Allelic and Ectopic Recombination Between Ty Elements in Yeast

Martin Kupiec and Thomas D. Petes

Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637

Manuscript received February 1, 1988
Revised copy accepted April 1, 1988

ABSTRACT

Allelic and nonallelic (ectopic) recombination events were analyzed in a set of isogenic strains that carry marked Ty elements. We found that allelic recombination between Ty elements occurred at normal frequencies both in meiosis and mitosis. The marked Ty elements were involved in a large variety of different types of ectopic recombination and this variety was greater in mitosis than in meiosis. Allelic and ectopic recombination events occurred at similar frequencies in mitosis, but allelic recombination predominated in meiosis. Some of the types of ectopic mitotic recombination indicated the common occurrence of concerted recombination events. The length of homology represented by a delta element (330 bp) seemed to be sufficient for some types of mitotic and meiotic recombination.

ALL eukaryotic organisms studied to date contain repetitive DNA sequences interspersed through their genomes. Although most of these sequences have no known function, they represent a potential source of genomic instability since recombination between dispersed repeated sequences can lead to a variety of chromosomal rearrangements. Recombination of this type has been extensively studied in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Scherer and Davis 1980; Potter, Winsor and Lacroute 1982; Mikus and Petes 1982; Roeder and Fink 1982; Sugawara and Szostak 1983; Kohli et al. 1984; Roeder, Smith and Lambie 1984; Jinks-Robertson and Petes 1985, 1986; Lichtten, Borts and Haber 1987). Recombination between repetitive sequences has also been demonstrated (Liskay and Stachelek 1983) or inferred in mammals (Slighmont, Blech and Smithies 1980; Lehrman et al. 1987; Nicholls, Fischel-Ghodsi and Higgs 1987; Rouyer et al. 1987).

One of the most common dispersed repeat in *S. cerevisiae* is the transposable element Ty (about 60-80 copies per diploid cell) which is similar in its structure to retroviruses (Boeke et al. 1985). This element consists of a 5-kb central element (ε) flanked by two direct repeats approximately 330 bp in length (δ); there are also approximately 200 δ elements in the diploid genome that are not associated with Ty’s (reviewed by Roeder and Fink 1983; Williamson 1983).

Ty elements can be divided into two main classes, Ty1 and Ty2. Although the organization of these two elements is similar, there are two regions that show a considerable amount of sequence divergence (Kingsman et al. 1981). Consequently, although Ty1 and Ty2 elements share certain restriction sites (for example, a BglII site near the 3’ end of the element), certain sites are characteristic of either Ty1 or Ty2. In addition, restriction site differences have been observed between different Ty1 elements (Williamson 1983). Ty elements transpose at low frequencies (about $10^{-8}$) through an RNA intermediate (Boeke et al. 1985) but can engage in homologous recombination with nonallelic Ty elements (ectopic recombination) at higher frequencies during vegetative growth (Roeder and Fink 1982). Both reciprocal exchanges and gene conversion events have been detected (Roeder and Fink 1983). These events have been shown to be involved in the generation of deletions, inversions and translocations in yeast (Rothstein 1979; Roeder and Fink 1980; Liebman, Shalit and Picologlou 1981; Roeder 1983; Breiman, Gafner and Ciiracy 1985; Rothstein, Helms and Rosenberg 1987). Meiotic ectopic recombination events involving Ty elements, both reciprocal (Roeder 1983) and nonreciprocal (M. Kupiec and T. D. Petes, unpublished data), have also been described. The mitotic recombination studies described above were done in haploid strains. Thus, any recombination events involving Ty elements were nonallelic (involving elements at different chromosomal loci), also termed “ectopic” (Lichten, Borts and Haber 1987). Allelic meiotic recombination events involving Ty elements have not been previously examined. Below, we describe our analysis of allelic and ectopic recombination in a series of isogenic diploid strains. We find that allelic recombination events involving Ty elements occur at frequencies similar to those observed for other types of genomic sequences. Ectopic exchanges were also observed in meiosis and
mitosis. Ectopic events involving more than one pair of interacting Ty elements were fairly common in mitosis. Our analysis of ectopic events indicates that 330 bp of homology is sufficient for this type of recombination in mitosis and meiosis.

**MATERIALS AND METHODS**

**Strains:** The yeast strains used in the present study are listed in Table I. All the strains are isogenic and are derivatives of strains MK28 and MK31, a pair of MATa and MATα isogenic strains derived from DBY921 (a ura3-50 leu2 hist4 met8-1 can1-1) (Botstein et al. 1979). Strain MK28 was constructed by transforming DBY931 with PstI-Bal31 treated DNA of the plasmid pM1, selecting for Leu+ transformants. The purpose of this transformation was to replace the normal Ty1-17 near LEU2 with TyCla, a Ty element in which the BglII site near the 3’ end of the element was replaced by a ClaI site as described below. A brief Bal31 treatment of the plasmid was necessary since there is a small amount of poly GC linker on one end of the fragment containing part of Ty1-17 (Klein and Petes 1984). The mating type of this strain (MK28) was then switched using the plasmid YCP50-HO (provided by I. Herskowitz), generating the strain MK31.

The Escherichia coli strain MC1066 (pyrF:: Tn5 leuB trpC9830 lac− gal− str− hisDr− , Casadaban et al. 1982) was used as a host in all cloning experiments.

**Plasmids:** Plasmid pM1 was constructed by partially digesting plasmid CV9 with BglII and filling-in the recessed ends with the Klenow fragment of DNA polymerase I. This procedure creates a 3′ overhang for the insert. The plasmid CV9 contains part of the Ty1-17 element in addition to LEU2 sequences (Klein and Petes 1984). The plasmid pM1, therefore, has a ClaI site replacing the unique BglII at the end of Ty1-17.

In order to replace Ty1-17 by a marked Ty carrying the URA3 gene, the plasmid pM29 was constructed. First, a 1.1-kb BamHI fragment carrying the URA3 gene (Goebel and Petes 1986) was inserted into the unique BglII site of plasmid pM4, which contains the whole Ty1-17 element (derived from k g5K17 (Kingsman et al. 1981)) cloned in the EcoRI site of pUC19. The Asp718 site in the adjacent LEU2 gene was eliminated by partially digesting with Asp718 and filling-in the recessed 3′ ends with the Klenow fragment of DNA polymerase I. This procedure creates a new SmaI site; we call this mutant allele leu2A. When the EcoRI fragment of pM29 was used to transform strain MK28, which carries a TyCla replacing Ty1-17, all of the Ura+ colonies were also Leu−. Southern analysis confirmed the presence of TyUra replacing TyCla and the presence of the SmaI site replacing the Asp718 site.

Three plasmids were used in the Southern analysis of recombination events near TyUra (see Figure 2). Plasmid pM35 contains pBR322 sequences and a 700 bp BamHI-BstEII fragment of chromosome III, distal to Ty900, that was derived from plasmid ASC (Newlon et al. 1986). Plasmid pM42 contains pBR322 sequences and a 800-bp EcoRI-SpeI fragment of chromosome III, located between Ty900 and Ty1-17, and derived from the plasmid pR274 (a generous gift from G. S. Roeder). Plasmid Ylp33 contains the SalI-Xho1 2.2-kb fragment containing the LEU2 gene (proximal to Ty1-17) cloned in the SalI site of pBR322 (Botstein et al. 1979).

**Media and growth conditions:** Yeast cells were grown vegetatively at 30°C and sporulated at room temperature. Standard media was used for mitotic growth (Sherman, Fink and Hicks 1986). Cells were induced to undergo meiosis in SM medium after vegetative growth in YPA medium (Jinks-Robertson and Petes 1986). Ura− cells were selected by plating on medium containing 5-fluoroorotic acid (5-FOA; Boeke, Lacroix and Fink 1984). E. coli cells were grown in LB medium at 37°C. Ampicillin (50 µg/ml) of tetracycline (10 µg/ml) were added when necessary.

**Physical analysis of yeast DNA:** Yeast DNA was isolated from 5 ml stationary phase cultures of yeast (Sherman, Fink and Hicks 1986). Following gel electrophoresis of DNA samples treated with various restriction enzymes, the fragments were transferred to Hybond membranes (Amerham) and hybridized to a 32P-labeled probe.

**Measurement of meiotic recombination frequencies:** Individual diploid cultures were grown at 30°C in YPA to a concentration of about 107 cells/ml, then washed and resuspended in SM medium. After 3 days of incubation at 23°C, ascis were treated with a reducing agent and Glusulase, sonicated and resuspended in water (Jinks-Robertson and Petes 1986). Ura− spores were selected by plating on medium containing 5-fluoro-orotic acid (5-FOA; Boeke, Lacroix and Fink 1984). E. coli cells were grown in LB medium at 37°C. Ampicillin (50 µg/ml) of tetracycline (10 µg/ml) were added when necessary.

**Measurement of mitotic recombination:** The rate of mitotic recombination events involving TyUra was measured by fluctuation tests. Twenty colonies of equal size grown on YPD were transferred to Eppendorf tubes containing 200 µl of sterile water. Samples were either plated directly on 5-FOA plates, or diluted and plated on YPD (to measure total cell number). After 5 days, colonies were counted and transferred to YPD plates. These plates were
Recombination Between Ty Elements

**RESULTS**

In order to examine both allelic and ectopic recombination events involving Ty elements in both meiosis and mitosis, we constructed three isogenic diploid strains MK38, MK49 and MK65 (Figure 1 and Table 1). The strains MK38 and MK49 (which differ only at the HIS4 and LYS2 loci) contain two marked Ty1-17 elements; Ty1-17 is a Ty2 element located close to the LEU2 gene on chromosome III (WARMINGTON et al. 1985). One of the elements (TyUra) has an insertion of a wild-type URA3 gene in the BglII site near the end of the element and the allelic Ty1-17 (TyCla) contains a ClaI site at the same position (see MATERIALS AND METHODS). Thus, TyUra and TyCla are uniquely marked since all Ty elements (both Ty1 and Ty2) examined in our and other studies contain the BglII site at the end of the element (WILLIAMSON 1983). The strain MK65 is homozygous for TyUra and is otherwise isogenic with MK49.

Since the URA3 insertion in TyUra is flanked by Ty sequences, gene conversion events between TyUra and Ty elements that do not contain the URA3 insertion should result in a Ura- phenotype. A similar approach has been previously used to detect mitotic interactions between Ty elements in haploid strains (ROEDER and FINK 1982). Below, we will first discuss experiments measuring the rate of mitotic and meiotic recombination events involving TyUra. We will then describe the molecular analysis of the different classes of recombination events.

**Rate of mitotic recombination events involving TyUra**

The rate of mitotic recombination events (both allelic and ectopic) involving TyUra was estimated in strain MK49 by measuring the rate of loss of the Ura- phenotype. Ura- diploid derivatives of MK49 can be conveniently identified using media containing 5-FOA. These derivatives were then screened for mating and sporulation ability, and for histidine prototrophy. Ura- derivatives of MK49 could arise in a number of ways: (1) loss of the chromosome containing TyUra (resulting in cells that have the a mating type, are incapable of sporulation and are His-), (2) reciprocal crossing over between TyUra and the centromere (resulting in cells that are non-naturers, capable of sporulation and His-) and (3) recombination events (either allelic or ectopic) between TyUra and other Ty elements that delete the URA3 insertion (resulting in cells that are nonnaturers, capable of sporulation and His+). Recombination events between the URA3 insertion and the ura3-50 allele present in chromosome V, as well as mutations within the insert, will also be scored in the third group. As will be discussed below, however, these classes are rare and can be distinguished by a molecular analysis.

We examined the frequency of each class of event described above in 20 independent cultures of MK49 (as described in MATERIALS AND METHODS). The rates (per division), calculated by the median method (LEA and COULSON 1948), were: 0.39 ± 0.13 × 10^{-6} for chromosome loss, 0.91 ± 0.23 × 10^{-6} for crossing over and 1.08 ± 0.54 × 10^{-6} for recombination events (plus mutation) involving TyUra.

**FIGURE 1.**—Schematic representation of chromosome III organization in strains MK49 and MK65. Ty elements are represented as open boxes flanked by triangles (8 elements). Ty1's are represented as having an EcoRI site (R), Ty2's as having a BamHI site (B). Hatched boxes represent the URA3 insertion, striped boxes the LEU2 gene, and gray boxes the HIS4 gene. G: BglII site.

then replica-plated to media lacking histidine and to lawns of tester strains to assess mating ability. Rates of recombination were calculated using the median method (LEA and COULSON 1948). Only one colony from each plate was used for DNA analysis.

**Papillation test:** Ura- colonies that showed a parental restriction pattern in the Ty1-17 region could be produced by either a conversion event involving TyUra and the ura3-50 mutant allele on chromosome V, or a new mutation within the URA3 insertion. In order to distinguish between these two possibilities, we plated these strains on media lacking uracil after treating them with ultraviolet light to stimulate recombination. If the Ura- phenotype is the result of a new mutation (which is not located very close to the ura3-50 alteration), Ura- papillae should be formed as the result of mitotic recombination between the new mutant ura3 gene and the ura3-50 allele on chromosome V. If the Ura- phenotype is the result of a conversion event, no Ura- papillae should be observed since the strains would contain two ura3-50 alleles and the ura3-50 allele does not revert to Ura+. As discussed in the RESULTS section, Ura- strains of both classes were detected.
Rate of meiotic recombination events involving TyUra

Two different types of experiments were done to examine meiotic recombination of TyUra. The rates of allelic and ectopic recombination events were examined by tetrad dissection of the diploid strains heterozygous for TyUra (MK38 and MK49). Random spore analysis was used to examine the rate of ectopic recombination in the diploid strain that was homozygous for TyUra (MK65). These results are further described below.

Meiotic recombination of TyUra was detected in dissected tetrads of MK38 and MK49 by following the Uracil phenotype of the spores. Gene conversion of TyUra should result in tetrads that segregate either 3 Ura+:1 Ura− or 1 Ura+:3 Ura− spores. In the strain MK49, gene conversion at the LEU2, HIS4, and LYS2 loci could also be monitored. Of 2704 tetrads analyzed, 20 had conversion of TyUra (Table 2). This frequency (0.74%), although low, is in the same range (0.63–18%) as that of other yeast genes (FOGEL, MORTIMER and LUSNAK 1981). In particular, this level of conversion is similar to that observed for LYS2 (0.95%). In 14 of the 20 TyUra convertant tetrads, the leu2 mutation, located 1 kb away, co-converted.

We analyzed DNA from the spores derived from convertant tetrads by Southern blotting. In 14 of the 20 tetrads, the conversion event involving TyUra was allelic (3 TyUra:1 TyCla spores or 1 TyUra:3 TyCla spores). Allelic conversion of the marked Ty elements showed approximate parity since the URA3 insertion was duplicated in nine and deleted in ten tetrads. In one tetrad, ectopic meiotic conversion occurred between TyUra and an unmarked Ty element since this tetrad segregated 1 TyUra:2 TyCla:1 unmarked Ty2.

Because the number of ectopic meiotic conversion events involving TyUra that could be analyzed in strain MK49 was small, we examined ectopic conversion of TyUra in the isogenic strain MK65. Since this strain is homozygous for TyUra (Figure 1), all recombination events deleting the URA3 gene from TyUra should be ectopic events. We sporulated MK65 and identified Ura− spores using 5-FOA plates. The frequency of Ura− spores was 1.62 ± 0.08 × 10−5 per spore (6.5 × 10−5 per tetrad). Thus, during meiosis the rate of allelic recombination of TyUra (7.0 × 10−3 per tetrad) is about 100-fold higher than the rate of ectopic recombination.

Since the rate of mitotic recombination events involving TyUra (both allelic and ectopic) is only 1 × 10−6, the rate of allelic recombination of TyUra in meiosis is at least three orders of magnitude higher than in mitosis. Since (as described below) we found that about half of the mitotic recombination events involving TyUra were allelic and half were ectopic, meiosis apparently enhances allelic conversion of TyUra more than ectopic conversion.

The experiments described above indicate that both the absolute rates of recombination of TyUra as well as the ratio of allelic and ectopic events involving TyUra are different in meiosis and mitosis. As discussed below, we also found that the classes of ectopic events were different in mitosis and meiosis.

Physical analysis of mitotic recombination events involving TyUra

The region of chromosome III containing TyUra and Ty900. Ty elements and URA3 gene are represented as in Figure 1. Black boxes represent sequenced used as probes in Southern analysis. The 8 elements are numbered starting from the one closest to the LEU2 gene. The 83 and 84 elements, which are shown arranged in tandem, may actually be integrated one into the other (WARMINGTON et al. 1986). Regions A through E are indicated in order to facilitate the description of the Southern analysis. B: BamHI site; R: EcoRI site; G: BglIII site; P: PstI site.

Figure 2.—Representation of the region of chromosome III containing TyUra and Ty900. Ty elements and URA3 gene are represented as in Figure 1. Black boxes represent sequenced used as probes in Southern analysis. The 8 elements are numbered starting from the one closest to the LEU2 gene. The 83 and 84 elements, which are shown arranged in tandem, may actually be integrated one into the other (WARMINGTON et al. 1986). Regions A through E are indicated in order to facilitate the description of the Southern analysis. B: BamHI site; R: EcoRI site; G: BglIII site; P: PstI site.
Recombination Between Ty Elements

**FIGURE 3.**—Restriction maps of Tyl-17 (Warmington et al. 1985) and Ty900 (from Southern analysis). Not all the sites for the restriction enzymes shown are drawn for Ty900; for each restriction enzyme, only the first site beyond the probes used in Southern analysis can be determined. A: Asp718; B: BamHI; Bc: BclI; Be: BstEII; C: ClaI; D: HindIII; G: BglII; P: PstI; R: EcoRI; S: SalI; Sa: Sad; Sn: SnaBI; V: PvuII; X: XbaI; Xb: XhoI.

derivatives of MK49 by Southern analysis, using plasmids YIp33, pM33 and pM42 as hybridization probes (Figure 2). In all cases, we found that the chromosome originally containing the TyUra allele had various structural alterations, whereas the chromosome containing the TyCla allele was unaltered by the recombination event. The Ura− His+ derivatives could be grouped into several classes, according to the organization of the Ty1-17 region. Ty elements containing BamHI sites, and lacking EcoRI and PstI sites were classified as TyS's, and those containing EcoRI and PstI sites, but lacking BamHI sites, as Tyl's. Figure 4 shows the general structure of the different classes obtained (see also Table 3). As described in detail below, class 1 represents allelic recombination events involving TyUra, classes 2–9 represent various types of ectopic interactions involving TyUra and other Ty or 6 elements, class 10 represents ectopic gene conversion between the URA3 insertion in TyUra and a mutant ura3 gene on chromosome V and class 11 represents new mutations in the URA3 gene in TyUra.

**Class 1:** This largest class of recombinants (36 of 83) contained two identical chromosomes, each carrying a TyCla. This pattern is the expected product of an allelic gene conversion event in which the URA3 insertion was replaced by sequences derived from the TyCla allele. No other changes were detected in the ~30-kb region surrounding Ty1-17 with the following enzymes: Asp718, BamHI, BglI, BglII, BstEII, ClaI, EcoRI, HindIII, PstI, PvuI, PvuII, SalI, and XhoI.

**Class 2:** This class accounted for a quarter of the ectopic mitotic recombination events involving TyUra. In class 2 strains, an unmarked Ty2 element replaced TyUra. No other restriction site changes were observed in this class with any of the restriction enzymes used. This arrangement of restriction sites suggests that class 2 strains result from a mitotic gene conversion between TyUra and an unmarked Ty element elsewhere in the genome, resulting in a deletion of the URA3 insert. The donor Ty could be either a Ty2 or a Ty1 (assuming that no co-conversion of diagnostic sites occurred in the Tyl-TyUra conversion) and could be located elsewhere on chromosome III (for example, Ty900) or on a nonhomologous chromosome.

**Class 3:** In strains of this class (10 isolates), the TyUra element has been deleted. More specifically, regions B, C, and D (Figure 2) have been replaced by a single Ty1, which now separates region A from region E. Seven of the derivatives contained a Ty1 element indistinguishable from Ty900 by restriction site analysis. Such strains could result from an intrachromosomal recombinational event involving 81 and 85 as shown in Figure 5A. Alternatively, the Ty900 element in the sister chromatid or in the homologous chromosome could serve as a donor of information in a conversion event involving repair of a double-stranded gap in the chromosome (Figure 5B); this mechanism has been invoked to explain conversion events involving delta elements in the
TABLE 3

<table>
<thead>
<tr>
<th>Class</th>
<th>Mitotic (MK49)</th>
<th>Meiotic (MK65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1 (allelic)</td>
<td>36</td>
<td>NA^a</td>
</tr>
<tr>
<td>Class 2</td>
<td>11 (26.2)</td>
<td>27 (54.0)</td>
</tr>
<tr>
<td>Class 3</td>
<td>10 (23.8)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Class 4</td>
<td>4 (9.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Class 5</td>
<td>4 (9.5)</td>
<td>13 (26.0)</td>
</tr>
<tr>
<td>Class 6</td>
<td>4 (9.5)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Class 7</td>
<td>3 (7.1)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Class 8</td>
<td>2 (4.8)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Total ectopic events involving TyUra</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>Class 10 (conversion between URA3 in TyUra and ura3-50 on chromosome V)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Class 11 (mutations of URA3)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>83</td>
<td>50</td>
</tr>
</tbody>
</table>

*Percentage of ectopic events involving TyUra in each class is indicated in parentheses.

^a NA, not applicable.

SUP4 region of the genome (ROTHSTEIN, HELMS and ROSENBERG 1987). It is also possible that the donor of information is a Ty element identical to Ty900 located elsewhere in the genome.

Three of the class 3 strains had Ty elements that were not identical to Ty900 in their restriction pattern. One strain (Z25) contains a Ty1 element identical to Ty900, except for the presence of SalI and BstEII sites at the 3' end of the element. These sites are present in Ty1-17, but absent in Ty900 (Figure 3). A second strain (Z63) has a single Ty element with the SacI and BstEII sites as in Ty1-17 but it lacks the SalI site located 3' of these sites in Ty1-17. The third strain (M5) has a single Ty element with a HindIII site at a position characteristic of Ty1 but SalI, BstEII and SacI sites at the positions expected for Ty2. All three of these strains, therefore, contain Ty elements that appear to be hybrids between Ty1 and Ty2.

There are two models that can explain the formation of such hybrid elements. First, a gene conversion event could involve heteroduplex formation between Ty900 and TyUra. Patchy correction of the resulting mismatches (including deletion of URA3) followed by resolution of the heteroduplex intermediate by crossing over could generate the resulting single hybrid Ty elements. Alternatively, the hybrid elements could result from a conversion intermediate involving repair of a double-stranded gap (similar to that shown in Figure 5B) in which the donor of information is a Ty element in the genome other than TyUra, TyCla or Ty900.

Class 4: In this class, regions B, C and D (Figure 2) of chromosome III were replaced by a Ty1 element (as classified using EcoRI, PvuII, PstI and BglIII) that contains a ClaI site, instead of the conserved BglII site at the 3' end of the element. This ClaI site could have only come from the TyCla in the homolog. This class could be explained by gene conversion mediated by gap repair (similar to that shown in Figure 5B) in which TyCla served as a donor to repair a gap spanning from 81 at the right to the 3' end of Ty900 at the left. Three of the four strains in this class, however, differ from Ty900 in some restriction sites (two have a BglII site at a different position, one carries a HindIII site, which Ty900 entirely lacks). Since the positions of these sites in these three strains is characteristic of neither TyCla nor Ty900, it is likely that these strains result from two concerted conversion events, one involving TyUra, Ty900 and TyCla and a second involving a different genomic Ty element.

Class 5: In this class of strains, TyUra was replaced by a single delta element, consistent with a recombination event involving 81 and 82. This event could involve recombination between delta sequences within one chromatid or an unequal sister-strand interaction. Since we cannot recover all of the interacting chromosomes in a mitotic recombination event,
we cannot determine whether the recombination event is reciprocal or nonreciprocal (gene conversion). In one of the four class 5 strains (Z67), we detected a restriction site change in the near-by Ty900 (the changed element does not contain PstI sites), indicating the possibility of two concerted recombination events.

**Class 6:** In this class, the TyUra element was replaced by a single delta element and there is a second Ty2 distal to this delta element (Figure 4). This class can be explained as the result of a conversion event initiated as a double-strand break in the URA3 gene of TyUra, followed by gap formation removing URA3 (Figure 6). The gap could be repaired using information derived from the other homolog (or the sister chromatid), such that one end of the broken chromosome is paired with Ty900 and the other end (61) is paired with 82. Alternatively, class 6 strains could result from a two-step process, in which an unequal crossover occurs between Ty900 and TyUra coupled with a 6-8 recombination in the second TyUra (Figure 6B). The four class 6 strains show differences in the restriction sites of the Ty2, which could reflect either different boundaries of conversion in the first model or different locations of the crossover in the second.

**Class 7:** In class 7 strains, the TyUra was replaced by a single delta element and the proximal 500 bp from region C (Figure 2) were missing. This structure is consistent with a recombinational event involving 81 and 83. The region distal to 83 remained unchanged in all class 7 strains.

**Class 8:** The structure of chromosome III in these strains was similar to that of class 7, except that another ~300 bp from region C were missing, consistent with a 61-84 recombinational event.

**Class 9:** In this class, a single delta element separated region A from region E, as would be expected from a recombinational event involving 61 and 86.

**Classes 10 and 11:** By Southern analysis, class 10 and class 11 Ura+ strains were identical to the parental strain. These strains, therefore, do not result from the deletion of the URA3 gene from TyUra by recombination. These classes represent gene conversion events between the URA3 insertion and the mutant ura3-50 allele on chromosome V of MK49 (class 10) or mutations of the URA3 allele (class 11). These two classes can be distinguished genetically (as described in MATERIALS AND METHODS).

In several of the classes of ectopic recombination described above, we could not determine whether the interacting Ty and δ elements were located close together on chromosome III (TyUra, Ty900 and associated δ elements), far apart on chromosome III or on nonhomologous chromosomes. Since gene conversion events in meiosis and mitosis are often associated with reciprocal exchange of flanking sequences

---

**Figure 6:** Two models for recombination events that generate class 6 strains. (A) A double-strand break in the TyUra is followed by nuclease degradation, creating a gap. Following pairing of 61 and 82 and the δ of TyUra with the ε of Ty900, the gap is repaired with information derived from the sister chromatid or homologous chromosome. (B) Two concerted events occur: a crossover between TyUra and Ty900 and a second crossover between 61 and 82.

(FOGEL, MORTIMER and LUSNAK 1981; FOGEL AND HURST 1963), ectopic conversion events involving Ty or δ elements could lead to various chromosomal aberrations (deletions, inversions, translocations, dicentric and acentric) depending on the orientation and location of the repeated elements. These chromosomal rearrangements can be recognized by two criteria: (1) poor (50% or less) viability of spores following meiotic segregation of the chromosomes and (2) lack of physical continuity between regions A and C (Figure 2).

We dissected at least six tetrads of each of the His+ Ura+ strains resulting from mitotic recombination of TyUra as well as the parent strain MK49. Spore viability in all strains exceeded 80%, indicating no gross chromosomal rearrangements. DNA was isolated from all strains, digested with either BglII, PstI or BamHI, and examined by Southern analysis. In all cases, the same DNA fragments hybridized to YIp33, pM42 or pM33, indicating physical continuity from region A to region E. In addition, only the expected sizes of bands were seen in DNA from all the strains with all the restriction enzymes used. Thus,
either the mitotic recombination events involving TyUra do not usually involve Ty or δ elements at widely dispersed regions of the genome or gene conversion events associated with this type of interaction are not usually resolved as reciprocal exchanges.

Our analysis of the mitotic recombination events involving TyUra indicated that this marked Ty element was involved in a large number of different types of interactions. As described below, we found that in meiosis, the number of different classes of interactions was considerably more limited.

**Physical analysis of ectopic meiotic recombination events involving TyUra**

Strain MK65, which is homozygous for the TyUra (otherwise isogenic with MK49), was induced to undergo meiosis and DNA from fifty independent Ura− spores was subjected to Southern blot analysis. Since this strain carries identical information on both copies of Ty1-17, only ectopic recombination events can create Ura− spores. All 50 haploid strains could be characterized as belonging to one of the classes described for the mitotic events (Table 3). The distribution of strains in these classes, however, was strikingly different from that observed for mitosis, with three-fourths of the strains being represented by one of two classes.

More than half of the strains belonged to class 2, carrying an unmarked Ty2 element replacing TyUra. Southern analysis failed to show any restriction site polymorphisms in any of the 27 strains belonging to this class. As for the mitotic recombination events, this class represents a gene conversion between TyUra and an unmarked Ty element. We cannot determine whether the donor element is on chromosome III or on a nonhomologous chromosome nor whether the donor was a Ty1 or Ty2 element.

The second class in importance (about 26% of the strains) was class 5, in which TyUra was replaced by a single δ element. This class could be explained by a crossover between δ1 and δ2 (a “pop-out”) or by a gene conversion event in which TyUra is degraded. In two of the 13 class 5 haploid strains, restriction sites changes were detected in Ty900, suggesting the possibility of two concerted conversion events.

The other classes observed mitotically were either absent or present in low numbers (Table 3) among the strains derived from meiosis. Since the frequency of ectopic meiotic events is less than one order of magnitude higher than the frequency of ectopic mitotic events, it is likely that some of these rare classes may be the result of preexisting mitotic events.

We also examined, by Southern analysis, DNA derived from all 50 strains for chromosomal rearrangements associated with gene conversion. Since these strains were haploid, only balanced chromosomal rearrangements could be recovered. No rearrangements were detected.

**DISCUSSION**

The Ty transposable elements represent the largest family of dispersed repeated sequences in yeast, comprising about 2% of the genome. Most previous studies of recombination between Ty elements have examined mitotic recombination in haploid strains. In our study, we analyze allelic and ectopic recombination in mitosis and meiosis in one set of isogenic strains. Our conclusions are: (1) allelic recombination between Ty elements is not greatly suppressed in either mitosis or meiosis, (2) TyUra can be involved in a large variety of different types of ectopic mitotic recombination events, (3) concerted mitotic recombination events involving different Ty elements are common, (4) allelic and ectopic recombination events involving TyUra occur at similar frequencies in mitosis but allelic recombination predominates in meiosis, (5) the number of different types of ectopic recombination events in meiosis is more limited than in mitosis and (6) the length of homology represented by a δ element (350 bp) seems to be sufficient for some types of mitotic and meiotic recombination events. Each of these conclusions will be discussed in more detail below.

Using yeast strains that contain duplications of selectable genes, we (and others) previously observed a high level of ectopic meiotic recombination (Jinks-Robertson and Petes 1985, 1986; Lichter, Borts and Haber 1987). The level of gene conversion of duplicated sequences on nonhomologous chromosomes was similar (within a factor of 20) to that observed for allelic sequences and about half of these conversion events were associated with reciprocal exchanges (Jinks-Robertson and Petes 1986; Lichter, Borts and Haber 1987). We recently found that ectopic meiotic gene conversion events involving TyUra inserted at the LYS2 locus are less frequent than expected on the basis of previous studies involving artificial duplications (M. Kupiec and T. D. Petes, unpublished data). One interpretation of this result is that meiotic recombination between Ty elements is suppressed by a trans-acting system analogous to the EDR1 system (which is involved in suppressing mitotic recombination between delta elements (Rothstein 1984)). Our observation that allelic recombination of TyUra occurs at normal frequencies in meiosis indicates that either no such trans-acting system exists for meiotic recombination between Ty elements or the suppression system primarily affects ectopic meiotic recombination.

Our analysis of mitotic conversion events indicates that the URA3 insertion was lost by allelic recombination at a rate similar to that observed for gene
conversion of other types of genomic sequences (reviewed by Esposito and W gestaff 1981). The most common class of ectopic recombination (class 2) represents the replacement of the URA3 insertion from TyUra with sequences derived from a nonallelic Ty; the remainder of the Ty retains the Ty2 sequences of the original TyUra. We cannot determine whether the donor Ty was a Ty2 element or a Ty1 element. If the donor was a Ty1 element, the conversion tract must be short since no co-conversion of Ty1-specific restriction sites occurred.

Most of the other mitotic recombination events involving Tyu represent recombination between delta elements similar to those described previously (Roeder and Fink 1982; Roeder, Smith and Lambie 1984; Rothstein, Helms and Rosenberg 1987). All possible combinations of recombination between 81 and 82-86 were observed. It is somewhat surprising that recombination between the 81 and 85 elements (class 3) was more frequent than recombination between 81 and 82 (class 5) since the 81 and 82 elements are identical in sequence (Warming et al. 1985) and are closer together. It is possible that this type of recombination involves pairing between TyUra and Ty900, an event that may be more frequent than pairing between d elements due to the bigger size of the homologous region. Alternatively, this pattern of recombination may reflect structural constraints in folding the chromosome into the conformation necessary for intrachromosomal exchange.

In experiments similar to ours, but involving a marked Ty1 located about 15 kb distal to Ty900 on chromosome III, Roeder, Smith and Lambie (1984) concluded that d elements are more important than the internal region of Ty elements in determining how sequences will be aligned and suggested that delta elements are the site of initiation of gene conversion. In their studies, 86 of Ty900 was the element most commonly involved in conversion with the marked Ty. They suggested that this preference may reflect either a special property of this element or proximity to the marked Ty. In our studies, 86 is involved in conversion events only rarely (class 9) and the interactions of d elements that delete TyUra do not appear to be distance-dependent in any obvious way. These differences in the two studies could be due either to the different systems used (in their study, the marked Ty element acts as donor of information; in ours, as recipient; or to the different locations of the marked Ty elements, which may impose different chromosomal constraints. Some physical constraints controlling the type of recombination events seem likely, since some of the expected alterations of the region near TyUra were never seen (for example, Ura+ strains similar to class 3 except containing a single Ty2). We also never recovered strains with two Ty elements in tandem, sharing one 5 element, although this class of alteration has been reported by others (Scherer and Davis 1980; Liebman, Shalit and Picololou 1981; Roeder, Smith and Lambie 1984; Downs, Brennan and Liebman 1985).

As in several previous studies (Chaleff and Fink 1980; Liebman and Picololou 1988; Rothstein, Helms and Rosenberg 1987), we observed strains apparently resulting from multiple concerted mitotic recombination events. For example, three of the class 4 strains contained a single Ty element with a Cla site replacing the URA3 insertion as well as other restriction site changes that did not appear to be derived from either TyUra or Ty900. Such strains are likely to be a consequence of two recombination events, one resulting in a deletion of portions of TyUra and Ty900 (using TyCla as a donor of information) and a second involving a different genomic Ty as a donor.

There were several differences between the mitotic and meiotic recombination events involving TyUra. First, the ratio of ectopic to allelic recombination events was about 1.2 for mitotic and 0.05 or lower for meiotic recombination. This difference can be interpreted in a number of ways. Since allelic exchanges involve long regions of perfect homology (except for allelic differences) and ectopic exchanges involve short regions of less-than-perfect homology, the observed difference may indicate that the meiotic recombination system is more sensitive to sequence homology than the mitotic system. Since it has been shown previously that non-Ty repeated sequences of 1.7 kb (Jinks-Robertson and Petes 1985) and 2.2 kb (Lichten, Borts and Haber 1987) located on nonhomologous chromosomes recombine efficiently in meiosis, the length of perfect homology does not have to be very large. Consistent with the hypothesis that efficient meiotic recombination requires nearly perfect homology, Roeder (1983) found high levels of unequal meiotic recombination between two very similar Ty elements located on chromosome III.

A related interpretation of the difference in the ratio of allelic and ectopic recombination concerns the structure of TyUra. Since the URA3 insertion is near the end of the e portion of the repeat, deletion of the insertion by ectopic conversion with another Ty element requires removal of a 1.1-kb insertion flanked by 350 bp of homology on one side of the element and 5.5 kb of homology (good homology with about 10–20 other Ty2s and patchy homology with 50–70 Ty1’s) on the other side. This type of recombination may be relatively easier in mitosis than in meiosis. In experiments in which ectopic meiotic conversion of a URA3 insertion in a non-Ty repeat was examined, however, no obvious effect on the frequency of conversion was seen (M. Kupec and T. Petes, in preparation).
A third interpretation of the data is that initiation of recombination might occur very rarely within Ty elements. Ty elements could show a nearly normal rate of allelic conversion as the result of conversion events initiated outside of the element. Since the formation of conversion tracts requires sequence homology at both ends of the tract, this same mechanism would not allow high levels of ectopic recombination between Ty elements.

One final interpretation is that the paucity of ectopic meiotic events reflects, in part, differences in the strains used to study mitotic and meiotic conversion. Since the strain MK65 (which was used in most of the meiotic analyses) contained two identical TyUra elements, it is possible that meiotic allelic interactions would be more frequent than in strain MK49 which had one TyUra element and one TyCla element. If allelic and ectopic exchanges were in competition, one might observe relatively fewer ectopic exchanges in MK65 than MK49. Although we cannot exclude this possibility, it is unlikely to represent a large effect since we observe a preference for allelic interactions even in strain MK49. In addition, allelic gene conversion rates for deletions are similar to those observed for point mutations (FOGEL, MORTIMER and LUSNAR 1981), indicating that deletions and insertions are unlikely to affect substantially allelic pairing.

A second difference between the mitotic and meiotic data is the number of different classes of ectopic recombination observed and the distribution of strains into these classes. As indicated in Table 3 and Figure 4, most of the meiotic ectopic recombination events (unlike the mitotic events) were either class 2 or class 5. This difference may reflect differences in the structure of meiotic and mitotic chromosomes. For example, the probability of recombination could be influenced by features of chromatin structure that are different in meiosis and mitosis. Alternatively, this difference may reflect a different specificity of the enzymes that catalyze meiotic and mitotic recombination; for example, as described above, the meiotic recombination system might be more sensitive to sequence homology. In this regard, it is interesting that class 5 events were common in meiosis since this type of event probably represents an interaction between 81 and 82, which share 332 bp of complete homology (WARMINGTON et al. 1985).

Since we observed class 5 strains generated in meiosis and mitosis, 332 bp of complete homology appears to be sufficient to allow ectopic recombination events in both meiosis and mitosis. As described previously, we do not know whether the recombination event resulting in class 5 was a simple crossover between 81 and 82 or a conversion event involving these elements. It is also possible that recombination events involving 8 elements are restricted to intra-chromosomal interactions.

In our study, as well as others, it is assumed that the recombination events that delete URA3 from TyUra and do not involve other changes in the chromosome structure are the result of gene conversion events involving TyUra and another genomic Ty; the alternative (and much less likely) possibility is that the URA3 insertion is lost by a double crossover event (see ROEDER and FINK 1982). Conversion events are often associated with reciprocal exchange of flanking DNA in both meiosis (17–64% association) and mitosis (10–55% association) as reviewed by FOGEL, MORTIMER and LUSNAR (1981) and ESPOSITO and WAGSTAFF (1981). A reciprocal exchange between the TyUra in chromosome III and any Ty element oriented in the same direction relative to the centromere in a nonhomologous chromosome would result in a translocation. A crossover involving TyUra and other Ty elements in chromosome III would generate deletions or inversions. None of the observed gene conversion events in our study was associated with chromosomal aberrations. This result suggests that either conversion of TyUra is not associated with reciprocal recombination or that association of the conversion event with crossing over is undetectable because of the viability of strains containing the alterations.

We assume that the donor of information in all the recombinational events is another chromosomal Ty element. Since we found no evidence for physical interaction between the elements involved we cannot rule out, however, recombinational events between TyUra and the product of transcription of Tys, either as RNA molecules or as DNA reverse transcripts. Since Ty RNA represents several percent of the total polyA mRNA of the cell (ROEDER and FINK 1983), these molecules must be present in many more copies than the chromosomal Ty elements.

We thank G. S. ROEDER and C. NEWLON for providing plasmids used in this study and all members of the PETES laboratory for useful discussions and comments on the manuscript. The research was supported by National Institutes of Health research grants GM24110 and GM34646.

LITERATURE CITED


Recombination Between Ty Elements


Communicating editor: M. Carlson