Ty*1* Transposition in *Saccharomyces cerevisiae* Is Nonrandom

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ABSTRACT

A large collection of Ty*1* insertions in the *URA3* and *LYS2* loci was generated using a *GAL1-Ty1* fusion to augment the transposition frequency. The sites of insertion of most of these Ty elements were sequenced. There appears to be a gradient of frequency of insertion from the 5′ end (highest frequency) to the 3′ end (lowest frequency) of both loci. In addition we observed hotspots for transposition. Twelve of the 82 Ty*1* insertions in the *URA3* locus were inserted in exactly the same site. Hotspots were also observed in the *LYS2* locus. All hotspots were in the transcribed part of the genes. Alignment of the sites of insertion and of the neighboring sequences only reveals very weak sequence similarities.

TRANSPOSABLE elements are characterized by their ability to insert copies of themselves into nonhomologous target sequences. In most cases, transposons duplicate a small number of base pairs of the target sequence as part of the transposition process; the number of base pairs duplicated is characteristic of that type of transposon. This duplication strongly implies that a specific transposase (or in the case of retrotransposons and retroviruses, integrase) encoded by a given type of element mediates the breaking and joining reactions that are the crux of a transposition event. Thus a study of the target sequences recognized during transposition reactions should yield information on the properties of transposition intermediates.

The genome of the yeast, *Saccharomyces cerevisiae*, contains three families of transposable elements, called Ty elements (reviewed by BOEKE 1989). Two of these families, consisting of the Ty*1* and Ty*2* elements, are about 6 kb long and are flanked by long terminal repeats (LTRs, also called δ) of approximately 340 bp. The third family, Ty*3*, has the same overall structure but is very different from the other Tys in sequence. Ty*3* elements and their solo LTR sequences are always found inserted 15 to 19 bp from the 5′ end of tRNA coding regions (HANSEN, CHALKER and SANDMEYER 1988; SANDMEYER *et al.* 1988). Transcription of Ty elements initiates in the 5′ LTR and terminates in the 3′ LTR; 45 nucleotides of the major Ty transcript are terminally reduntant (ELDER, LOH and DAVIS 1983). These structural features, as well as sequence comparisons between Ty elements and retrotransposons from other eucaryotes, suggested an evolutionary link with the retroviruses of higher eucaryotes. Functional evidence for this relationship was obtained when Ty*1* elements were shown to transpose through an RNA intermediate, to encode a reverse transcriptase activity and to produce Ty-specified virus-like particles (Ty-VLPs) (BOEKE *et al.* 1985; GARFINKEL, BOEKE and FINK 1985; MELLOR *et al.* 1985). The full-length Ty transcript is packaged into Ty-VLPs, where it is converted into double-stranded DNA by the Ty-encoded reverse transcriptase. The double-stranded reverse transcript is then integrated into the host genome as the final step in the transposition process. The integration reaction results in the duplication of five bp of target DNA at each end of the new copy of the Ty element (FARABAUGH and FINK 1980; GAFNER and PHILIPPSEN 1980). A DNA-containing form of the Ty-VLP appears to be an intermediate in the transposition process (EICHINGER and BOEKE 1988).

The precise sites of insertion of several Ty*1* and Ty*2* elements have previously been determined at several loci. These results pointed to the possibility that Ty*1* and Ty*2* elements insert preferentially in the 5′ region of genes. For example, eight of nine randomly selected Ty insertions in the *LYS2* locus were located in the 5′ region (EIBEL and PHILIPPSEN 1984; SIMCHEN *et al.* 1984). Similarly, the *his4-912* and *his4-917* mutations are caused by the insertion of Ty elements upstream of the *HIS4* gene (ROEDER *et al.* 1980). Many examples of Ty*1* and Ty*2* insertions that activate the expression of silent genes have also been studied; not surprisingly, these insertions are all [with one exception (BACH, 1984)] in 5′ noncoding regions (ERREDE *et al.* 1980; reviewed in BOEKE 1989). Thus

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the existing set of sequenced Ty insertions is far from being a random sample. In fact, in most cases, these mutations were chosen for their remarkable genetic properties such as the ability to yield extragenic revertants or the ability to alter the regulation of adjacent genes.

In this paper we describe the molecular characterisation of a large number of Ty*1* transposition events and related events that occurred at the *URA3* and *LYS2* loci. In an attempt to select unbiased sets of Ty insertions, we made use of the positive selections that exist for the loss or reduction of *URA3* and *LYS2* gene function. We describe the isolation, cloning and sequencing of the site of insertion of nearly one hundred Ty*1* elements in the *URA3* and the *LYS2* loci of yeast.

MATERIALS AND METHODS

Media and strains: Yeast growth media have been described (SHERMAN, FINK and LAWRENCE 1978). The 5-fluoroorotic acid (5-FOA) containing medium was described by BOEKE, LACROUTE and FINK 1984).

The strains we used are JBX169-10B (**a** *lys2, trp1Δ1*), JBX169-11B (*α lys2, trp1Δ1*), JB282, a yeast transformant of BWG1-7a (**a** *ade1-100, his4-516, ura3-52, leu2-3,112*) carrying the plasmid pGTy1-H3, and GNX109 (**a**/*α leu2Δ1/+, ura3-52/ura3-52, his3Δ200/+, ade2-101/+, lys2-801/+*).

Plasmid constructions: The plasmid pGTy1-H3 has been described previously (BOEKE *et al.* 1985). Plasmid pJEF1114 was constructed by replacing the *Sma*I-*Bam*HI fragment containing the *URA3* gene of plasmid pJEF724 (=pGTy1-H3) with an *Eco*RI-*Bam*HI fragment containing the *TRP1* gene (the *Eco*RI end had been previously filled in using the Klenow fragment). Plasmid pGN801 was constructed as follows: pSK179 (kindly provided by SAM KUNES, MIT) consists of a 13-kb *Eco*RI yeast genomic fragment containing the *URA3* gene cloned in the *Eco*RI site of pBR322 [see ROSE, GRISAFI and BOTSTEIN (1984) for a *Bgl*II restriction map of this region]. pSK179 was digested with *Bg*II, filled in with Klenow large fragment enzyme, and ligated to *Not*I linkers. The largest fragment was redigested with *Not*I, *Eco*RI and *Sal*I. We isolated a 2-kb *Sal*I-*Not*I fragment from the 5′ flanking region of *URA3* and a 4-kb *Eco*RI-*Not*I fragment from the 3′ flanking region of *URA3*. These two fragments were ligated to the pBM453 vector that had been previously digested with *Eco*RI and *Sal*I. pBM453 was kindly provided by MARK JOHNSTON (Washington University). It is a pBR322 derivative carrying *ARS1, TRP1* and *CEN3* sequences. Thus the resulting *CEN* plasmid, pGN801, carries about 7.6 kb of *URA3* flanking regions surrounding a unique *Not*I site in place of 5.4 kb of DNA that contains the *URA3* gene itself.

DNA procedures: Plasmid isolations, restriction analysis, gel electrophoresis and Southern blot analysis were described by MANIATIS, FRITSCH and SAMBROOK (1982). Radiolabeled probes were prepared using random oligonucleotide primers as described by FEINBERG and VOGELSTEIN (1983).

Most of the sites of Ty element insertion were sequenced on cloned double stranded plasmid DNA using either Sequenase (U.S. Biochemicals) or reverse transcriptase, using the method of HUIBREGSTE and ENGELKE (1986). A few of the insertion points could be unambiguously identified by sequencing genomic DNA directly with oligonucleotide primers (labeled with $\gamma[^{32}P]$ATP using polynucleotide kinase) and AMV reverse transcriptase (HUIBREGSTE and ENGELKE 1986). We synthesized three primers complementary to *URA3* coding sequences. All *URA3* numbering in this paper is according to the sequence of ROSE, GRISAFI and BOTSTEIN (1984). The sequences of the primers used are as follows:

URA3-1: TAACTGTGCCCTCCATGG (432–449)
URA3-2: GTCGCTCTTCGCTCCCTG (734–751)
URA3-3: AGTTTTGCTGGCCGCATC (1010–1028)

Most of the *URA3* gene can be sequenced using one of these primers. URA3-3 tended to give poor sequence results, particularly on total genomic DNA. In some cases we used primers reading out from the 5′ end or the 3′ end of the LTR. All Ty*1* numbering is according to BOEKE *et al.* (1988). The primers used were as follows:

U3OUT: AACACCGTATATGATAATAT (52–72)
U5OUT: AATGGAATCCCCAACAAT (294–309)

When U3OUT and U5OUT were used as primers, and when the template plasmid carried a whole Ty, we sequenced a purified restriction fragment carrying a single LTR. Most Ty element junction sequences were obtained from only one end, however, in all cases where both ends were sequenced, a target site duplication of 5 bp was observed.

Testing the phenotype of a deletion 5′ to the *URA3* Gene: The diploid strain GNX109 (*ura3-52/ura3-52*) was transformed with a linear piece of DNA containing the wild-type *URA3* gene but lacking the *Hin*dIII (−650) to *Hin*dIII (+1) fragment found just upstream of the *URA3* gene. Ura$^+$ diploid transformants were selected and sporulated. Fifteen asci were dissected; all 15 tetrads had four viable spores. The Ura$^+$ phenotype segregated $2^+/2^-$. For two tetrads we confirmed by Southern blotting that the Ura$^+$ spores carried the 0.65-kb deletion.

RESULTS

In order to study the target specificity of the Ty*1* transposon as well as the general properties of an extensive collection of transposition events, we isolated a large number of independent Ty*1* insertion mutations in the *URA3* and the *LYS2* genes. Mutations inactivating these two genes can be selected easily because *ura3* strains are resistant to 5-FOA (BOEKE, LACROUTE and FINK 1984), and *lys2* and *lys5* strains are resistant to α-aminoadipate (α-aa) (CHATTOO *et al.*1979). Only a very small fraction of the spontaneous α-aa resistant mutants are caused by Ty insertions in the *LYS2* gene (EIBEL and PHILIPPSEN 1984; SIMCHEN *et al.* 1984). Likewise, spontaneous 5-FOA resistant mutants do not often contain Ty insertion mutations at the *URA3* locus (J. D. BOEKE, unpublished data). In order to increase the fraction of mutations caused by Ty insertions, we induced the high frequency transposition of Ty*1* elements by means of a pGTy1-H3 plasmid (BOEKE *et al.* 1985) prior to imposing selection on 5-FOA or α-aa containing media. pGTy1-H3 plasmids consist of the transcribed region of a Ty element (Ty*1*-H3) fused to the yeast *GAL1* promoter on a 2-μm based, high copy number vector. High levels of transcription from the *GAL1*

TABLE 1

Types of *ura3* mutants obtained after 5-FOA selection of transposition induced cells

Type	Number of isolates
Ura$^+$, wild-type restriction pattern	1
Ura$^-$, simple Ty insertion in direct orientation	44
Ura$^-$, simple Ty insertion in opposite orientation	34
Ura$^-$, double Ty insertion	2
Ura$^-$, solo-delta insertion	2
	83

TABLE 2

Types of *lys2* mutants obtained after α-aminoadipate selection of transposition induced cells

	lys2 mutants			*lys5* mutants	
Phenotype	Simple Ty insertion	Other rearrangements	Not rearranged		Total
Lys$^-$	29	2	16[a]	12	59
Lys$^+$	12[b]	0	7[c]	0	19

[a] All are *lys2* mutants.
[b] All 12 simple Lys$^+$ Ty insertions are in the promotor region. Lys$^+$ strains have various degrees of Lys prototrophy.
[c] Could be a *lys2* or a *lys5* mutant.

promoter are induced by growth on galactose-containing medium. Earlier work demonstrated that when the *GAL1* promoter is induced in a strain carrying pGTy1-H3, transposition frequencies increased by at least 20–100-fold (BOEKE *et al.* 1985; BOEKE, STYLES and FINK 1986).

Ty insertion mutations in the *URA3* and the *LYS2* genes: Strains JBX169-10B and JBX169-11B, which are both *URA3*, *trp1*, were transformed with plasmid pJEF1114 [a derivative of pGTy1-H3 (BOEKE *et al.* 1985) that carries the *TRP1* selectable marker in the vector rather than the *URA3* selectable marker]. 5-FOA-resistant (*ura3*) derivatives were isolated from these transformants. Cells (not previously exposed to galactose) were streaked out for single colonies on SC-TRP plates containing galactose as a sole carbon source. Colonies were grown for 5 days at 22° and then replicated to SC+ 5-FOA medium at 30°. Each mutant isolated was derived from a different single parent colony. Eighty-three independent drug-resistant mutants were picked after 4 days. The mutants were colony purified and their 5-FOA-resistant phenotype was confirmed. The properties of the mutants are summarized in Table 1. Eighty-two of these were uracil auxotrophs. The uracil auxotrophs were studied by genomic Southern blotting as follows. Genomic DNAs digested with *Nco*I were hybridized to the 1.1-kb *Hin*dIII fragment (containing the *URA3* gene) probe see Figure 1 for restriction map). We confirmed this analysis of the *URA3* locus and its flanking DNA in these mutants by additional Southern blotting analysis using the enzymes *Hin*dIII and *Pst*I (data not shown). Almost all of the mutations were caused by insertion of a single Ty element. In two instances we observed the insertion of two Ty elements into the *URA3* region. In both of these cases only one of the two Ty elements was inserted in the 1.1-kb *Hin*dIII fragment containing the *URA3* gene itself; we conclude that the second Ty element is inserted in flanking regions in both of these cases. In addition, we observed two solo-δ insertions in the *URA3* region, in the collection of mutants.

A similar approach was used to select Ty insertions at the *LYS2* locus. A *LYS2* strain, JB282, carrying pGTy1-H3, was subjected to the same procedure as outlined for the previous experiment except that after the induction of transposition on galactose the colonies were replica-plated onto α-aa medium. Seventy-eight independent resistant colonies were picked and colony purified. We determined the Lys phenotype of the α-aa resistant colonies and subjected them to Southern blot analysis. DNAs were digested with *Eco*RI, *Eco*RV, *Bgl*II, *Nco*I and *Bam*HI alone and in appropriate combinations; the *Eco*RI-*Hin*dIII and *Bgl*II-*Xho*I fragments containing the *LYS2* gene were used as hybridization probes (see Figure 1 for restriction map). The results are summarized in Table 2. Fifty-nine strains were Lys$^-$; 31 of these showed alterations of the *LYS2* locus. The Southern hybridization patterns of 29 of these DNAs are consistent with their being simple Ty insertions. In addition, we detected one insertion of a 2-kb piece of DNA of unknown origin and one small deletion of approximately 0.2 kb. The 28 remaining Lys$^-$ strains showed a wild-type *LYS2* restriction pattern. Complementation tests with *lys2* and *lys5* tester strains indicated that 12 of these strains were *lys5* mutants and 16 were *lys2* mutants. Nineteen of the 78 α-aa resistant strains were Lys$^+$. Twelve of these bore rearrangements of the *LYS2* locus whose properties were consistent with a single Ty insertion in the promoter region of the gene. The remaining seven showed a wild-type pattern of hybridizing bands when their DNA was cut with four different restriction enzymes and analysed by Southern blot analysis. Because the latter strains had a Lys$^+$ phenotype we could not use complementation tests to distinguish between the *LYS2* and *LYS5* loci in these cases (Table 2).

The approximate position of the insertions in both genes was determined by Southern blotting (Figure 1). We observed that in both genes the 5′ end of the coding region is a preferred target compared to the rest of the coding sequence (*Eco*RV-*Bgl*II fragment in *LYS2* and *Pst*I-*Nco*I fragment in *URA3*). An even higher frequency of transposition occurs in the *LYS2* 5′ noncoding region. In contrast, only 2 out of the 82

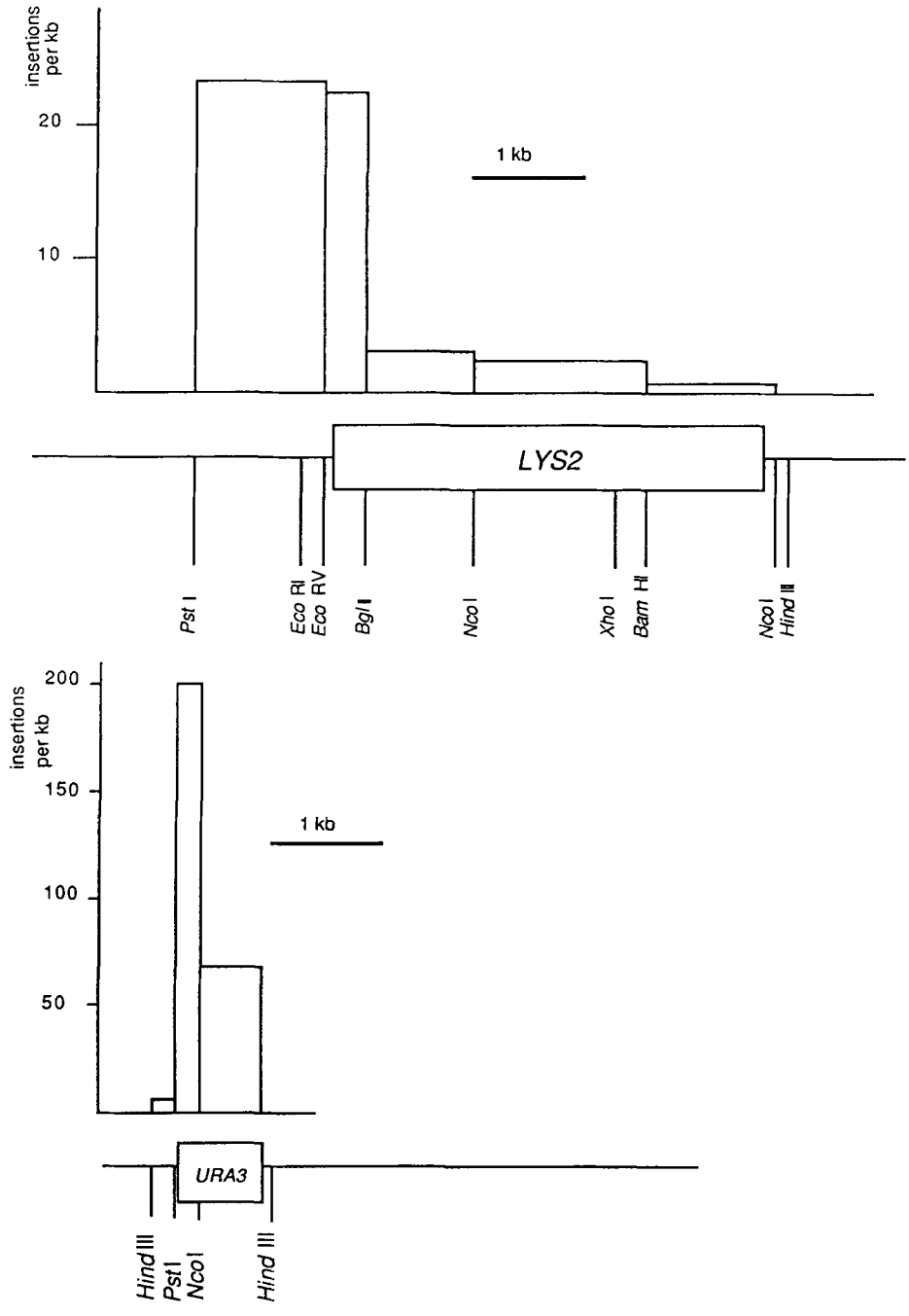

FIGURE 1.—Distribution of Ty insertions at the *URA3* and *LYS2* genes. Histograms indicate the number of insertions per kb. Coding regions are boxed. Landmark restriction sites referred to in the text are indicated.

URA3 insertions occurred in the *Hin*dIII-*Pst*I 5′ noncoding region fragment. Because the distribution of Ty insertions throughout both of these target genes was not random, we cloned and sequenced the exact insertion point of all of the *URA3* insertions and ten of the *LYS2* insertions.

Cloning of Ty insertions in the *URA3* and the *LYS2* loci: The *URA3* region of each mutant was cloned using the gap-repair procedure (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1980). To facilitate this cloning, we constructed a plasmid (pGN801) carrying the regions flanking *URA3* joined by a *Not*I linker (a 5.4-kb region containing the *URA3* gene is missing from this plasmid). This *CEN* plasmid carries the *TRP1* selectable marker. Every *ura3* mutant was transformed with pGN801 linearized at the *Not*I site (Figure 2). The plasmid is able to recombine with the homologous chromosomal region (*i.e.*, *URA3* carrying a Ty element insertion). The double-strand gap is repaired from the chromosomal information and the recombination intermediate is resolved either by integrating the plasmid or by producing an autonomous plasmid. In the first case the transformant is presumably inviable because the chromosome in which the recombination occurred is now dicentric. In the second case, an unstable Trp^+ transformant can be recovered.

The above procedure was carried out for each of

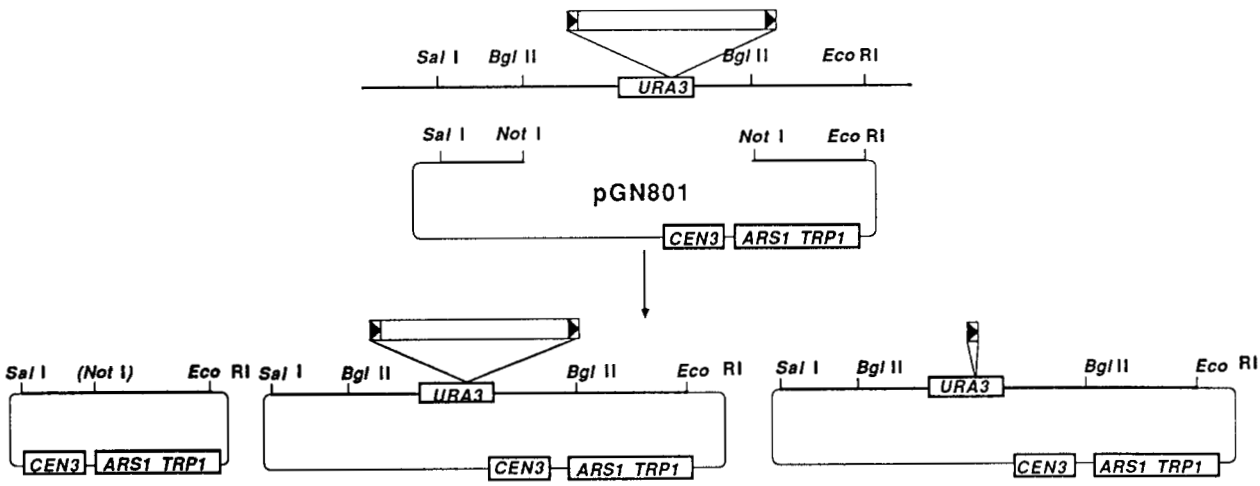

FIGURE 2.—The top part of the diagram shows the plasmid pGN801, linearized at the *Not*I site, recombining with the chromosomal *URA3* locus. The thick lines in pGN801 represent regions flanking the *URA3* gene. The bottom panel shows the three classes of plasmids recovered (at approximately equal frequencies) from Trp$^+$ transformants. The *Not*I site in the leftmost plasmid is indicated in parentheses because we did not determine whether it was retained in these plasmids.

the *ura3* mutants. The autonomous plasmids responsible for the Trp$^+$ phenotype were recovered in *E. coli*. Three classes of plasmids were obtained at roughly equal frequencies. The first class consists of plasmids identical to pGN801, which probably arose by incomplete digestion of pGN801 by *Not*I or by circularization of the linear plasmid in vivo. The second class of plasmids carried the whole *URA3* region including the Ty insertion, demonstrating that more than 11 kb of chromosomal information can be recovered by the gap-repair procedure. Plasmids of the third class also contained the whole *URA3* region but they only carried a single δ in place of the Ty. These plasmids arise even when it is clear from the Southern blot analysis that the initial lesion is an intact Ty element insertion. In this case, the loss of the internal region of the Ty is presumably due to an elevated frequency of recombination between the two δ elements during the gap-repair transformation procedure.

We used the eviction method (WINSTON, CHUMLEY and FINK 1983) to clone 10 Ty insertions in the 5′ region of the *LYS2* gene, as this region suffered an unusually large number of transposition events. We selected 5 Ty insertions in the *Eco*RI-*Eco*RV (*LYS2* 5′ non-coding region) fragment and 5 Ty insertions in the *Eco*RV-*Bgl*II (*LYS2* 5′ coding region) fragment for sequence analysis because this region of the wild-type *LYS2* gene had previously been sequenced (FLEIG, PRIDMORE and PHILIPPSEN 1986).

Sequence analysis of the sites of Ty insertion: The endpoints of eighty-two transposition events in *URA3* and ten in *LYS2* were sequenced using a variety of different techniques. Except in two cases, where we subcloned the insertion in an M13 phage, the templates used were the double stranded plasmids obtained from the cloning experiment.

The results (Figure 3 and Table 3) are completely consistent with the Southern blot data. Few Ty insertions were recovered in the *URA3* 5′ noncoding region (2 out of 82 insertions). Furthermore, more insertions occurred in the first 205 bp of the coding region than in the last 599 bp. Within each of these regions the distribution of the insertions is nonrandom. Position 341 in the *URA3* gene [the *URA3* numbering system used in this paper refers to the first bp of the five bp duplication generation by the Ty insertion in reference to the published *URA3* sequence of ROSE, GRISAFI and BOTSTEIN (1984)] is a hot spot for transposition, containing 12 of the 82 Ty insertions. The Ty elements inserted at position 341 consisted of 7 Ty elements inserted such that their transcript would be in the same direction as that of the *URA3* transcript, and 5 in the opposite orientation. A few less prominent hot spots were also observed, including five Ty elements inserted at position 665 and four Ty elements inserted at positions 244, 741, 949 and 959.

The sequenced insertions in the *LYS2* gene showed an even greater degree of nonrandomness. The five Ty elements that had inserted downstream of the *Eco*RV site were located at position 226 [the number refers to the first bp of the 5-bp duplication generated by the insertion in reference to the sequence published by FLEIG, PRIDMORE and PHILIPPSEN (1986)]. Four of the five Ty elements that were inserted upstream of the *Eco*RV site were located at position −42. Both of these preferred sites included examples of Tys inserted in both orientations.

Sequence comparison of the sites of Ty element insertion: The nonrandom pattern of Ty insertions into the target genes suggested that there might be conservation of a sequence motif at or near the site of insertion. To search for this, we aligned the sequence

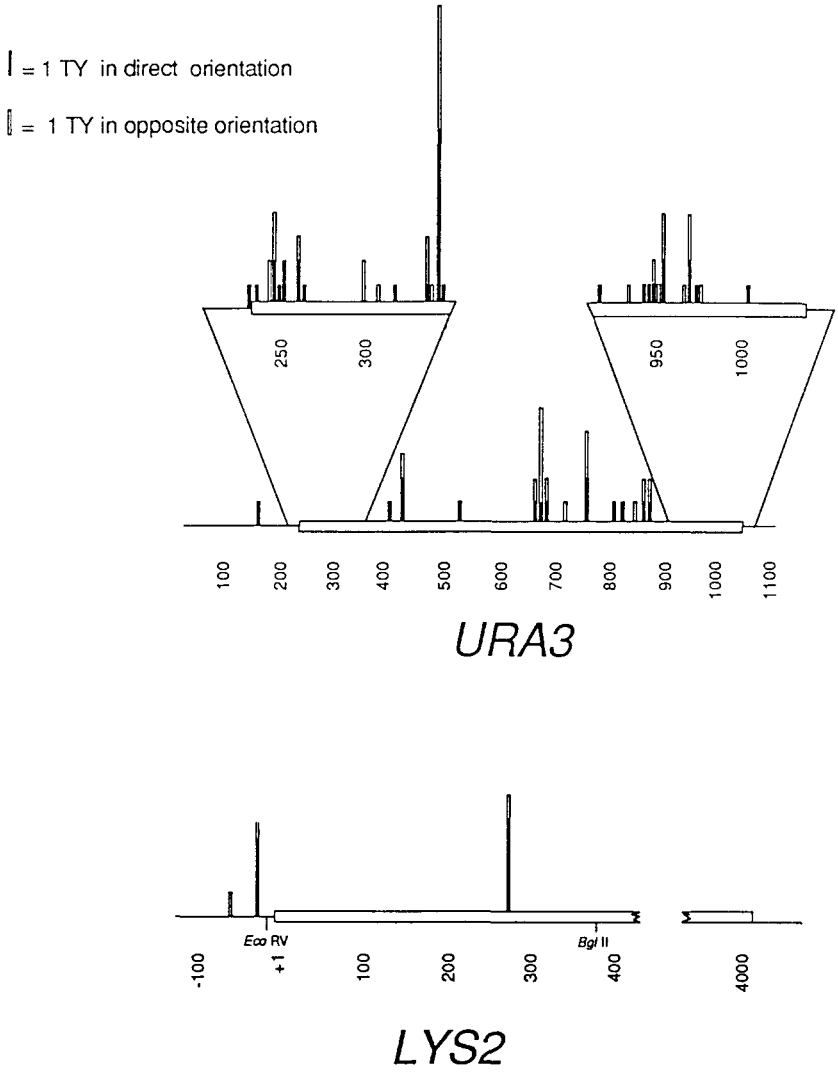

FIGURE 3.—Distribution of insertions in the *URA3* and *LYS2* genes. The coding sequences are boxed. Insertions are represented by histograms. The filled part of the histogram's bars represent Tys whose transcription is in the same direction as the transcription of the target gene. The open part represents Tys in the opposite orientation. Nucleotide positions in *URA3* are numbered according to ROSE, GRISAFI and BOTSTEIN (1984). The *URA3* ATG is in position +227 and the two major transcription start sites are in positions 189 and 194. In *LYS2*, positions are numbered from the ATG (FLEIG, PRIDMORE and PHILIPPSEN 1986). The *LYS2* transcription start site is located in position −82.

of the sites of insertion in the *URA3* gene and of the flanking 200 bp (from −100 to +100). We aligned the coding strand of *URA3* (5′ to 3′) when the Ty was inserted in direct orientation and the noncoding strand of *URA3* (5′ to 3′) when the Ty was inserted in the opposite orientation. In other words the flanking sequences were arranged for comparison in such a way that the Ty sequences were always perfectly aligned. This will allow the detection of a nonsymmetrical consensus sequence. In cases when multiple insertions had occurred, we entered that sequence as many times as there were insertions at that position. The alignment is centered on the central (third) base of the 5-bp duplication. For every position we determined the frequency at which each of the 4 bases occurs. We then looked for positions where particular bases would be significantly overrepresented (≫25%) or underrepresented (≪25%). We did not find any such sequences in the DNA flanking the insertions. Within the 5-bp duplication, however, we found that T was underrepresented in position 1 (3.7%) and A was underrepresented in position 5 (9.8%). At the central position (3) of the five bp duplication nucleotides C and G are also somewhat underrepresented (9.8% and 11%, respectively) (Table 5). The extremely degenerate consensus sequence obtained for the five base pair target site duplication is (G/A/C)-X-(A/T)-X-(G/T/C). An unweighted analysis of only the hotspots does not significantly increase the quality of the consensus (Table 5).

Is there an essential gene immediately 5′ to *URA3*? The dearth of insertions in the 5′ noncoding region of *URA3* prompted us to consider possible explanations for the different behavior of the two 5′ noncoding regions studied. One possible explanation for the lack of transposition events mapping within the *URA3* 5′ non-coding region is the existence of an essential gene immediately 5′ to the *URA3* gene. The *URA3* 5′ noncoding region might overlap with some essential part of such an adjacent gene. Alternatively, the Ty enhancer might affect the expression of an adjacent gene in a lethal manner. This might explain

TABLE 3

Position of Ty1 insertions in the *URA3* gene

Position	Allele	Orientation	Number of isolates
152	*ura3-184*	→	
222	*ura3-145*	→	
232	*ura3-153*	→	
243	*ura3-189*	←	2
244	*ura3-109*	→	2
244	*ura3-134*	←	2
245	*ura3-158*	→	
247	*ura3-178*	→	2
258	*ura3-116*	→	2
258	*ura3-167*	←	
260	*ura3-157*	→	
293	*ura3-108*	←	2
306	*ura3-154*	←	
316	*ura3-190*	→	
335	*ura3-111*	→	
335	*ura3-106*	←	2
336	*ura3-143*	←	
341	*ura3-104*	→	7
341	*ura3-101*	←	5
342	*ura3-103*	→	
393	*ura3-133*	→	
414	*ura3-155*	→	2
414	*ura3-136*	←	
519	*ura3-191*	→	
652	*ura3-150*	→	
652	*ura3-137*	←	
665	*ura3-149*	→	
665	*ura3-117*	←	4
673	*ura3-148*	→	
673	*ura3-165*	←	
712	*ura3-162*	←	
741	*ura3-113*	→	2
741	*ura3-127*	←	2
798	*ura3-128*	→	
817	*ura3-166*	→	
839	*ura3-182*	←	
854	*ura3-107*	→	
854	*ura3-173*	←	
865	*ura3-185*	→	
865	*ura3-118*	←	
906	*ura3-122*	→	
928	*ura3-110*	←	
943	*ura3-120*	→	
944	*ura3-196*	→	
947	*ura3-135*	→	
947	*ura3-147*	←	
948	*ura3-181*	←	
949	*ura3-112*	→	2
949	*ura3-102*	←	2
957	*ura3-146*	→	
959	*ura3-121*	→	2
959	*ura3-100*	←	2
965	*ura3-144*	→	
966	*ura3-179*	←	
995	*ura3-180*	→	

Positions are given according to ROSE, GRISAFI and BOTSTEIN (1984). Number of isolates are given only when more than one independent Ty was isolated in that position and in that orientation.

why Ty insertions in the *URA3* 5′ non-coding region were not recovered by our experimental procedure.

Of the two insertions that we did isolate in the *URA3* 5′ noncoding region, one is an intact Ty element inserted immediately 5′ to the ATG; the second is one of two solo-δ insertions; it is located 75 bp 5′ to the ATG. As the only insertion that occurred more than a few base pairs from the ATG is a solo-δ sequence, it could be argued that the presence of a whole Ty element at that position has a deleterious effect.

TABLE 4

Statistical analysis of the distribution of insertions in the *URA3* gene

x	$P(x)$	$NP_{(x)}$		$n_{(x)}$		$\frac{(n_{(x)} - NP_{(x)})^2}{NP_{(x)}}$
0	0.947	1464		1491		0.49
1	$5.08\ 10^{-2}$	78.5		38		20.9
2	$1.36\ 10^{-3}$	2.10		14		68
3	$24.3\ 10^{-6}$	$3.7\ 10^{-2}$		0		
4	$3.25\ 10^{-6}$	$5.02\ 10^{-4}$		1		
5	$3.49\ 10^{-9}$	$5.3\ 10^{-6}$	0.0375[a]	1	3[a]	234[a]
6	$3.11\ 10^{-11}$	$4.8\ 10^{-8}$		0		
7	$2.38\ 10^{-13}$	$3.67\ 10^{-10}$		1		
						323

We estimated the probability that the observed distribution of Ty element insertions was non-random as follows. The number of possible targets was assumed to be $n = (995\text{-}222) \times 2$ orientations $= 1546$. Thus with random insertion, the probability of inserting into a given target is $p = 1/1546$. We used the binomial distribution to calculate the probability $P(x)$ of observing $0, 1, 2, \ldots x$ insertions in q given positions after y repetitions (in our case $y = 82$ independent Ty insertions). Using the formula $P(x) = y!/x!(y - x)!\, p^x (1 - p)^{y-x}$ we obtained the probabilities shown in the first column of Table 4. Multiplication of these values by 1546 produces the expected total number of unspecified positions at which $0, 1, 2, \ldots$, Ty elements would have inserted in the complete absence of target site specificity. The chi-square test was used to compare the difference between the observed ($n(x)$) and expected frequencies ($NP(x)$). The chi square value obtained (323) is much greater than that corresponding to a 99.9% degree of confidence (13.8).

[a] Numbers next to brackets are summations of the values for $x = 3$–7.

TABLE 5

Base composition and consensus sequence of sites of Ty1 insertion in *URA3*

	Position				
Nucleotide	1	2	3	4	5
%G	32/21	20/21	11/14	18/28	31/42
%A	33/35	32/35	45/57	34/28	10/0
%T	4/7	34/35	34/21	23/21	30/35
%C	32/35	20/7	10/7	18/21	31/21
	A		A		T
	C	×		×	G
	G		T		C

Consensus sequence analysis of sites of Ty1 insertion in *URA3*. The first entry refers to a weighted analysis of all 82 insertions, the second refers to an unweighted analysis of only those insertions recovered more than once in a particular position and orientation. The percent of each nucleotide at each position of the 5-bp duplication generated by insertion is indicated. Deduced preferred site is shown below (using the weighted analysis). The *URA3* *Hin*dIII fragment is 66% A/T.

We tested for the possible existence of an essential adjacent gene 5′ to *URA3* by deleting a 650-bp *Hin*dIII fragment immediately upstream of *URA3* in a diploid strain. Haploid derivatives bearing the deletion are viable, Ura$^+$, and their growth is indistinguishable from that of the wild-type spores (see MATERIALS AND METHODS). Thus, the low frequency of Ty transposition in the 5′ non-coding region of *URA3* is unlikely to be due to the presence of an important sequence immediately upstream of the *URA3* gene.

DISCUSSION

We have studied the properties of Ty*1* transposition events at two defined and selectable target loci: the *URA3* and the *LYS2* genes. We obtained a rather homogeneous collection of 5-FOA resistant mutants; 82 of the 83 5-FOA resistant strains studied showed evidence of Ty transposition or related events in the *URA3* gene. Nearly all of these mutations consisted of simple Ty insertions at the *URA3* locus. Insertions in both orientations were obtained at approximately equal frequencies.

Two strains had two Ty elements inserted in the *Eco*RI restriction fragment containing the *URA3* locus. In both cases, Southern blot analysis showed that in fact only one Ty element was inserted in the *URA3* gene itself, whereas the other Ty element was inserted in the region flanking the gene. Knowing that there are, on average, 5 unselected transpositions in each of the 83 strains that we analysed, that the size of the yeast genome is approximately 15 megabases and that the *URA3* region we probed is approximately 15 kb, we calculated (using the binomial distribution) the probability to observe one or more unselected transpositions in the *URA3* region. The probability of not observing such an event is 66%, the probability of observing it once is 27% and the probability of observing it twice is 5%. This suggests that a double insertion is not that unlikely to occur in the region that we probed. Thus we do not think that the transposition of a Ty in this particular region of the chromosome significantly increases the probability that a second Ty will transpose in the neighboring DNA, nor that the *Eco*RI fragment containing the *URA3* locus is a particularly "hot" target for transposition in general.

In two cases we observed solo δ insertions in *URA3*. Solo δ insertions have recently been isolated by another group studying spontaneous mutagenesis at the yeast *SUP4-o* locus (GIROUX *et al.* 1988) without the benefit of a pGTy1-H3 plasmid. WILKE *et al.* (1989) have isolated a solo δ insertion in a similar study of the *CAN1* locus. Hence, solo δ insertions appear to occur occasionally as mutagenic lesions at various target loci. If solo δs are incapable of transposing independently, as seems likely as δ elements lack sites to prime reverse transcription, these insertions are presumably due to two separate events, namely an intact Ty transposition event followed by recombination between the two δ sequences, looping out the internal region of the recently transposed Ty element. The frequency at which we observe solo-δ insertions among the collection of transposition events (2.4%) is at least 1000-fold higher than the previously measured frequencies of mitotic δ-δ recombination in a marked Ty near the *HIS4* locus (10^{-5}) (WINSTON *et al.* 1984). This raises the possibility that δ sequences recombine at a very high rate during or immediately after transposition. Perhaps Ty transposition leaves some DNA damage at the site of transposition, greatly stimulating homologous recombination in the region of the terminal δ elements. An alternative, but less likely explanation for the solo δ insertions is that (−) and (+) strong stop DNA molecules, which are putative intermediates in Ty element reverse transcription, might anneal within the Ty-VLP, which is thought to be a transposition intermediate (EICHINGER and BOEKE 1988). Extension of such an annealed product would yield a linear solo δ piece of DNA that bears all of the signals required for integration of the DNA (D. EICHINGER and J. D. BOEKE, in preparation).

The α-aa^r selection yielded both *lys2* and *lys5* mutants, of which many remained Lys$^+$. This is not surprising, since a relatively small decrease in *LYS2* or *LYS5* expression can result in a strain becoming α-aa^r without becoming Lys$^-$ (for example, *LYS2/lys2* diploids are completely Lys$^+$ but are α-aa^r). Ty insertions in the 5′ noncoding region of the *LYS2* gene that result in a Lys$^+$ phenotype have been described previously (EIBEL and PHILIPPSEN 1984; SIMCHEN *et al.* 1984). In these strains the Ty is oriented such that its transcription is divergent from that of *LYS2* and their Lys$^+$ phenotype is apparently under mating-type regulation (*i.e.*, these *lys2* alleles are Lys$^+$ in haploids but Lys$^-$ in **a**/α diploids) (G. NATSOULIS and J. BOEKE, unpublished data). In this configuration the Ty enhancer, located near the 5′ end of the Ty, apparently influences the transcription of the neighboring *LYS2* gene.

By Southern blot and sequence analysis, we examined the distribution of Ty transposition events at the *URA3* and *LYS2* loci. The results for these two genes differed in that we observed only two insertions in the 5′ noncoding region of *URA3* but many in the *LYS2* noncoding region. We found no evidence for an essential gene upstream of *URA3* that might prevent our recovery of insertions in the *URA3* 5′ noncoding region. Thus it seems likely that the reason for the lack of *URA3* 5′ noncoding region insertions is the fact that the 5-FOA selection requires close to complete disruption of function in order to obtain a drug resistant phenotype. Of note, the two insertions we

isolated that map in the *URA3* 5′ noncoding region cause a slower growth rate on 5-FOA medium when compared with insertions in the coding region. Furthermore, known leaky *ura3* mutations that we have isolated grow very poorly on 5-FOA medium. Therefore, we suspect that mutations in the *URA3* 5′ noncoding region may well have occurred, but may not have been recovered. Alternatively, it is possible that the *URA3* 5′ noncoding region is indeed a poor target.

More than half of the Ty insertions in *LYS2* occurred in the 5′ region of the locus. These include both insertions in the noncoding DNA as well as the first 390 bp of the coding region. As one proceeds 5′ to 3′ along the gene, the number of insertions per kb decreases steadily to near zero at the 3′ end of the gene.

Transposable elements vary widely in the extent of their target site specificity. Perhaps the element with the highest target site specificity is the bacterial element Tn7 (reviewed by CRAIG 1989). This element always transposes to a single site in the *Escherichia coli* genome. Tn*10* recognizes a degenerate interrupted palindromic sequence (HALLING and KLECKNER 1982). Tn*3*, on the other hand, recognizes a very wide variety of sites; these are usually AT-rich and sometimes resemble the sequence of the end of the element, but no consensus sequence has been reported (GRINSTED *et al.* 1978; TU and COHEN 1980). As described below, Ty*1* transposition shows less sequence specificity than Tn*10*, but more than Tn*3*. Certain other retrotransposons appear to recognize a consensus sequence, based on the sequencing of unselected preexisting insertions. A group of Drosophila retrotransposons, *gypsy*, *17.6*, and *297*, apparently prefers the target sequences TAC/TA and ATAT (reviewed by BINGHAM and ZACHAR 1989). Ty*3*, a recently discovered yeast retrotransposon, does not recognize a consensus sequence but is probably highly specific for insertion within 15–19 bp of tRNA coding regions, because all copies of Ty*3* and its solo LTR derivatives (σ elements), are found at these sites (HANSEN, CHALKER and SANDMEYER 1988; SANDMEYER *et al.* 1988).

Within each of the two targets studied, insertions are not randomly distributed. In the case of *URA3*, only 38 out of 82 insertions were represented by unique isolates; in the remainder, more than one Ty had inserted in the same position and in the same orientation. The most dramatic example is at position 341 where 7 insertions in one orientation and 5 in the reverse were recovered. This insertion site is one base pair away from the site of Ty element insertion in the well-known allele *ura3-52* (ROSE and WINSTON 1984).

We tested the distribution of insertions recovered to determine the probability that the observed pattern of multiple insertions at the same position were the result of random chance alone. We known that Ty element insertions in both orientations between positions 222 (corresponding to 5 bp before the ATG, we did not include the 152–222 region because this region is entirely within the promotor and we do not expect that every insertion in that interval will yield a 5-FOA^r phenotype) and 995 (the position of the most 3′ insertion recovered) can inactivate the *URA3* gene. For the purpose of this calculation, we assumed that any Ty element inserted between positions 222 and 995 would be recovered as a 5-FOA resistant strain (in fact it is likely that insertions between 995 and the 3′ end of the coding region would also give a 5-FOA resistant phenotype). Chi square analysis of the observed and expected distributions (Table 4) indicated that the distribution of Ty element insertions observed is not due to random chance at a confidence level $\gg$99.9%. Hence we reject the hypothesis that the observed distribution of Ty element insertions is a sample from a randomly distributed population.

In the case of the small number of insertions sequenced at the *LYS2* locus the bias toward specific sites is even greater. All five insertions in the 5′ coding region of *LYS2* that we sequenced were inserted at the same position (*i.e.*, the same 5 bp were duplicated), and again, both orientations of the Ty element relative to *LYS2* were recovered.

Thus we conclude that there is indeed target site specificity in Ty transposition. However, the features that define a good target site have not been defined. We aligned the 82 regions where Tys had inserted in the *URA3* gene, and looked for a consensus sequence. A very degenerate consensus (G/A/C/-X-A/T-X-T/G/C), centered on the 5-bp duplication, was identified. No similarities were found in the regions flanking the five base pair duplication. It is interesting to note that this consensus is palindromic. This is a reflection of the observation that the transposition hot spots nearly always contain insertions in both orientations. The 5-bp duplications generated at the two *LYS2* hot spots (position −42: AAAAC; position +226: GTTCT) fit this consensus, as does the hotspot found in *his3Δ4* (BOEKE, STYLES and FINK 1986). However, the significance of this consensus is tempered by the fact that this sequence occurs by random chance approximately once per three base pairs when the base composition of *URA3* is taken into account. Alignment of only hotspot target sequences also failed to reveal a convincing consensus sequence. Alignment of all published Ty*1* target sequences produced a similar but not identical consensus sequence (WILKE *et al.* 1989). Hence the primary sequence at the site of insertion seems to deviate little from randomness. Thus Ty*1* transposition is characterized by an extreme sequence degeneracy of the sites used, but nevertheless a good deal of target specificity.

The 5′ regions of two target genes, *URA3* and *LYS2*, appear to contain preferred target sites for Ty element transposition. The three highly preferred sites are all within the transcribed part of the DNA. An abundant literature suggests that the 5′ end of genes contains nuclease hypersensitive sites in chromatin that presumably reflect a different, "looser" organization of nucleosomes and other chromatin components. Avian and murine retroviruses have been shown to integrate in the vicinity of DNAse I hypersensitive sites (ROBINSON and GAGNON 1986; VIJAYA, STEFFEN and ROBINSON 1986: ROHDEWOHLD *et al.* 1987). However a large portion, albeit not all, of these insertions had been cloned from retrovirus-induced tumors or lymphomas so that this is not a random sample of insertions. It has recently been shown that Ty-VLPs, which are about 60 nm in diameter, are apparent intermediates in the transposition process (EICHINGER and BOEKE 1988). If these bulky entities are indeed immediate precursors to the integration event, it supports the observed pattern of insertions seen in vivo; tight chromatin might sterically restrict access of target DNA to a bulky particulate intermediate; loose chromatin would presumably provide less of an impediment.

Highly preferred insertion sites for Rous sarcoma virus (a retrovirus) have been observed by SHIH, STOYE and COFFIN (1988). These authors generated a collection of approximately 15,000 proviral insertions over the whole genome. They identified four positions at which more than one independent proviral integration event had occurred and calculated that this represented a 10^6-fold bias for these sites. For each of the four positions, the insertions all occurred in the same orientation. The analysis of 300 bp on either side of two of the four hotspots revealed no obvious sequence similarity.

Although the targets we used were much smaller our results are comparable to those of SHIH, STOYE and COFFIN (1988). We observe high biases at the hotspots in both *LYS2* and in *URA3* over what would be expected if insertions occurred randomly. Except for the extremely loose consensus sequence mentioned earlier, we do not detect significant sequence similarities between the regions flanking the insertion points. However, our results differ from the above in that in the cases where more than one Ty element was isolated at a particular position, insertions in both orientations were usually observed. Because at this point it would be interesting to analyse the target specificity of Ty*1* over larger regions of the genome, we are currently generating a random collection of Ty*1* insertions into the chromosome *III* of yeast (D. MOORE, G. NATSOULIS and J. D. BOEKE, unpublished results).

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