MOLECULAR AND CELLULAR BIOLOGY, Apr. 1984, p. 703–711
0270-7306/84/040703-09$02.00/0
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Vol. 4, No. 4

Intrachromosomal Movement of Genetically Marked *Saccharomyces cerevisiae* Transposons by Gene Conversion

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Received 28 July 1983/Accepted 22 December 1983

In this paper, we describe the movement of a genetically marked *Saccharomyces cerevisiae* transposon, Ty912(*URA3*), to new sites in the *S. cerevisiae* genome. Ty912 is an element present at the *HIS4* locus in the *his4-912* mutant. To detect movement of Ty912, this element has been genetically marked with the *S. cerevisiae URA3* gene. Movement of Ty912(*URA3*) occurs by recombination between the marked element and homologous Ty elements elsewhere in the *S. cerevisiae* genome. Ty912(*URA3*) recombines most often with elements near the *HIS4* locus on chromosome III, less often with Ty elements elsewhere on chromosome III, and least often with Ty elements on other chromosomes. These recombination events result in changes in the number of Ty elements present in the cell and in duplications and deletions of unique sequence DNA.

Haploid cells of *Saccharomyces cerevisiae* carry more than 30 copies of a transposable element known as Ty (transposon yeast). Ty elements are approximately 6,000 base pairs in length and are terminated by direct repeats of a 330-base-pair sequence called δ (1). Ty elements are capable of transposition to new sites in the yeast genome. Insertion of a Ty element into or near an *S. cerevisiae* gene often results in a mutant phenotype (15).

The Ty insertion mutation of interest in this paper is the *his4-912* mutation. This mutation is the result of insertion of a Ty element, known as Ty912, into the regulatory region at the 5′ end of the *HIS4* gene (2, 13). His^+ revertants of the *his4-912* mutation most often result from excision of Ty912 by reciprocal recombination between the terminally repeated δ sequences (3, 13). Excision of the Ty element leaves behind a solo δ in the *HIS4* regulatory region.

To facilitate the study of Ty912, we have constructed *S. cerevisiae* strains in which this Ty element is genetically marked with the *S. cerevisiae URA3* gene (14). In this paper, we describe the movement of the genetically marked Ty912 element to new sites in the *S. cerevisiae* genome. We demonstrate that these movements result from recombination between Ty912(*URA3*) and Ty elements elsewhere in the *S. cerevisiae* genome. We assume, but cannot prove, that the recombination events sustained by the *URA3*-marked Ty912 element are similar to those sustained by the unmarked Ty912 element.

MATERIALS AND METHODS

Genetic analysis. Methods of tetrad analysis and media used were as described by Sherman et al. (16).

Southern analysis. Methods of DNA preparation, gel electrophoresis, and Southern hybridization have been described previously (13).

Construction of strains carrying a Ty912(*URA3*) tandem duplication. The construction of *S. cerevisiae* strains carrying a *URA3*-marked Ty912 element has been described previously (14). In this construction, a plasmid containing a single Ty912(*URA3*) element was introduced into the *S. cerevisiae* cell by transformation. Not all transformants obtained carry a single Ty912(*URA3*) element at *HIS4*. Many carry a tandem duplication or triplication of Ty912(*URA3*) elements overlapping with each other by a δ sequence. This structure was determined by Southern hybridization with *HIS4* and *URA3* DNA to probe total *S. cerevisiae* DNA digested with a variety of restriction enzymes.

Strains. The *S. cerevisiae* strains used in this study are listed in Table 1 and described below.

Transformations. Transformation of *Escherichia coli* was carried out as described by Morrison (9). *S. cerevisiae* transformations were carried out as described by Hinnen et al. (5) or by Ito et al. (6).

Cloning of Ty elements and adjacent sequences. The Ty elements and their flanking DNA were cloned by integration and excision as described previously (12, 13; see Winston et al. [17] for a general description of the approach). The cloning of Ty900, Ty1-17, and the flanking DNA has been described previously (11).

Chromosomal segments containing a *URA3*-marked Ty element were cloned as follows. The plasmid used to transform *S. cerevisiae* contained pBR322 DNA, a 2,200-base-pair *Sal*I-*Xho*I fragment of *LEU2* DNA and a 1,200-base-pair *Hin*dIII fragment of *URA3* DNA. The *Bam*HI site of this plasmid was destroyed by digestion with *Bam*HI, generation of blunt ends with reverse transcriptase, and ligation. Before transformation into *S. cerevisiae*, the plasmid was cleaved with *Sma*I, which cuts the plasmid once in *URA3* DNA. Cleavage at the *Sma*I site causes the plasmid to integrate specifically into *URA3* DNA (10). The linearized plasmid was used to transform a *leu2*$^-$ *S. cerevisiae* strain carrying the *URA3*-marked Ty element; Leu^+ transformants were selected. Transformants in which a single copy of the plasmid had integrated into the *URA3*-marked Ty were identified by Southern hybridization with a pBR322 plasmid containing the *URA3* gene as a probe. DNA from such transformants was digested with *Bam*HI, ligated, and used to transform *E. coli* to ampicillin resistance.

The unmarked Ty elements between *PGK1* and *MAT* (see Fig. 4a) were cloned as follows. A *Hin*dIII fragment of

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TABLE 1. His$^+$ Ura$^+$ revertants of *his4-912* (*URA3*)

Class	Revertant	Parent	Ty912 (*URA3*) in parent
I-1	MS108	SR160-2C	Single Ty
	MS215	SR112-1A	Tandem Tys
	MS225	SR112-1A	Tandem Tys
I-2	MS101	SR160-2C	Single Ty
	MS109	SR160-2C	Single Ty
	MS113	SR160-2C	Single Ty
	MS118	SR160-2C	Single Ty
	MS216	SR112-1A	Tandem Tys
I-3	MS115	SR160-2C	Single Ty
	MS602	SR160-2C	Single Ty
I-4	MS114	SR160-2C	Single Ty
	MS472	SR112-1A	Tandem Tys
	MS478	SR112-1A	Tandem Tys
I-5	MS474	SR112-1D	Tandem Tys
I-6	MS222	SR112-1A	Tandem Tys
II	MS100	SR160-1C	Single Ty
	MS212	SR112-1A	Tandem Tys
III	MS106	SR160-2C	Single Ty
	MS107	SR160-2C	Single Ty
IV	MS117	SR160-2C	Single Ty
V	MS119	SR160-2C	Single Ty
	MS120	SR160-2C	Single Ty
	MS224	SR112-1A	Tandem Tys
	MS477	SR112-1D	Tandem Tys

unique sequence DNA to the right of the Ty elements shown in Fig. 4b was inserted into a pBR322-derived plasmid containing the *S. cerevisiae LEU2* gene. This plasmid was used to transform SR112-1A, and Leu$^+$ transformants were selected. DNA from several of these transformants was digested with *Bam*HI, ligated, and used to transform *E. coli* to ampicillin resistance.

RESULTS

Detection of Ty movement events. The movement of genetically marked Ty elements was detected in *S. cerevisiae* strains carrying derivatives of the *his4-912* mutation. The construction of strains carrying a Ty912 element which is genetically marked with the *S. cerevisiae URA3* gene has been described previously (14). The construction of strains carrying a tandem duplication of *URA3*-marked Ty912 elements is described above; in these strains, the two Ty912(*URA3*) elements share one δ sequence. The strains used in this study carry, in addition to the genetically marked Ty912 element(s), *ura3*$^-$, *leu2*$^-$, and *spt2*$^-$ mutations. The *spt2*$^-$ mutation is necessary for growth in the absence of histidine of cells which have undergone Ty912 excision and which contain a solo Ty912 δ at *HIS4* (F. Winston, D. T. Chaleff, B. Valent, and G. R. Fink, Genetics, in press).

Strains of the genotype *his4-912*(*URA3*) *ura3-52 spt2*$^-$ revert to His$^+$ at a frequency of 1 in 10^3 to 10^4 cells. Reversion results from excision of Ty912(*URA3*) and usually leads to a Ura$^-$ phenotype. To isolate cells in which Ty912(*URA3*) had moved to a new site in the genome, we selected cells that had reverted to His$^+$, but that were still Ura$^+$. Cells of this phenotype occur at a frequency of 1 in 10^7 to 10^8 cells.

As a first step in the characterization of the His$^+$ Ura$^+$ revertants, we carried out Southern hybridizations with a *Sal*I fragment of wild-type *HIS4* DNA as a probe (13). As indicated in Fig. 1a and c, strains carrying the *his4-912*(*URA3*) mutation carry two *Sal*I fragments that hybridize to the *HIS4* probe. The 3,100-base-pair fragment corresponds to the centromere-proximal *HIS4*-Ty912 junction fragment; the 1,400-base-pair fragment corresponds to the centromere-distal junction fragment. About 40% of the His$^+$ Ura$^+$ revertants carry a single *Sal*I fragment of 1,900 base pairs in length (Fig. 1b and d); this fragment is characteristic of a *HIS4* gene containing a solo Ty912 δ (3, 13). Revertants containing the solo δ are described below as class I through class IV revertants. About 15% of the revertants carry the *HIS4* fragment containing a solo δ, but also carry the centromere-proximal *HIS4*-Ty912 junction fragment (Fig. 1e); these revertants are described below as class V revertants. The remaining 45% of the revertants (class VI) carry both of the fragments characteristic of the *his4-912*(*URA3*) mutation as well as the *HIS4* fragment containing a solo δ (Fig. 1f). This result suggests that the class VI revertants contain two copies of chromosome III, one carrying the *his4-912*(*URA3*) mutation and one carrying a solo δ at *HIS4*. These strains could be true $2N$ diploids or they could be $N + 1$ aneuploids for chromosome III. Some of these strains were crossed to haploid strains of opposite mating type, and tetrads were dissected and analyzed. Less than 5% spore viability was observed in these crosses. This is the pattern of spore viability observed when a triploid is sporulated, indicating that the class VI revertants carry two copies of most, if not all, chromosomes. These revertants will not be discussed further.

Twenty-four independently isolated class I through class V revertants have been analyzed in detail. As indicated in Table 1, 14 of these revertants have been isolated from strains carrying a single Ty912(*URA3*) at *HIS4*, and 10 have been isolated from strains carrying a tandem duplication of Ty912(*URA3*) elements. The genetic and molecular analysis of these revertants is described below.

Class I revertants. In the 15 class I revertants, the *URA3* gene is very tightly linked to *LEU2* (see Fig. 2 for a map of chromosome III); *URA3* and *LEU2* are separated by less than 2 centimorgans (cM) in these revertants. Southern

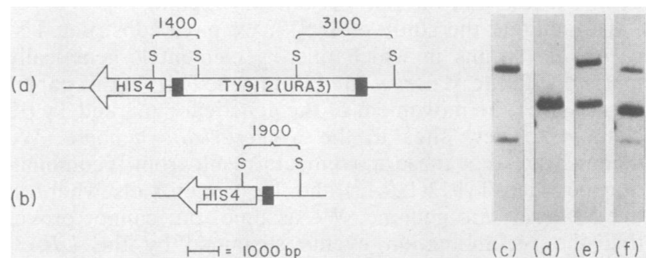


FIG. 1. Restriction maps and Southern hybridizations of *his4-912*(*URA3*) and revertants. (a) *Sal*I restriction map of *HIS4* region from *his4-912*(*URA3*) mutant. (b) *Sal*I restriction map of *HIS4* region after excision of Ty912(*URA3*) by δ-δ recombination. (c through f) Southern hybridization of total *S. cerevisiae* DNA digested with *Sal*I and probed with a 1,650-base-pair *Sal*I fragment of DNA from the wild-type *HIS4* gene (12). DNA for Southern hybridization was derived from *his4-912*(*URA3*) (c), class I haploid revertant (d), class V haploid revertant (e), and diploid revertant (f). The open box represents the internal region of Ty912(*URA3*); the black boxes represent δ sequences. The arrow represents the *HIS4* gene; the arrowhead indicates the direction of *HIS4* transcription. The chromosome III centromere lies to the right of the *HIS4* gene as diagrammed. S indicates a site for restriction by *Sal*I; the numbers above the brackets indicate the sizes in base pairs of *Sal*I restriction fragments. In (c) through (f), the 3,100-, 1,900-, and 1,400-base-pair *Sal*I restriction fragments are represented by the top, middle, and bottom bands, respectively.

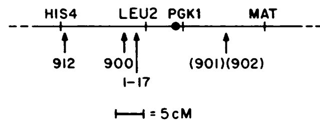


FIG. 2. Genetic map of chromosome III. The figure shows a segment of chromosome III DNA containing the genetic markers *HIS4*, *LEU2*, *PGK1*, and *MAT*. The numbers and arrows below the line indicate the positions of the Ty elements Ty912(*URA3*), Ty900, Ty1-17, Ty901, and Ty902. The black dot represents the chromosome III centromere.

hybridization analysis with *LEU2* and *URA3* DNA as probes indicates that these revertants fall into six different subclasses. The chromosomal segment containing the *URA3* gene has been cloned from at least one representative of each of these subclasses, and the cloned segments have been analyzed by restriction mapping. The restriction maps of the *LEU2* region from one of the parental strains, SR160-2C, and from the six subclasses of revertants are shown in Fig. 3.

The parental strain carries two Ty elements just to the left of the *LEU2* gene—Ty900 and Ty1-17 (Fig. 3a). Ty900 is a member of the Ty912 or Ty1 class of elements (1, 15). Ty912 and Ty900 share extensive sequence homology throughout their entire length, but can be distinguished from each other by several restriction site differences. Ty912 carries two *Sal*I sites and one *Hin*dIII site in the internal region of the element; Ty900 carries only one *Sal*I site and no *Hin*dIII site. Ty900 carries *Xho*I sites in both δ sequences; Ty912 lacks these sites. Ty1-17 was originally observed by Kingsman et al. (7) and is similar to the Ty917 element described by Roeder et al. (12). Ty1-17 differs from Ty900 and Ty912 by substitution mutations (see Fig. 6) covering two-thirds of the internal region of the Ty (7, 15). Ty1-17 and Ty900 lie in the same orientation on the chromosome; in this orientation, Ty transcription initiates in the left δ sequence and proceeds to the right (Fig. 3).

In revertants of subclass 1 (Fig. 3b), there is a tandem duplication of Ty912-like elements at the position of the original Ty900 element. The Ty on the left carries the *URA3* marker, and the Ty on the right is unmarked. In subclass 2, Ty900 is replaced by a *URA3*-marked Ty912-like element (Fig. 3c). In subclass 3, Ty900 and the region between Ty900 and Ty1-17 are replaced by a *URA3*-marked Ty912-like element (Fig. 3d). These strains carry a tandem duplication of Ty elements—a *URA3*-marked Ty912-like element on the left and Ty1-17 on the right. In subclass 4, Ty900, Ty1-17, and the region lying between these elements is replaced by a single *URA3*-marked Ty912-like element (Fig. 3e). In subclass 5, there is a tandem triplication of Ty912-like elements at the position of the original Ty900 element (Fig. 3f). The Ty on the left carries the *URA3* marker; the other two are unmarked. In subclass 6, Ty900, Ty1-17, and the region between these Ty elements are replaced by a tandem duplication of *URA3* marked Ty912-like elements (Fig. 3g).

Class II revertants. In revertants MS212 and MS100, the *URA3* marker has remained on chromosome III, but it is not tightly linked to the *LEU2* gene. Genetic analysis of these revertants is presented in Table 2. In revertant MS212, the *URA3* marker is approximately equidistant between the *PGK1* and *MAT* markers on the right arm of chromosome III (Fig. 2). In revertant MS100, the *URA3* marker is also on the right arm of chromosome III, about 10 cM centromere proximal of the *MAT* locus. It is not known whether the *URA3* marker in MS100 and the marker in MS212 are at the same or nearby sites on the chromosome.

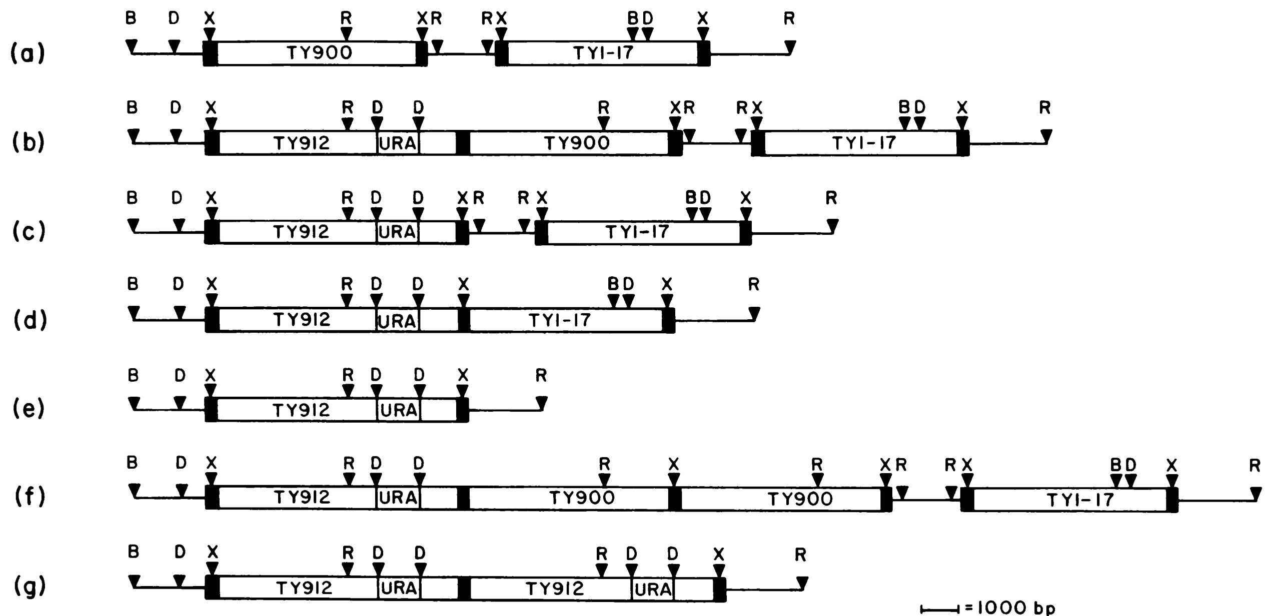


FIG. 3. Restriction maps of *LEU2* region from *his4-912*(*URA3*) and class I revertants. *LEU2* region from (a) *his4-912*(*URA3*), (b) subclass 1 revertant, (c) subclass 2 revertant, (d) subclass 3 revertant, (e) subclass 4 revertant, (f) subclass 5 revertant, and (g) subclass 6 revertant. The open boxes represent the internal regions of Ty elements; the black boxes represent δ sequences. The solid lines represent chromosome III DNA adjoining the Ty elements. The *LEU2* gene and the chromosome III centromere lie to the right; *HIS4* is to the left. The arrows indicate sites for restriction by *Bam*HI (B), *Hin*dIII (D), *Eco*RI (R), and *Xho*I (X). The 1,500-base-pair fragment between Ty900 and Ty1-17 contains three or four *Xho*I sites whose positions have not been accurately determined and which are not included in the restriction maps. Note that Ty1-17 and Ty900 both carry *Xho*I sites in their δ sequences, whereas Ty912 does not. All *URA3*-marked elements are labeled as Ty912, and all unmarked Ty912-like elements are labeled as Ty900; some of these elements may in fact be hybrids between Ty912 and Ty900.

TABLE 2. Linkage data for MS212 and MS100

Revertant	Interval	Tetrad type[a]			Map distance (cM)
		PD	NPD	TT	
MS212	*PGK1-URA3*	21	0	8	13.8
	MAT-URA3	19	0	8	14.8
	PGK1-MAT	13	0	17	28.3
MS100	*LEU2-URA3*	15	0	14	24.1
	MAT-URA3	24	0	6	10.0
	LEU2-MAT	13	0	15	26.8

[a] PD, Parental ditype; NPD, nonparental ditype; TT, tetratype.

TABLE 3. Linkage data for MS106 and MS107

Revertant	Interval	Segregation[a]		Tetrad type[b]			Map distance (cM)
		FDS	SDS	PD	NPD	TT	
MS106	*HIS4-URA3*			10	7	11	Unlinked
	LEU2-URA3			15	12	1	Unlinked
	MAT-URA3			9	7	12	Unlinked
	CEN-URA3	27	1				1.8
MS107	*HIS4-URA3*			2	4	19	Unlinked
	LEU2-URA3			5	2	18	Unlinked
	MAT-URA3			5	2	18	Unlinked
	CEN-URA3	8	17				Unlinked

[a] FDS, First-division segregation; SDS, second-division segregation. These were determined by using the centromere-linked markers *TRP1* and *LEU2*.

[b] PD, Parental ditype; NPD, nonparental ditype; TT, tetratype.

We have cloned the chromosomal region containing the *URA3* gene from revertant MS212. In addition, we have cloned the corresponding chromosomal region from the parental strain, SR112-1A. The parent carries a tandem duplication of Ty912-like elements (Fig. 4a); these Ty elements are indicated in Fig. 2 as Ty901 and Ty902. These elements are not at the same position as the Ty1-161 element found on the right arm of chromosome III by Kingsman et al. (7). Ty901 and Ty902 are nonidentical, differing from each other by at least a *Hin*dIII restriction site. In MS212 (Fig. 4b), the Ty carrying the *Hin*dIII site is replaced by a *URA3*-marked Ty.

Movement of the *URA3* gene in MS100 also involved recombination between Ty912(*URA3*) and another Ty element. This conclusion is based on Southern hybridization of *Xho*I digests of total *S. cerevisiae* DNA with *URA3* DNA as a probe. Almost all Ty elements in the yeast genome contain *Xho*I restriction sites in their δ sequences; however, the Ty912(*URA3*) element at the *HIS4* locus lacks these sites. MS100 DNA contains a 7,400-base-pair *Xho*I fragment that hybridizes to the *URA3* probe; this fragment is exactly the size predicted for a Ty element that carries the *URA3* marker and which has *Xho*I sites in its δ sequences. Double digests with *Xho*I and other restriction enzymes provide further evidence that the *URA3* gene in MS100 is contained within a Ty element that has *Xho*I sites in its δ sequences.

Class III revertants. Two His$^+$ Ura$^+$ revertants, MS106 and MS107, resulted from the movement of the *URA3*-marked Ty912 element to another chromosome. The genetic analysis of these revertants is presented in Table 3. The *URA3* gene MS106 is unlinked to chromosome III markers, but it is very tightly linked to a centromere; the distance between *URA3* and its centromere is only 2 cM. Tetrad data for MS107 indicate that *URA3* is not linked to chromosome III markers or to a centromere.

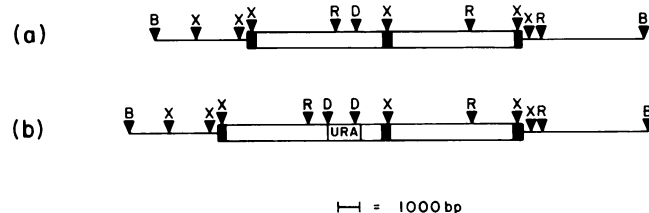


FIG. 4. Restriction maps of Ty elements between *PGK1* and *MAT* from *his4-912(URA3)* and revertant MS212. Ty elements between *PGK1* and *MAT* from (a) *his4-912(URA3)* and (b) revertant MS212. The open boxes represent the internal regions of the Ty elements. The black boxes represent δ sequences. The solid lines represent the adjoining chromosome III DNA. The orientation of the Ty elements relative to the chromosome III centromere is unknown. The arrows indicate sites for restriction by *Bam*HI (B), *Hin*dIII (D), *Eco*RI (R), and *Xho*I (X). The *Eco*RI-*Bam*HI fragment at the extreme right contains four or five *Hin*dIII sites whose positions have not been accurately determined and which are not included in the restriction maps.

Southern hybridization analysis of revertants MS106 and MS107 indicates that the *URA3*-marked Ty912 elements have moved by recombination with Ty elements on other chromosomes. DNA from both MS106 and MS107 contains a 7,400-base-pair *Xho*I restriction fragment that hybridizes with a *URA3* probe. As described above, this fragment corresponds to a Ty element flanked by δ sequences containing *Xho*I sites. Double digests with *Xho*I and other enzymes provide additional evidence that the *URA3* marker in MS106 and MS107 is contained within a Ty element whose δ sequences differ from those of the parental Ty912(*URA3*) element.

Class IV revertant. In one of the His$^+$ Ura$^+$ revertants examined, *URA3* function is no longer associated with a Ty element. As mentioned above, the strains used in these experiments carry a *ura3*$^-$ mutation (*ura3-52*) at the normal *URA3* locus on chromosome V. In revertant MS117, this mutant gene has been converted to the wild-type allele by using the *URA3* gene in Ty912 as a template for correction. Both genetic and physical evidence support this interpretation.

MS117 was mated with a strain which carries a wild-type gene at the *URA3* locus. When 20 tetrads were dissected and analyzed, only Ura$^+$ spores were found. If the *URA3* gene in MS117 and the normal *URA3* gene were at different chromosomal locations, then they should segregate independently and Ura$^-$ spores should be generated. Thus, these data indicate that the *URA3* gene in MS117 is at the same genetic location as the wild-type *URA3* gene on chromosome V.

Further evidence for gene conversion at the *URA3* locus comes from Southern hybridization analysis with *URA3* DNA as probe. Strains that carry the *ura3-52* mutation contain two *Eco*RI fragments that hybridize to *URA3* DNA; these fragments are approximately 9 and 10 kilobase pairs in length. Strains with a wild-type *URA3* gene contain a single *URA3*-hybridizing *Eco*RI fragment of 11 kilobase pairs in length. MS117 carries the 11-kilobase-pair fragment found in wild-type strains.

Class V revertants. In 4 of the 24 His$^+$ Ura$^+$ revertants examined, a segment of chromosome III DNA has been duplicated. When DNA from revertants MS119, MS120, MS224, and MS477 was digested with *Sal*I and probed with *HIS4* DNA by Southern hybridization, two fragments were detected. As described previously, one of these fragments

corresponds to a *HIS4* gene containing a solo δ, and one corresponds to the centromere-proximal Ty912-*HIS4* junction fragment (Fig. 1e). Similar results were obtained with several other restriction enzymes. These results indicate that class V revertants carry an intact *HIS4* gene that contains a solo δ and an extra segment of DNA that has one endpoint in Ty912(*URA3*) and one endpoint somewhere to the right (centromere proximal) of *HIS4*. This extra segment of DNA must include at least 12 kilobase pairs of DNA to the right of *HIS4* since it includes a *Bam*HI restriction site that is known to be 12 kilobase pairs centromere proximal of the site of Ty912 insertion.

Southern hybridization analysis indicates that the duplication of chromosome III DNA may have involved recombination between Ty912 (*URA3*) and a *Xho*I site-containing δ sequence elsewhere in the genome. The parental *his4-912*(*URA3*) strain contains a 9.1-kilobase-pair *Xho*I fragment that hybridizes to a *URA3* probe; this fragment is defined by *Xho*I sites which lie outside Ty912 in the flanking *HIS4* DNA. Class V revertants contain, instead of the 9.1-kilobase-pair fragment, an 8.8-kilobase-pair *Xho*I fragment (data not shown). This fragment is the size that would be predicted if the centromere-distal δ of Ty912(*URA3*) had acquired an *Xho*I site.

Class V revertants were also analyzed genetically. In crosses with haploid strains, good spore viability was obtained. In the tetrads derived from these crosses, chromosome III markers showed normal 2:2 segregation. These results indicate that the duplication of chromosome III DNA is not associated with diploidy or with aneuploidy for chromosome III. The distance between *HIS4* and *URA3* is 14 cM, and the distance between *LEU2* and *URA3* is 2 cM.

Ty movement without excision. Is the movement of Ty912 (*URA3*) to a new site in the genome always accompanied by its excision from *HIS4* or can movement occur before excision? To answer this question, we looked for cells in which Ty912 (*URA3*) had moved to a new site in the genome, but was also still present at the *HIS4* locus. Such cells are distinguishable on the basis of the frequency of reversion to His$^+$ Ura$^+$. Whereas the starting strains revert to His$^+$ at a frequency of 10^{-4} and to His$^+$ Ura$^+$ at a frequency of 10^{-7}, a strain carrying Ty912 (*URA3*) at *HIS4* and at a second chromosomal location should revert both to His$^+$ and to His$^+$ Ura$^+$ at a frequency of 10^{-4}.

Cells of a strain carrying a single Ty912(*URA3*) at *HIS4* were plated for single colonies on complete medium. The colonies were replicated to medium lacking histidine and to medium lacking both histidine and uracil. After 3 days at 30°C, His$^+$ or His$^+$ Ura$^+$ revertants were apparent as small papillae within each of the colonies. Most colonies displayed 10 to 20 papillae on the medium lacking histidine and no papillae on the medium lacking both histidine and uracil. Of 16,000 colonies examined, 18 showed 10 to 20 papillae on both kinds of medium. These colonies were purified, grown in liquid complete medium, and plated on medium lacking hisitidine and on medium lacking histidine and uracil. These tests confirmed that the strains revert to both His$^+$ and His$^+$ Ura$^+$ at a frequency of 10^{-4}.

DNA from the 18 strains was analyzed by Southern hybridization with *URA3* DNA as probe. Sixteen of the strains showed only the bands present in the parent strain and characteristic of Ty912(*URA3*) at *HIS4*. By analogy to the class VI revertants described above, these strains are probably diploids that revert to His$^+$ Ura$^+$ when the Ty912 (*URA3*) element on one chromosome III is excised and the Ty912(*URA3*) on the other chromosome is retained. The remaining two strains show the bands characteristic of Ty912(*URA3*) and an additional *URA3*-hybridizing band. Southern hybridization with *HIS4* DNA confirmed that these two strains contain only the fragments characteristic of the *his4-912*(*URA3*) mutation and no fragment corresponding to a *HIS4* gene containing a solo δ. These two strains represent cells in which the *URA3* gene has moved to a new genomic location without excision from the *HIS4* locus.

DISCUSSION

Ty movement. In this paper we describe the movement of a genetically marked transposon, Ty912(*URA3*), throughout the yeast genome. Two general features of Ty912(*URA3*) movement arise from these studies. First, movement almost always occurs by homology-dependent recombination events. Second, movement is preferentially intrachromosomal and distance dependent.

Homology dependence. Ty912(*URA3*) movement is almost always the result of recombination between homologous DNA sequences. In 19 of 24 revertants examined, Ty912(*URA3*) moved by recombination with Ty elements elsewhere. In one case, the *URA3* gene in Ty912 recombined with the mutant *URA3* gene on chromosome V. The recombination events responsible for the four class V revertants are poorly understood, but it is likely that these revertants are also the results of recombination between homologous Ty or δ elements (see below and Fig. 7). The recombination events described here are most likely unrelated to the ability of Ty elements to transpose into nonhomologous DNA sequences and cause insertion mutations. The Ty elements are probably acting as dispersed repetitive DNA sequences that can function as substrates for the generalized recombination system in *S. cerevisiae*. As is the case for bacterial transposons, this ability to act as portable regions of homology is an important aspect of the fluidity of genomic DNA effected by transposable elements (8).

Chromosome specificity. Ty912(*URA3*) movement is preferentially intrachromosomal. In 21 of the 24 cases examined, the *URA3*-marked Ty element remained on chromosome III. Furthermore, recombination between Ty elements appears to decrease with increasing distance between the elements. Ty912(*URA3*) recombines with Ty elements near *LEU2* (approximately 15 cM away) more than seven times as often as it recombines with the Ty elements on the other side of the centromer (approximately 35 cM away). There are more than 20 Ty912-like elements in the *S. cerevisiae* genome that do not reside on chromosome III. Many of these are even more similar to Ty912(*URA3*) in their restriction maps than is Ty900. Thus, it seems unlikely that Ty912(*URA3*) recombines preferentially with the Ty elements on chromosome III simply because it shares more homology with these elements than with the Ty elements on other chromosomes. The intrachromosomal migration more likely reflects increased interactions, and therefore opportunities for recombination, between DNA sequences on the same chromosome. Indeed, it has been shown that interconversion between *S. cerevisiae* mating type cassettes occurs more frequently when the cassettes reside on the same chromosome than when they occupy sites on different chromosomes (4).

Gene conversion versus integration. The recombination events responsible for class I, II, and III revertants fall into two classes—those in which Ty912(*URA3*) replaces another Ty element or DNA sequence (Fig. 3c, d, e, and g; Fig. 4b) and those in which the *URA3*-marked Ty element becomes inserted into another element (Fig. 3b and f). Ty replace-

ments have been described previously and are believed to be the results of gene conversion (14). Two Ty elements align with each other, and the sequence of one element is corrected by using the other element as a template. The Ty element that is used as a template could be either the Ty912(*URA3*) element resident at the *HIS4* locus or a Ty912(*URA3*) element that has been excised from *HIS4*.

Ty insertions could occur by three different kinds of recombination events. First, Ty912(*URA3*) could be excised from the *HIS4* locus by a reciprocal crossover between the terminally repeated δ sequences; the excised circular Ty could then engage in a single reciprocal crossover with a Ty element elsewhere to become reintegrated into the genome (Fig. 5a). Second, insertion could occur by a double reciprocal crossover involving both δ sequences of Ty912(*URA3*) and one of the δ sequences of another Ty (Fig. 5b). According to this model, Ty912(*URA3*) excision is closely coupled to its movement to a new site. The third possibility is that Ty912(*URA3*) is inserted into a single δ by gene conversion (Fig. 5c). In this case, the left δ and the right δ of Ty912(*URA3*) align with a single δ from another Ty element. The single δ is then converted to the DNA sequence with which it is paired; the result is that Ty912(*URA3*) is duplicated and inserted into the δ of the second Ty element.

The isolation of strains carrying a Ty912(*URA3*) element at the *HIS4* locus and also at a second genomic location indicates that Ty movement need not always precede or be tightly coupled to its excision. Our experiments indicate that the frequency of Ty912(*URA3*) movement unaccompanied by excision is 1 in 10^4 cells. As described previously, Ty912(*URA3*) excises at a frequency of 10^{-3} to 10^{-4}, and the frequency of reversion to His$^+$ Ura$^+$ of the *his4-912*(*URA3*) mutation is 10^{-7} to 10^{-8}. Thus, the frequency of His$^+$ Ura$^+$ revertants (10^{-7} to 10^{-8}) is equal to the frequency of Ty excision (10^{-3} to 10^{-4}) multiplied by the frequency of Ty movement unaccompanied by excision (10^{-4}). Therefore, it is possible that all of the revertants described in this paper are derived from cells in which Ty912(*URA3*) first moved to a new site in the genome and then excised from *HIS4*.

Movement of tandem duplications. In subclasses 1 and 2 of class I revertants. (Fig. 3b and c), a single *URA3*-marked Ty element has moved to the *LEU2* region. Some of these revertants are derived from strains carrying a tandem duplication of Ty912(*URA3*) elements at the *HIS4* locus. These results indicate that one of the two Ty elements within a tandem duplication can move independently of the other element. In two cases, however, the elements have moved in concert. In the strain represented in Fig. 3g, Ty900, Ty1-17, and the region between these elements have been replaced by the tandem duplication of *URA3*-marked elements originally present at *HIS4*. In the revertant diagrammed in Fig. 3f. there is a tandem triplication of Ty912-like elements at the position of Ty900. Surprisingly, only the leftmost of these carries the *URA3* marker. This strain most likely resulted from the insertion of a tandem duplication of Ty912(*URA3*) elements followed by a gene conversion event in which one of the *URA3*-marked Ty elements was corrected with Ty900 as a template.

Endpoints of gene conversion. When two segments of DNA engage in gene conversion, recombination must both start and stop in a region of homology between the recombining molecules. For the *URA3* marker in Ty912 to move to the Ty elements near the *LEU2* locus, Ty912 sequences to the left of the *URA3* marker and Ty912 sequences to the right of the marker must both interact with homologous sequences near *LEU2*. In the *LEU2* region, there are six regions homologous to Ty912 sequences to the left of (*URA3*); these are region A of Ty900, δ2 of Ty900, regions C, D, and E of Ty1-17, and δ4 of Ty1-17 (Fig. 6). There are four regions homologous to Ty912 sequences to the right of *URA3*; these are δ1 of Ty900, region B of Ty900, δ3 of Ty1-17, and region F of Ty1-17 (Fig. 6). For the *URA3* marker to move to the Ty element near *LEU2*, the left end of Ty912 must interact with one of the four possible regions of homology, and the right end of Ty912 must interact with the same region or with a region to the right. When all possible combinations of left and right endpoints are considered, a total of 12 different classes of gene conversion events are possible (Fig. 6). Even if all class

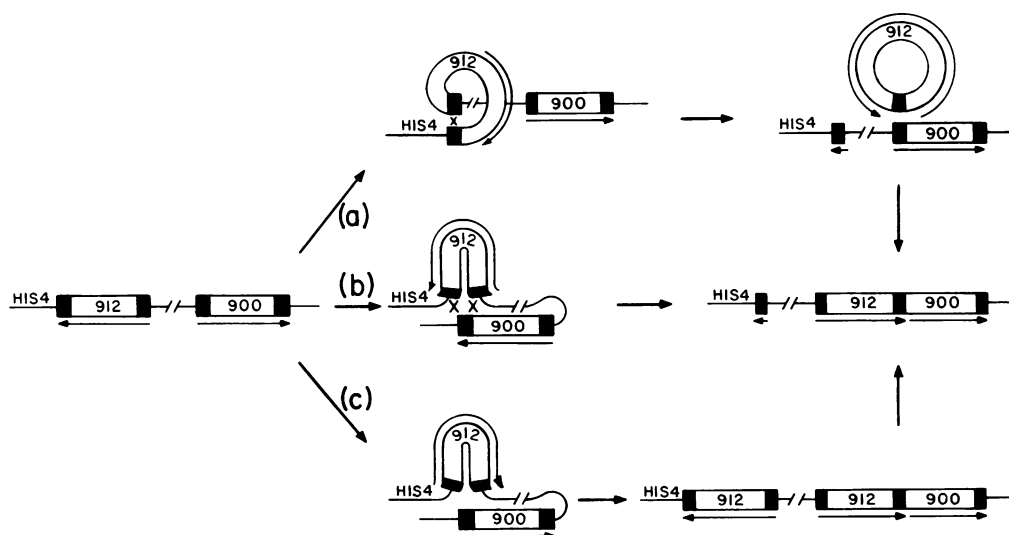
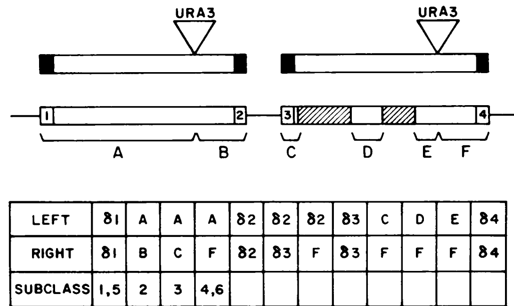


FIG. 5. Models for insertion of Ty912(*URA3*) into Ty900. Shown on the left is the chromosomal region containing Ty912(*URA3*) and Ty900 before Ty912(*URA3*) movement. (a) Movement by excision and reintegration. (b) Movement by a double-reciprocal crossover. (c) Movement by gene conversion. The models are described in detail in the text. The open boxes represent the internal region of the Ty elements, and the black boxes indicate δ sequences. The element indicated as 912 carries the *URA3* marker. The arrows below the Ty elements indicate the direction of Ty transcription. The Xs indicate the sites of reciprocal crossovers.



LEFT	δ1	A	A	A	δ2	δ2	δ2	δ3	C	D	E	δ4
RIGHT	δ1	B	C	F	δ2	δ3	F	δ3	F	F	F	δ4
SUBCLASS	1,5	2	3	4,6								

FIG. 6. Regions of homology between Ty elements and endpoints of gene conversion. Shown in the center of the figure are Ty900 (left) and Ty1-17 (right). Shown above each of these elements is a Ty912(*URA3*) element. The boxes numbered 1 through 4 indicate the left and right δ sequences of Ty900 and the left and right δ sequences of Ty1-17, respectively. The black boxes represent the Ty912 δ sequences. The open boxes represent the internal regions of the Ty elements; the slashed boxes represent the regions of heterology between Ty1-17 and the Ty912 and Ty900 elements. The triangle above Ty912 indicates the position of the *URA3* insert. The brackets and letter designations below Ty900 and Ty1-17 indicate the regions of homology with Ty912(*URA3*). Regions A, B, C, D, E, and F are 4,500, 1,500, 400, 900, 700, and 1,500 base pairs in size, respectively. The chart at the bottom indicates all possible combinations of left and right endpoints of gene conversion when Ty912 (*URA3*) recombines with the Ty elements near *LEU2*. The chart also indicates the types of gene convertants that have been detected and the class I revertants representing each type.

I revertants are assumed to result from gene conversion events (Fig. 5b), only 4 of the 12 possible classes of convertants have been observed.

All class I revertants have one endpoint near the left end of Ty900; convertants having their left endpoints in any of the other three possible regions of homology have not been observed. In contrast, the right endpoints of conversion are more or less equally distributed among the four possible regions of homology. These observations suggest that a sequence near the left end of Ty900 is a preferred site for the initiation of recombination events. The left end of Ty900 may be preferred simply because it is closest to Ty912(*URA3*); alternatively, the left end of Ty900 may contain a special DNA sequence that is recognized by recombination enzymes as an initiation site for mitotic recombination events.

Synapsis of Ty elements. An examination of class I revertants suggests that δ sequences may be the preferred sites for the alignment or synapsis of Ty elements before recombination. If Ty912(*URA3*) and the elements near *LEU2* paired with each other in such a way as to maximize the interactions between homologous DNA sequences, then Ty900 and Ty912(*URA3*) would always align with each other. The *URA3* DNA would have no homologous region with which to pair; otherwise, the elements would be paired completely from one end to the other. Gene conversion would result in the replacement of Ty900 by Ty912(*URA3*) to generate a subclass 2 revertant. The other subclasses of revertants result from interactions between sequences that differ from each other by extensive regions of heterology. In some cases, δ sequences are the only regions of homology present at one or both ends of the recombining molecules. These observations suggest that the δ sequences are more important than the internal regions of the Ty elements in determining how sequences will be aligned. One way to account for the importance of the δ sequences is to propose that the δ sequences are the preferred sites of action of an enzyme that initiates recombination events. The δ sequences may be the sites of single-strand or double-strand endonucleolytic cleavages leading to free ends which can then invade an homologous sequence elsewhere. The enzyme which recognizes and cleaves the δ sequences may play a role in the transposition of Ty elements by a homology-independent process as well as in their movement by homology-dependent means.

Duplications associated with Ty excision. Class V revertants carry a duplication of a large segment of chromosome III DNA lying centromere proximal to *HIS4*. In Fig. 7, we present a model for the generation of these duplications. The first step in this model is the excision of a large segment of chromosome III DNA by a reciprocal crossover between one of the δ sequences of Ty912 (*URA3*) and a δ sequence somewhere in the region between Ty912(*URA3*) and its centromere. The δ sequences in Ty900 and Ty1-17 are not in the correct orientation for such a crossover. However, the region between Ty900 and Ty1-17 contains two or three solo δ sequences containing *Xho*I sites; one of these may lie in the same orientation as the Ty912 δ sequences. Recombination between the centromere-distal δ of Ty912 and a δ between Ty900 and Ty1-17 would result in the generation of a circular molecule containing approximately 20 kilobase pairs of chromosome III DNA. We propose that this circle engages in recombination with the Ty900 element of a sister chromatid that has not sustained an excision event. The resulting chromosome contains a duplication of a large segment of chromosome III DNA. When the Ty912(*URA3*) element at the *HIS4* locus is excised by δ-δ recombination, this chromosome will contain an intact *HIS4* gene containing a solo δ and also the centromere-proximal *HIS4*-Ty912(*URA3*) junction fragment. This model accounts for the observed duplication of chromosome III DNA, the tight linkage of *URA3* to the *LEU2* locus, and the acquisition of an *Xho*I site by one of the δ sequences of Ty912(*URA3*).

An alternative version of the model shown in Fig. 7 eliminates the need for a circular intermediate. If chromosome III sister chromatids aligned with each other in opposite orientations, then the region between Ty912 and the solo δ could be inserted into Ty900 by gene conversion or by two reciprocal crossovers.

Ty transposition. None of the Ty movements described here resulted from the insertion of Ty912(*URA3*) into a nonhomologous DNA sequence. This is not surprising given the high frequency of homology-dependent recombination events as compared to the relatively low frequency of Ty transposition. In this study, movement of Ty912(*URA3*) by gene conversion occurred at a frequency of 1 in 10^4 cells. The frequency of Ty transposition has not been accurately determined; however, estimates based on the frequency of insertion mutations at particular genetic loci are available. These estimates range from 10^{-8} to 10^{-9} insertions per cell at a single genetic locus (15). If the entire *S. cerevisiae* genome is considered, these estimates give an overall frequency of cells that have undergone Ty transposition of 10^{-4} to 10^{-5} per cell. If all Ty elements are assumed to transpose equally frequently, a frequency of 3×10^{-6} to 3×10^{-7} per element is obtained. This frequency is 2 to 3 orders of magnitude lower than the frequency of movement of Ty912(*URA3*) by gene conversion. These calculations suggest that 100 to 1,000 His^+ Ura^+ revertants would have to be examined before a transposition event would be detected. It is also possible that the insertion of the *URA3* marker has rendered Ty912 incapable of transposition and that no transpositions

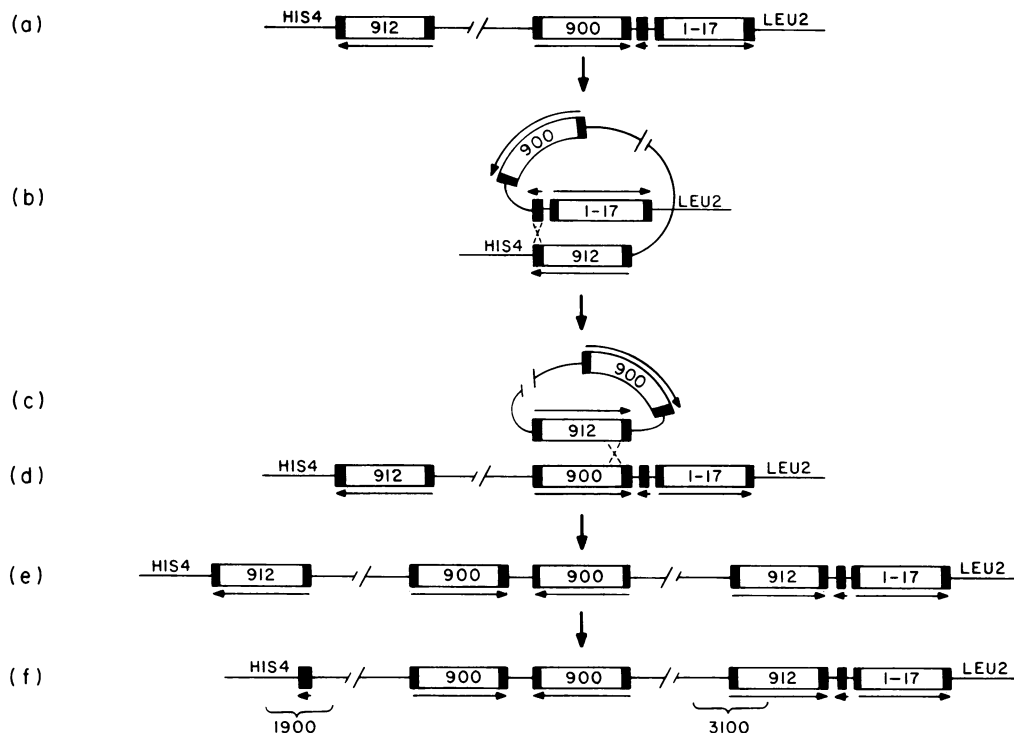


FIG. 7. Model for duplication of chromosome III DNA and generation of class V revertants. (a) Segment of chromosome III DNA from *HIS4* to *LEU2*. (b) Pairing and reciprocal crossing over between the centromere-distal δ of Ty912(*URA3*) and a solo δ between Ty900 and Ty1-17. (c) The circular molecule resulting from the crossover depicted in (b); the orientation of the circle is reversed relative to (b). (d) Chromosome III chromatid that has not sustained a deletion. (e) Chromosome III resulting from integration of (c) into (d). (f) Chromosome shown in (e) after excision of Ty912(*URA3*) from *HIS4*. The final product (f) contains a *HIS4* gene containing a solo δ, a *URA3*-marked Ty element near *LEU2*, and a duplication of a large segment of chromosome III DNA. Note that the newly inserted chromosome III DNA is in inverted orientation relative to the normal chromosome III DNA. The open boxes represent the internal regions of the Ty elements. The element indicated as 912 carries the *URA3* marker. The black boxes represent δ sequences. The solo δ between Ty900 and Ty1-17 (a) carries an *Xho*I site as described in the text. The arrows below the Ty elements indicate the direction of Ty transcription. The Xs indicate the sites of reciprocal crossovers. The numbers below the brackets (f) indicate the sizes in base pairs of *Sal I* restriction fragments hybridizable with a *HIS4* probe (Fig. 1). Diagram is not to scale.

of this element would be detected even if a large number of revertants could be examined.

Conclusions. The experiments described in this paper demonstrate that movement of a genetically marked Ty element occurs at high frequency in mitotically dividing haploid *S. cerevisiae* cells. These movements most often result from recombination between the genetically marked Ty and a homologous Ty element elsewhere in the genome. Ty recombination events occur more often between Ty elements that are close together on the same chromosome than between elements that are far apart on the same chromosome or on different chromosomes. We have shown previously that recombination between Ty elements can result in the replacement of the DNA sequence of one element by the sequence of another element (14). In this paper, we demonstrate that recombination between Ty elements can also result in an increase or a decrease in the total number of Ty elements present in the cell and in the deletion or duplication of unique sequence DNA.

ACKNOWLEDGMENTS

We thank Karen Engst and Vicki Evangelidis for excellent technical assistance and Ralph Keil and Ronald Pearlman for critical reading of the manuscript.

This investigation was supported by Public Health Service grant GM28904 from the National Institutes of Health. M.S. is a Career Investigator of the Medical Research Council of Canada; this work was done during an extended study leave supported by the Medical Research Council of Canada.

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