 4). Nearly half of the Tys which arose in the *SPT3* strain that contained sequence changes were polymorphic at more than one site, suggesting that these sequence changes often occur in blocks.

Almost all of the Ty insertions were found in one orientation with respect to *HIS3*. Their orientation was such that *HIS3* and the Ty would be divergently transcribed (Fig. 2A). The same divergent orientation has been observed when other yeast genes were activated by Ty element insertion (11, 36). A few elements recovered in the *SPT3* strain were inserted in the opposite orientation (Fig. 2B). These constitute a rare class of Ty elements which can activate an adjacent gene in the tandem transcriptional orientation.

Position of insertion with respect to *HIS3*. The distance between the sites of Ty insertion and the start of the *HIS3* gene was determined by high-resolution restriction mapping combined in some cases with DNA sequencing (see Materials and Methods). All (40 of 40) of the insertions selected in the *SPT3* strain and 69% of those selected in the *spt3-101* strain were located within 175 bp of the *HIS3* initiator ATG codon (Table 4, Fig. 3). The remaining 31% of the insertions recovered in the *spt3-101* strain fell as far as 240 bp 5′ to the *Bam*HI site, which was located 186 bp upstream of the *HIS3* ATG. All the members of the first (*HIS3*-proximal) group were able to confer a His^+ phenotype regardless of the constitution of the *SPT3* locus, whereas the members of the second (*HIS3*-distal) group were His^+ in the *spt3-101* strain but His^- in the $SPT3^+$ strain.

There is a pronounced hot spot for Ty insertion in the *SPT3* strain at 126 nucleotides 3′ to the *Bam*HI site. The hot spot was much less pronounced in the *spt3-101* strain, in which only a few transposons were recovered at this position. In general, the transposons recovered from the *spt3-101* strain tended to lie farther from the *HIS3* gene. Presumably, the two patterns of insertion sites were shaped by selection for a His^+ phenotype and do not necessarily indicate different target specificities in the two strains.

DISCUSSION

Ty elements, like retroviruses, encode a reverse transcriptase that promotes transposition via reverse transcription of an end-to-end Ty transcript. Nevertheless, transcription of Ty is dependent on functions provided by the yeast host. Previous work suggests that RNA polymerase II (19) and the product of the *SPT3* gene (38; Durbin, Ph.D. thesis) and perhaps additional *S. cerevisiae* genes (F. Winston, J. Clare, P. Farabaugh, P. L. Minehart, and C. Dollard, Genetics, in press) are required for Ty transcription. Although the exact nature of the *SPT3* gene product is unknown, the simplest explanation for the phenotype of *spt3* mutations is that *SPT3* encodes a positive transcription factor required only for initiation at δ (LTR) promoters (38; Durbin, Ph.D. thesis). If the *SPT3* gene is destroyed by frameshift or deletion mutations, the normally abundant LTR-LTR transcript disappears. Instead, there is a small amount of Ty transcript which is about 800 bp shorter at the 5′ end. The shorter transcript is unlikely to provide an adequate substrate for transposition for two reasons. (i) It lacks the 45-bp terminal redundancy required for the generation of a complete reverse transcript (14, 34). (ii) It lacks the AUG codon of the first reading frame, which is presumably essential for the expression of both Ty reading frames (6, 24).

We have shown that the absence of full-length Ty transcript in *spt3-101* strains leads to the inability of chromosomal Ty elements to transpose. Under these circumstances the frequency of transposition is diminished at least 20-fold, in good agreement with estimates of the reduction in amount of full-length Ty transcript. Introduction of the pGTyH3 plasmid into the same *spt3-101* strain, however, restored transposition, presumably because transcription of the plasmid-borne TyH3 element is *SPT3* independent. Moreover,

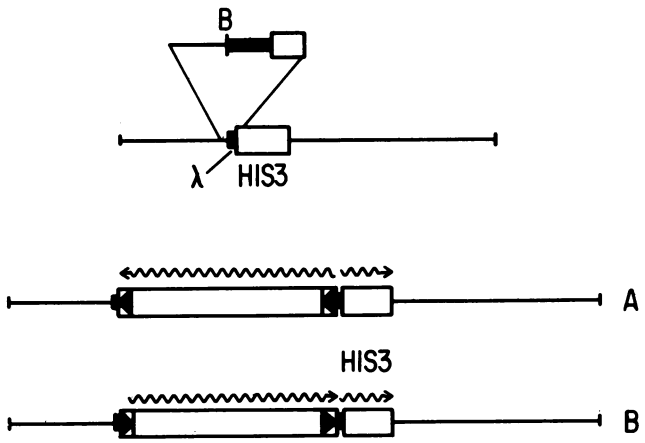

FIG. 2. Activation of the *his3Δ4* gene by Ty transpositions in both orientations. (Top) Open box, *HIS3* coding sequences; solid box, λ DNA sequences; lines, remainder of pAB100 linearized at the *Eco*RI site; B, *Bam*HI site. (Bottom) A and B are two His^+ revertants of pAB100 with the Ty inserted so that its transcription is away from (A) or towards (B) the *HIS3* gene. Solid triangles are δ sequences. Wavy lines symbolize transcripts. Transpositions of the A type were far more common than those of the B type (Table 4).

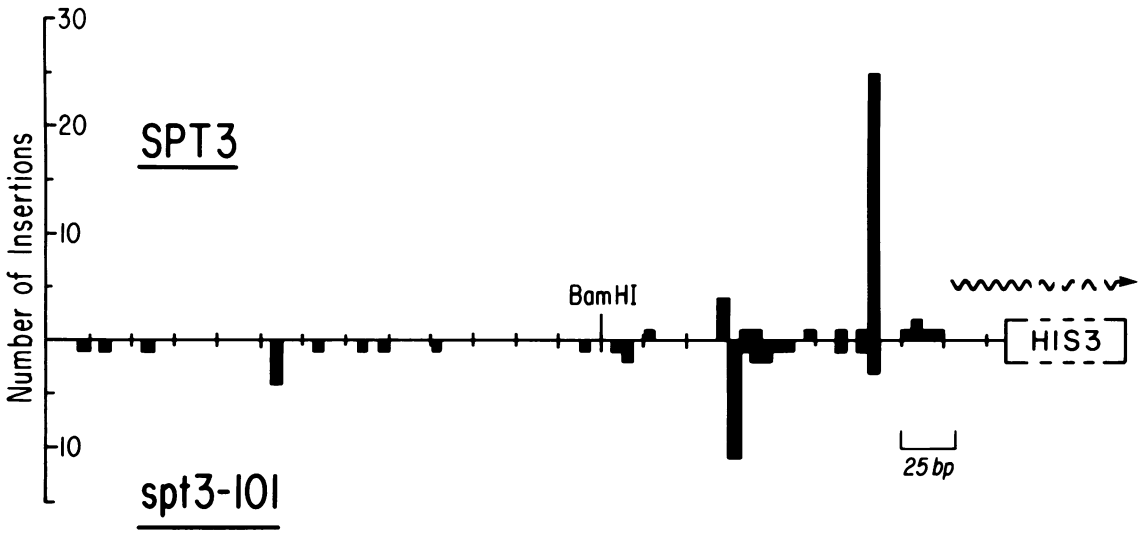

FIG. 3. Histogram of Ty insertion sites. The number of insertions falling within 5-bp segments of the target DNA 5′ to the *HIS3* gene in pAB100 in *SPT3* (above line) and *spt3-101* (below line) strains is indicated by the solid bars. The position of insertion (center of 5-bp duplication) was determined as described in Materials and Methods. The position of the *HIS3* transcript (wavy line) and coding sequence (box) are shown (from K. Struhl, personal communication). The broken lines indicate a segment of the diagram which is not drawn to scale.

nearly all of the transposition events we saw in the *spt3-101* strain derived from the plasmid-borne Ty, as judged by experiments with marked elements. The few unmarked Ty elements which were recovered in *spt3-101* could be the result of a very low level of full-length transcripts of chromosomal Ty elements present in strains carrying the *spt3-101* mutation (i.e., the mutation may be slightly leaky). Alternatively, these transpositions may derive from one or more "renegade" Ty elements which are independent or partially independent of *SPT3* control or from rare transcripts initiating outside certain Tys which read through the adjacent Ty and may sometimes serve as a template for reverse transcription. Yet another possibility is that the shortened chromosomal transcripts can participate in transpositional recombination. Finally, it is possible that these transposons result from rare homologous recombination events between chromosomal Ty elements and the pGTyH3-*lacO* plasmid at the DNA level prior to transposition. In any case, the vast majority of the elements recovered in the *spt3-101* strain derived from the marked Ty plasmid.

The structure of the marked Ty elements that have experienced a transposition in both *SPT3* and *spt3-101* strains provides an explanation for the sequence polymorphisms reported earlier for marked Ty transposition (2). Many of the marked transposons corresponded imperfectly to the parental pGTyH3 plasmid; although they carried the *lacO* marker fragment, they differed from the parental Ty element at one or more restriction sites. Two possible mechanisms were proposed for the generation of these polymorphisms: (i) misincorporation of nucleoside triphosphates by an inherently error-prone reverse transcriptase (15) or (ii) some form of recombination between the marked (plasmid-derived) and chromosomal Tys. By analogy with retroviruses, the most likely mechanism for such recombination would be copy choice, in which the reverse transcriptase switches templates (7) or facilitates strand displacement and subsequent assimilation of the displaced strand into a heteroduplex during the act of reverse transcription (1, 20). Our data strongly support the conclusion that a recombinational mechanism generates most if not all of the sequence changes which occur during transposition. In the *SPT3* strain, in which chromosomal Ty elements were transcribed abundantly, about 20% of the marked elements recovered showed one or, more often, multiple restriction site polymorphisms. The other 80% appeared normal with the enzymes used. By contrast, in the *spt3-101* strain, in which there was little or no chromosomal Ty transcription, all of the marked elements recovered had restriction patterns identical to those of the parent. In other words, in the absence of variant Tys as recombination partners, all the newly transposed elements were nonrecombinant. A more exhaustive analysis will be required to determine whether misincorporation also plays a role in generating Ty diversity. To date, we have detected no sequence changes or polymorphisms within marker segments we have inserted into the Ty, only changes within the element itself, and these changes are at sites known to be polymorphic among the chromosomal ensemble of Tys.

Reverse transcription takes place in isolated Ty viruslike particles (13, 25) as it does in retrovirus particles. Presumably, reverse transcription also takes place in retroviral core particles in vivo. The in vitro experiments of Junghans et al. (20) showed that recombination intermediates form in vitro in preparations of reverse-transcribing retrovirus particles, suggesting that at least some retrovirus recombination occurs in the particles. In our system recombination occurred at high frequency during transposition (at least 20% of the transpositions which arose in *SPT3* strains were recombinant), suggesting that multiple Ty RNA templates can interact during reverse transcription. We propose that, as in retroviruses, two (or more) Ty RNA molecules are packaged per viruslike particle.

The spectrum of insertion sites in the target plasmid was different in the *spt3-101* strain than in the *SPT3* strain. Specifically, the group of *spt3-101*-derived insertions contained a set of elements occupying a region of DNA where insertions were not found in the *SPT3* strain. When plasmids of this set were transformed into the *SPT3* strain, they did not confer a His^+ phenotype. The best explanation for these data is that there is a position effect: insertions less than 175 bp from the *HIS3* ATG are capable of producing enough

HIS3 transcript to confer a His^+ phenotype in either the *SPT3* or *spt3-101* background, but insertions further away from the *HIS3* ATG are able to confer a His^+ phenotype only in the *spt3-101* strain background. We propose that this effect may be due to promoter competition. Although the *his3Δ4* "gene" does not have a promoter per se, we suspect that the Ty LTRs are not solely responsible for the His^+ phenotype and that the *HIS3* and perhaps even the λ DNA sequences participate as well. It is unlikely that the *HIS3* transcripts intiate in the LTR because any transcript emanating from a point more than 50 bp within the LTR would contain AUG and terminator codons which would presumably prevent proper *HIS3* translation. One way of looking at this is that in the *HIS3*-proximal insertions, the *HIS3* and Ty promoters may be close enough together that *HIS3* can "compete" effectively for some limiting transcription factor, perhaps polymerase itself. When the powerful Ty promoter is moved some distance away (in the *HIS3*-distal insertions), *HIS3* can only compete for this factor effectively in the absence of *SPT3* gene product, i.e., when the Ty element is not actively transcribed. In any case, the "enhancer" within the Ty appears to be able to operate at distances of more than 500 bp from the *HIS3* gene.

SPT3 provides a critical "host function" required for Ty transposition. The release of Ty transcription from *SPT3* dependence which occurs in the *GAL1*-TyH3 construction suggests that the *SPT3* gene product acts within the U3 sequences of the LTR to determine correct transcription initiation. In addition, Ty transposition requires RNA polymerase II and probably a tRNA primer such as initiator methionine tRNA (9, 31; A. Bystrom and G. R. Fink, unpublished data). Other host functions may be required for various steps in transposition, such as the processing of Ty gene products, the "frameshifting" event thought to allow expression of the *tyb* gene (6, 18, 24) and integration of the reverse transcript into the DNA (26). Alternatively, Ty may be fairly independent of other specific host functions, as in the case of some retroviruses which are able to replicate in diverse cell types with presumably very diverse genetic backgrounds.

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