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Meiotic Recombination between Repeated Transposable Elements in *Saccharomyces cerevisiae*

MARTIN KUPIEC AND THOMAS D. PETES†*

Department of Molecular Genetics and Cell Biology, University of Chicago, 920 E. 58th Street, Chicago, Illinois 60637

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We have measured the frequency of meiotic recombination between marked Ty elements in the *Saccharomyces cerevisiae* genome. These recombination events were usually nonreciprocal (gene conversions) and sometimes involved nonhomologous chromosomes. The frequency of ectopic gene conversion among Ty elements appeared lower than expected on the basis of previous studies of recombination between artificially constructed repeats. The conversion events involved either a subset of the total Ty elements in the genome or the conversion tract was restricted to a small region of the Ty element. In addition, the observed conversion events were very infrequently associated with reciprocal exchange.

Most individuals within a phylogenetic species have the same karyotype. The frequency of spontaneous chromosomal alterations, such as translocations, deletions, duplications, and inversions, is usually low. Chromosomal aberrations can be regarded as the product of illegitimate recombination events. In some cases, the event producing the altered chromosome appears to be a site-specific type of recombination (for a review, see reference 9). For example, the translocations observed in certain non-Burkitt B-cell tumors appear to be the result of errors made during V-D-J joining in the immunoglobin gene cluster (51). A possible alternative mechanism for the production of chromosomal alterations is homologous recombination between dispersed repeated genes. Such exchanges have been shown to produce deletions and duplications in higher eucaryotes. For example, a duplication of a region of the human LDL receptor gene as the result of a recombination event involving repeated Alu elements has been reported previously (30). In higher eucaryotes, however, no spontaneous translocations (other than those involved in tumor formation) have been analyzed at the level of DNA sequence. It is unclear, therefore, whether translocations can also involve homologous recombination between dispersed repeats.

In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, recombination between dispersed repeated genes has been observed in meiosis and mitosis. Such events have been termed ectopic recombination (31). Both reciprocal and nonreciprocal recombination events have been detected (12, 23, 24, 27, 31, 34, 37, 39, 41, 45, 49, 50); the nonreciprocal transfer of DNA sequences from one repeat to another represents a gene conversion event. Most of these studies have involved duplications of genes that are normally present in a single copy per haploid genome, although recombination between naturally occurring repeats has also been observed.

One surprising feature of these studies is that meiotic recombination (both gene conversion and crossovers) between artificially duplicated genes was found to occur at high frequencies (23, 24, 31). The frequency of meiotic gene conversion between a single pair of dispersed repeats does not differ significantly from that observed for normal allelic gene conversion events. As with allelic conversion events (17), approximately half of the ectopic conversion events were associated with reciprocal recombination, producing chromosomal translocations (24, 31).

One of the most abundant dispersed class of repeated sequences in the yeast genome are the Ty elements, which are found in approximately 30 to 50 copies per haploid genome (40). These elements have a structure similar to that of retroviruses, consisting of a 5-kilobase (kb) central element (epsilon) that is flanked by 338-base-pair (bp) direct repeats (delta elements). Ty elements have been shown to transpose into target loci at low frequency (about 10^{-8}) through a reverse transcription process (1).

Since there are many Ty elements in the genome and since (as described above) duplicated genes on nonhomologous chromosomes have high levels of reciprocal meiotic recombination, the relative stability of the yeast karyotype is difficult to explain. One obvious possibility is that meiotic recombination between Ty elements is strongly suppressed. Yeast strains with a mutation in the *EDR1* gene have increased levels of mitotic recombination between delta elements (43). It seems likely that similar mechanisms may exist to reduce the frequency of Ty-Ty recombination in meiosis. In mitotically dividing cells, the repression of recombination mediated by the *EDR1* system is not absolute, since both gene conversion events as well as reciprocal recombination events (involving either Ty elements within one chromosome or Ty elements on nonhomologous chromosomes) have been observed between Ty elements (4, 7, 32, 41). It should be noted, however, that a very efficient system that suppresses mitotic exchange between Ty elements is probably not necessary, since mitotic exchanges in general are 3 to 4 orders of magnitude less frequent than meiotic exchanges (for a review, see reference 13). Although no systematic study of the frequency of meiotic recombination between Ty elements has been done, Roeder (38) found a high frequency of unequal reciprocal meiotic recombination between two Ty elements located on chromosome III.

As described below, we measured directly the frequency of meiotic gene conversion and associated reciprocal exchange between marked Ty elements. We found that the frequency of such recombination events was low. One interpretation of this result is that there is a Ty-specific system that represses meiotic ectopic exchange.

* Corresponding author.
† Present address: Department of Biology, University of North Carolina, Chapel Hill, N.C.

MATERIALS AND METHODS

Media and growth conditions. Yeast cells were grown vegetatively at 32°C and sporulated at room temperature. Standard media were used for mitotic growth (47). Cells were induced to undergo meiosis in SM medium after vegetative growth in YPA medium (24). For selecting *ura3* strains, we used plates containing 5-fluoro-orotate (5-FOA) (2); we added 50 mg of uracil and 1 g of 5-FOA per liter of SD complete medium (47). To select *lys2* strains, we used plates containing the following: 0.17% yeast nitrogen base without ammonium sulfate (Difco Laboratories), 2% glucose, 0.2% α-aminoadipate (Sigma Chemical Co.), 30 mg of lysine per ml, and other amino acids as required (8).

Escherichia coli strains were grown in LB medium at 37°C (33). Ampicillin (50 μg/ml) or tetracycline (10 μg/ml) was added when necessary.

Plasmid constructions. *E. coli* MC1066D (genotype, *recA pyrF*::Tn*5 leuB6 trpC9830 lac gal* Str^r *hsdR* [6]) was used as a host in all cloning experiments; standard cloning procedures were used (33). Restriction enzymes were purchased from New England BioLabs, Inc., IBI, or Boehringer Mannheim Biochemicals.

In order to generate a uniquely marked Ty element, we changed a conserved *Bgl*II site within the Ty1-17 element to a *Cla*I site. The plasmid CV9 (which contains a portion of the Ty1-17 element, as well as the adjacent *LEU2* gene [26]) was partially digested with *Bgl*II, and the recessed ends were filled-in with the Klenow fragment of DNA polymerase. This plasmid (pM1), therefore, had lost the *Bgl*II site from the end of Ty1-17 and had a *Cla*I site at the same position.

We also constructed a plasmid (pM28) that contained a wild-type *URA3* gene inserted into a copy of Ty1-17 that was inserted into a *lys2* gene. This plasmid was constructed in several steps. First, an *Eco*RI fragment containing a complete Ty1-17 element (derived from lambda gtKG17 [25]) was inserted in the *Eco*RI site of pUC9, generating the plasmid pM4. A 1.1-kb *URA3* gene with *Bam*HI linkers (20) was inserted into the unique *Bgl*II site in pM4 which is near the end of the Ty element, generating plasmid pM14. In order to insert this marked Ty element into the *lys2* gene, we did a second series of constructions. The plasmid YK22A (provided by Ker Yu, University of Chicago) contains a delta element derived from the D15 Ty element (19) on a *Bgl*II-*Hin*dIII fragment; the *Bgl*II site in this plasmid is 21 bp from the beginning of the delta element, and the *Hin*dIII site is immediately adjacent to the end of the delta element. YK22A was cut with *Hin*dIII, and the recessed end was filled-in using the Klenow fragment. The plasmid was then treated with *Bgl*II, and the resulting fragment containing the D15 delta was inserted into the plasmid pSL42-2 (48). The original plasmid pSL42-2 contains an *Eco*RI-*Hin*dIII fragment that includes the wild-type *LYS2* gene, inserted into the vector YIp5. In order to insert the delta element derived from YK22A, we cut pSL42-2 with *Xho*I, filled-in the recessed end, and then cut it with *Bgl*II. This resulting fragment was ligated to the *Bgl*II-*Hin*dIII (filled-in) fragment containing the D15 delta element, generating the plasmid pM11. The net result of this construction thus far is that a 3-kb *Bgl*II-*Xho*I fragment from within the *lys2* gene has been replaced by a 330-bp delta element derived from D15. In order to insert the marked Ty1-17 into the *lys2* gene, we cut pM14 with *Xho*I (which cuts within both delta elements that flank the marked Ty1-17) and pM11 with the same enzyme (which cuts within the D15-derived delta element that is in the *lys2* gene). The resulting fragments were mixed and treated with ligase, generating the plasmid pM28. Thus, the pM28 plasmid has a marked Ty element (TyUra) that is identical to Ty1-17, except for the insertion of *URA3* and the hybrid nature of the flanking delta elements; other than the Ty1-17 element, no other sequences derived from chromosome III are present in the *lys2* gene. In pM28, the genes are oriented such that *lys2*, *URA3*, and the Ty element are transcribed in the same direction.

Plasmid pM46 was constructed in several steps. First, a *Pvu*II fragment of plasmid p3L6, which carries the *LYS2* gene (obtained from C. Falco), was subjected to a BAL 31 treatment and cloned into the unique *Sma*I site of pUC19. This plasmid (pM43) was then digested with *Bam*HI and *Bgl*II and self-ligated to generate plasmid pM44. Thus, this plasmid (pM44) carries a fragment of DNA with 3.9 kb of homology to the *LYS2* sequences to the right of the *Bgl*II site, and 180 bp of homology to the left of the *Bam*HI site (see Fig. 1). A *Spe*I-*Sac*I fragment of plasmid C1G (obtained from C. Newlon), carrying the *HIS4* gene, was then ligated to a *Bam*HI-*Hin*dIII-digested pM44 (after filling-in the four DNA ends with either T4 DNA polymerase or the Klenow fraction of the *E. coli* DNA polymerase), resulting in the plasmid pM46.

Two other plasmids were used in these studies. The plasmid pM38 was constructed by inserting a 5.5-kb *Bam*HI fragment containing *URA3* (from pSR13 [24]) into *Bgl*II-*Bam*HI-treated pSL42-2. Since the *Bam*HI site in pSL42-2 is about 3 kb from the *Bgl*II site, in pM38, a 3-kb fragment from within the *lys2* gene was replaced with the 5.5-kb *URA3* fragment. The plasmid pM35 was constructed in the same way as pM38, except that the 1.1-kb *URA3 Bam*HI fragment was used instead of the 5.5-kb fragment. In both plasmids, the transcriptional orientation of *URA3* is the same as for *lys2*. Restriction maps of plasmids pM1, pM28, pM38, pM35, and pM46 are shown in Fig. 1.

Yeast strain constructions. We used three diploid strains for the meiotic studies of Ty recombination (MK42, MK54, and MK60) and one strain (MK44) for the mitotic studies. All strains were isogenic, except for the changes introduced by transformation. The diploid MK42 was homozygous for an altered Ty1-17 on chromosome III (TyCla) and homozygous for a Ty element with a *URA3* insertion (TyUra) integrated into *lys2* (chromosome II). In order to construct this strain, we transformed (22) the haploid strain DBY931 (a *ura3-50 leu2 his4 met8-1 can1-1* [3]) with *Pst*I-BAL 31-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 brief BAL 31 treatment of the Pst fragment derived from pM1 was necessary, since there is a small amount of poly(G · C) linker on one end of the fragment containing Ty1-17 (26). The mating type of the resulting strain (MK28) was then switched using the plasmid YCp50-HO (provided by I. Herskowitz), generating the strain MK31. Since YCp50-HO contains a wild-type *URA3* gene, in addition to the cloned homothallism gene, we selected Ura$^+$ transformants of MK28 and then screened for Ura$^-$ derivatives that had lost the plasmid and switched mating types.

The strains MK28 and MK31 were then transformed with pM28 DNA that had been cut to completion with *Eco*RI and partially digested with *Bam*HI; Ura$^+$ transformants were selected. These transformants were examined by Southern analysis in order to detect those strains in which the wild-type *LYS2* gene was replaced by the *lys2* gene containing the insertion of TyUra. The transformed haploid derived from

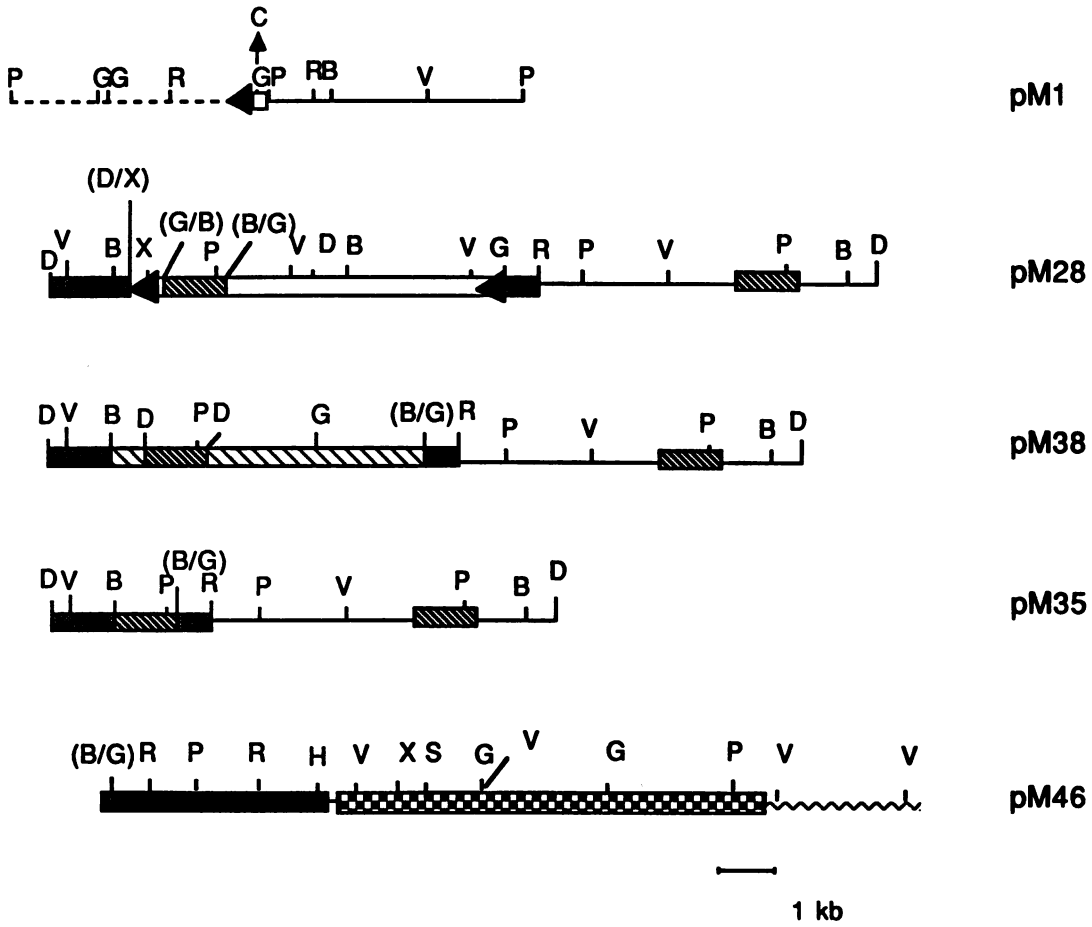

FIG. 1. Plasmids used in the present study. In plasmid pM1, a *Bgl*II site in Ty1-17 was changed to a *Cla*I site (see Materials and Methods for details). Plasmids pM28, pM38, pM35, and pM46 are drawn aligned at the *Bam*HI site in their *LYS2* sequences. Symbols: ——, pBR322 sequence; ∿∿∿, pUC19 sequence; □, Ty sequence; ◀, delta element; ▨, 5.5-kb fragment from chromosome V that contains the 1.1-kb *URA3* gene; ▩, 1.1-kb *URA3* gene; ▬, *LYS2* sequence; ------, sequence, adjacent to Ty1-17 on chromosome III, containing the *LEU2* gene; ▭, *HIS4* sequence. Abbreviations: B, *Bam*HI; C, *Cla*I; D, *Hin*dIII; G, *Bgl*II; H, *Hpa*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; V, *Pvu*II; X, *Xho*I.

MK28 (MK40) was mated to the haploid derived from MK31 (MK39) to yield the diploid MK42.

The diploid MK54, which was homozygous for the 1.1-kb *Bam*HI *URA3* gene inserted within *lys2*, was constructed by mating haploid strains MK50 and MK52. The MK50 and MK52 strains were made by transforming MK28 and MK31 to uracil prototrophy with *Eco*RI-*Pvu*II-treated pM35 plasmid DNA. The transforming fragment contains the 1.1-kb *URA3* insertion, flanked by *lys2* sequences. The diploid MK68 was constructed by transforming MK54 with the plasmid pM46 digested with *Sal*I. The purpose of this construction was to insert *lys2* homology at the *HIS4* locus. The diploid MK60, which is homozygous for the 5.5-kb *Bam*HI *URA3* insertion within *lys2*, was constructed by mating MK58 to MK59. The MK58 and MK59 strains were obtained by transforming MK28 and MK31 to uracil prototrophy with *Eco*RI-*Pvu*II-treated pM38 DNA. The diploid used in the mitotic recombination experiments, MK44, was constructed by mating MK39 with MK28. This strain is homozygous for TyCla but heterozygous for TyUra. A summary of the yeast strains used in this study is given in Table 1.

Measurements of meiotic recombination. Individual diploid colonies were grown at 32°C in YPA medium to a concentration of about 10^7 cells per ml, then washed, and suspended in SM medium. After 3 days of incubation at 23°C, asci were treated with a reducing agent and glusulase and sonicated, as described previously (24). This treatment kills all the nonsporulated diploid cells and disrupts asci, leaving a population of free spores. Since preliminary experiments indicated that Ura^- spores derived from a Ura^+ diploid would not germinate if plated on 5-FOA plates directly, the spores were allowed to grow for 6 h in rich (YPD) medium before plating on 5-FOA plates and SD complete plates. During this incubation, all of the spores germinated and budded once, and about 10% of the spores mated. Reconstruction experiments with Ura^- and Ura^+ haploids showed that we could accurately measure the number of Ura^- cells in a mixed population by this technique. The frequency of 5-FOA-resistant cells was estimated by scoring colonies on the 5-FOA plates after 7 days of incubation at 32°C. From each individual plate, we analyzed DNA from only one colony of each mating type to ensure their independent origin.

Measurement of mitotic recombination. The rate of mitotic recombination between Ty elements was measured by fluctuation tests. In different experiments, 10 to 30 colonies of equal size grown on YPD plates (47) were transferred to Eppendorf tubes containing 200 μl of sterile water. From these tubes, samples were either plated directly to 5-FOA

TABLE 1. Yeast strains

Strain	Description
DBY931	**a** *ura3-50 leu2 his4 met8-1 can1-1*, a gift from D. Botstein
MK28	**a** *ura3-50 his4 met8-1 can1-1* TyCla replacing Ty1-17, obtained by transforming DBY931 with pM1
MK31	α *ura3-50 his4 met8-1 can1-1* TyCla replacing Ty1-17, mating type-switched derivative of MK28
MK39	α *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::TyUra[a], obtained by transforming MK31 with pM28
MK40	**a** *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::TyUra, obtained by transforming MK28 with pM28
MK42	Diploid formed by mating MK39 with MK40
MK44	Diploid formed by mating MK28 with MK39
MK50	**a** *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::1.1-kb *URA3*, obtained by transforming MK28 with pM35
MK52	α *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::1.1-kb *URA3*, obtained by transforming MK31 with pM35
MK54	Diploid formed by mating MK50 with MK52
MK58	**a** *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::5.5-kb *URA3*, obtained by transforming MK28 with pM38
MK59	α *ura3-50 his4 met8-1 can1-1* TyCla *lys2*::5.5-kb *URA3*, obtained by transforming MK31 with pM38
MK60	Diploid formed by crossing MK58 and MK59
MK68	Diploid obtained by transforming MK54 with pM46; homozygous for the *lys*::1.1-kb *URA3*, fragment of *LYS2* integrated at *HIS4*

[a] The designation *lys2*::TyUra indicates that a *lys2* mutation was generated by insertion of TyUra into the *LYS2* gene.

plates or diluted and plated on YPD (in order to measure total cell number). Colonies were counted after 5 days. Rates of recombination were calculated using the median method of Lea and Coulson (29). Only one colony from each plate was used for DNA analysis.

Physical analysis of yeast DNA. Yeast DNA was isolated from 5 ml of stationary-phase cultures of yeast (47). Following gel electrophoresis of DNA samples treated with various restriction enzymes, the fragments were transferred to Hybond membranes (Amersham Corp.) and hybridized to a ^{32}P-labeled YIp333 DNA (which has *LYS2* and pBR322 sequences).

RESULTS

Construction of strains and experimental rationale. The recombinant plasmids and yeast strains used in our study are shown in Fig. 1 and 2 and Table 1 and are described in the Materials and Methods section. We detected meiotic recombination involving Ty elements by using the diploid strain MK42. This strain is homozygous for an insertion in the *LYS2* gene on chromosome II of a Ty element marked with a wild-type *URA3* gene (Fig. 2). This gene was inserted into a conserved *Bgl*II site near the end of the element, and we will refer to it as TyUra. This site was chosen because it is outside the open reading frames of the Ty and it is near the end of the transcript. Since the *URA3* insertion contains transcription terminators (42), transcription in this marked element may terminate before the delta element (the normal termination site). These marked Ty elements were derived from a Ty element (Ty1-17) located near the *LEU2* gene on chromosome III, as described in Materials and Methods. The strain MK42 is also homozygous for an altered version

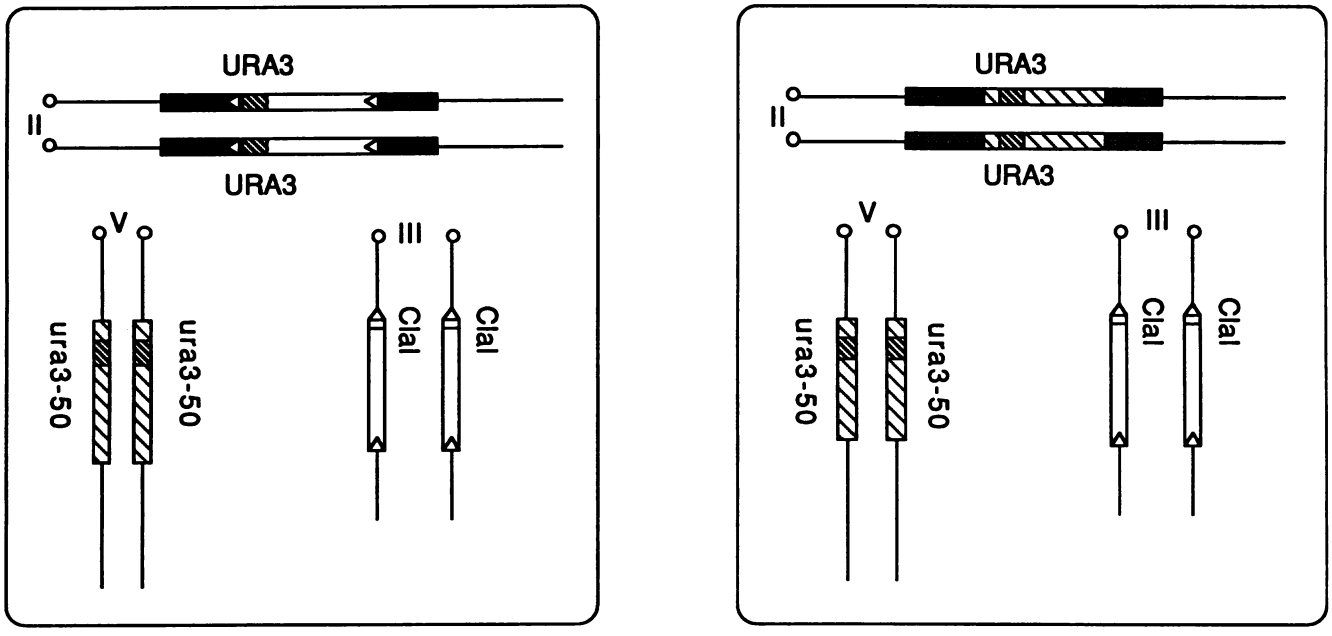

FIG. 2. Schematic representation of the relevant chromosomes of yeast strains. TyUra elements are on chromosome II, and TyCla elements are on chromosome III. Both strains carry 60 to 80 unmarked Ty elements randomly distributed in the genome. Symbols: ○, centromere; ▨, 5.5-kb fragment from chromosome V that contains the 1.1-kb *URA3* gene; ▨, 1.1-kb *URA3* gene; ■, *LYS2* sequences.

of the Ty1-17 element (TyCla) at its normal location; the *Bgl*II site normally present at the end of the Ty element was changed in vitro into a *Cla*I restriction site. In addition, MK42 is homozygous for a nonrevertible allele of the *URA3* gene, *ura3-50*, at its normal location on chromosome V. Lastly, MK42 contains the usual level of approximately 60 to 80 unmarked Ty elements (data not shown), most of which have a *Bgl*II site near the end (53).

We detected meiotic recombination events involving the Ty elements marked by the *URA3* gene insertion by sporulating MK42 and looking for Ura$^-$ spores. A similar approach was previously used to detect mitotic gene conversion events involving Ty elements (39). Spores with a Ura$^-$ phenotype can be selected by growth on medium containing 5-FOA (2). Several different types of genetic mechanism would be expected to result in a Ura$^-$ spore: (i) meiotic gene conversion between TyUra and any of the unmarked Ty elements in the genome, resulting in loss of the *URA3* insertion and insertion of a *Bgl*II site at the same position, (ii) gene conversion between TyUra and TyCla, resulting in deletion of *URA3* information and insertion of a *Cla*I site at the same position, (iii) gene conversion between the *URA3* in TyUra and *ura3-50*, resulting in a *ura3-50* insertion in TyUra, (iv) mutation of the *URA3* allele in TyUra to a new mutant *ura3* allele, and (v) reciprocal recombination of the delta elements flanking TyUra, resulting in a single delta insertion replacing the TyUra insertion. In the gene conversion events, since we select for loss of the *URA3* insertion, the TyUra gene is acting as a recipient for DNA sequences derived from other donor elements in the genome (Ty elements or the *URA3* gene).

All of these alternatives, except for alternatives iii and iv, can be distinguished by Southern analysis of DNA isolated from the Ura$^-$ spores by using a cloned fragment of *LYS2* DNA as a hybridization probe (Fig. 3). Although the patterns of Southern hybridization at the *LYS2* locus for alternatives iii and iv should be undistinguishable from that observed for a spore that has not undergone a recombination event, these alternatives could be distinguished genetically as described below.

Three further points should be made about the experimental system. First, the system was designed to detect meiotic gene conversion events involving the Ty elements that result in nonreciprocal loss of the *URA3* insertion; in *S. cerevisiae*, deletions and insertions have rates of meiotic gene conversion that are similar to the rates observed for conversion of point mutations (15). Second, the system was designed to determine whether gene conversion between Ty elements that have essentially the same DNA sequence (TyUra and TyCla) occurs more frequently than conversion between TyUra and a random Ty element. Previous studies have indicated that Ty elements are quite heterogeneous in DNA sequence (53) and, in principle, this sequence heterogeneity could prevent recombinational interactions. Third, since meiotic gene conversion events are often associated with reciprocal recombination of flanking DNA sequences (16), even when the interacting sequences are on nonhomologous chromosomes (24, 31), conversion events involving TyUra might also be associated with reciprocal recombination. We constructed the strain MK42, therefore, such that the TyUra, the TyCla, the *URA3* insertion in TyUra, and the *ura3-50* gene on chromosome V were all in the same orientation with respect to their centromeres. Thus, reciprocal exchanges associated with gene conversion events would be expected to produce reciprocal translocations (if nonhomologous chromosomes are involved), deletions, or inversions. Such chromosomal alterations should be detectable by patterns of Southern hybridization, viability patterns in genetic crosses (36) or by orthogonal-field alternation gel electrophoretic analysis of intact chromosomal DNA (5, 46).

In theory, the TyUra element in the strain MK42 can recombine with any of the other Ty elements in the genome. In order to estimate the frequency of recombination between non-Ty repeated elements in the same strain, we constructed the diploid MK60 (Fig. 2). This strain is isogenic with MK42, with one difference. In MK60, we inserted a 5.5-kb fragment, containing a wild-type *URA3* gene, into the *lys2* locus; this insertion was chosen for the control strain because it is approximately of the same size as TyUra. In this strain, therefore, the frequency of spores with the Ura$^-$ phenotype should reflect the frequency of meiotic gene conversion between the *URA3* gene inserted into *lys2* and the *ura3-50* gene on chromosome V. The *URA3* insertion in MK60 was in the same orientation as the *URA3* insertion in MK42.

Meiotic recombination between dispersed Ty elements. In order to determine the frequency of meiotic recombination between Ty elements, we caused the sporulation of strain MK42 (as well as the control strain MK60) and selected Ura$^-$ spores on plates containing 5-FOA. The frequency of Ura$^-$ spores in the strain MK42 (average of seven experiments) was 3.5×10^{-6} per spore (Table 2). The frequency of Ura$^-$ spores in the strain MK60 was 1.6×10^{-5} (Table 2). Although the *URA3* gene in MK60 should be capable of recombining with only one other sequence in the genome and the TyUra element in MK42 should be capable of recombining with 60 to 80 other Ty elements, the observed frequency of Ura$^-$ spores is about 4.5-fold less in MK42 than in MK60.

One possible explanation for the low frequency of Ura$^-$ spores in MK42 is that the ectopic gene conversion event involving TyUra and other Ty elements (removal of a 1.1-kb insertion) is mechanistically more difficult than conversion between the *URA3* genes in MK60, which does not involve conversion of a large heterologous sequence. In addition, the *URA3* insertion in MK42, although flanked by 5.6 kb of homology on one side, has only 350 bp of homology on the other. Although deletions and insertions have normal frequencies of allelic meiotic conversion (15), no study has examined the ectopic conversion of large insertions. In order to show that these factors were not responsible for the low frequency of conversion of TyUra in MK42, we constructed the strain MK68. This strain is homozygous for the insertion of a 1.1-kb *URA3* fragment in the *LYS2* gene. In addition, MK68 carries a copy of plasmid pM46 integrated at the *HIS4* gene on chromosome III. This plasmid (Fig. 1) carries a fragment of DNA with 3.9 kb of homology to the *LYS2* sequences to the right of the *URA3* insert and 180 bp of homology to its left. In this strain, Ura$^-$ derivatives are the result of ectopic gene conversion events that involve removal of the *URA3* insertion, analogous to those observed in MK42. The difference between the conversions in MK68 and MK42 is that only one donor of information is available (the *LYS2* sequences on chromosome III), and Ura$^-$ spores are the result of a conversion event involving non-Ty sequences.

Strain MK68 was sporulated and the frequency of Ura$^-$ spores was $1.04 \pm 0.09 \times 10^{-5}$. This frequency is three times higher than that of strain MK42 (3.5×10^{-6}) and comparable with that of strain MK60 (1.6×10^{-5}). Southern analysis indicated that most (30 of 35 strains examined) of the Ura$^-$ spores derived from MK68 were the result of removal of the *URA3* insertion (data not shown). We conclude that neither the size of the insertion nor its proximity to one end of the

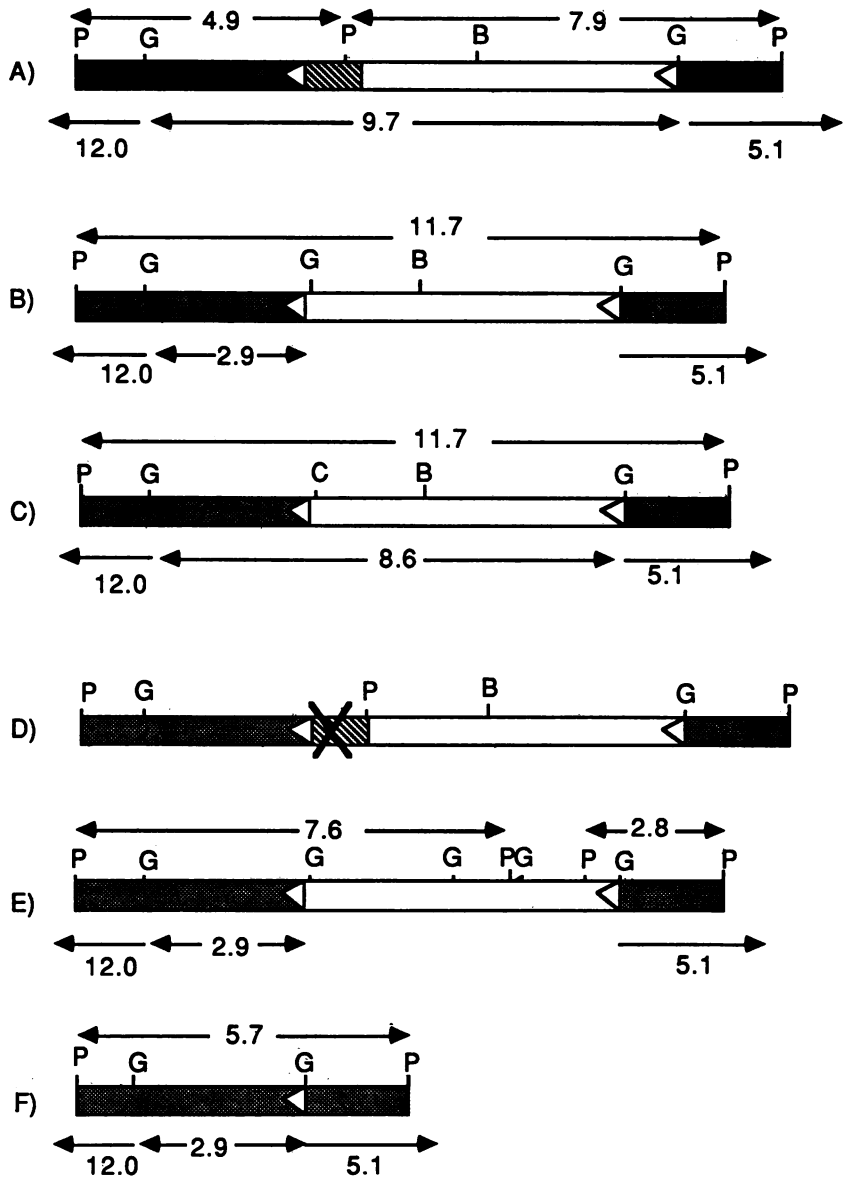

FIG. 3. Predicted restriction maps of the *LYS2* region of chromosome II resulting from various recombination events that affect the marked Ty element TyUra. For all maps, the sizes of the fragments are indicated in kilobases, and the abbreviations are as follows: P, *Pst*I; G, *Bgl*II; B, *Bam*HI. Other symbols are defined in the legends to Fig. 1 and 2. (A) Parental (Ura^+) restriction map; (B) map expected as the result of conversion of TyUra by an unmarked Ty2 element (class 1), resulting in deletion of the *URA3* gene; (C) map expected as the result of conversion of TyUra by TyCla (class 2); (D) map expected as the result of inactivation of *URA3* gene (×) in TyUra by either a new mutation or a gene conversion event involving the *ura3-50* allele on chromosome V (class 3); (E) map expected as the result of gene conversion of TyUra by an unmarked Ty1; (F) map expected as the result of recombination between the terminal delta elements of TyUra.

element is the main cause for the lower frequency of recombination found for TyUra.

A comparison between the results obtained with strains MK42, MK60, and MK68 suggests that meiotic recombination between Ty elements is suppressed by approximately 200-fold (3 × 70, MK68 and MK42) to 300-fold (4.5 × 70, MK60 and MK42). Caveats concerning this conclusion will be summarized in the Discussion.

We analyzed 144 independent Ura^- spores derived from MK42 by Southern analysis (using a cloned *LYS2* gene as a hybridization probe). The preliminary classification of the Ura^- spores was done using the enzymes *Pst*I and *Bgl*II. As expected from the restriction map shown in Fig. 3, we found that the DNA from the control Ura^+ spores hybridized to two *Pst*I fragments (4.9 and 7.9 kb) and three *Bgl*II fragments (5.1, 9.7, and 12 kb). On the basis of the *Pst*I and *Bgl*II hybridization patterns, most (95%) of the Ura^- spores could be classified into one of three classes (Fig. 3 and Table 3). The five Ura^- spores that could not be placed into one of these categories were put into class 4. The sizes of the *Pst*I and *Bgl*II fragments for members of class 4 are given in Table 3. The recombination events that resulted in these classes are described below.

Analysis of class 1 spores. Class 1 spores (103 of 144; frequency of 2.5×10^{-6}) had the restriction pattern expected as the result of a gene conversion event between TyUra and an unmarked Ty element, which resulted in a complete deletion of the *URA3* insertion (Fig. 3B). The diagnostic

TABLE 2. Meiotic frequency of Ura$^-$ spores

Strain and culture[a]	No. of Ura$^-$/10^6 spores[b]
MK42	
1	6.9
2	8.4
3	7.0
4	4.3
5	1.9
6	3.2
7	5.6
MK60	
1	20.5
2	33.2
3	21.2
4	14.9
5	11.5
6	25.3
7	11.4
MK54	
1	0.70
2	0.51
3	0.50
4	0.38
5	0.41
6	0.35
7	0.33

[a] Each independent meiotic culture was started from a single colony.
[b] The mean ± standard deviation (24) was 3.49 ± 0.65 for MK42, 16.16 ± 3.68 for MK60, and 0.43 ± 0.08 for MK54.

feature of this class of spore is the substitution of the *URA3* insertion in TyUra with a *Bgl*II site. Two other points concerning this class should be mentioned. First, the conversion events represented by class 1 spores were not associated with reciprocal recombination. If a gene conversion event occurred between TyUra and an unmarked Ty that was associated with a reciprocal exchange, after treatment of the DNA with *Pst*I, one would expect to see at least two bands of hybridization to a *LYS2*-specific probe instead of the single 11.7-kb band that was observed.

A second point is that the class 1 events either involve only a subset of the total Ty elements in the cell or involve only a small portion of the element. The Ty elements within the yeast genome can be divided into two classes, Ty1 and Ty2 elements (25). These elements have large regions of sequence homology interspersed with two regions of considerable sequence divergence (18). Although the *Bgl*II site near the end of the element into which the *URA3* gene is inserted in TyUra is conserved in both Ty1 and Ty2 elements (18), Ty1 elements usually have two additional *Bgl*II sites and two *Pst*I sites that Ty2 elements lack (Fig. 3E). In addition, Ty2 elements have a *Bam*HI restriction site that Ty1 elements lack. Ty1-17, from which both TyUra and TyCla are derived, is a Ty2 element. Southern analysis has shown that the strain MK42 has approximately 40 to 60 Ty1 elements and 24 to 28 Ty2 elements (data not shown). In principle, the Ty element that acts as a donor in the conversion event that deletes the *URA3* gene from TyUra could be either a Ty1 or Ty2 element. Since sites within a gene are often coconverted (17), a conversion event between TyUra and a Ty1 element could result in a hybrid element with sequences derived from both TyUra and Ty1. Class 1 spores do not represent such hybrid elements, since the Ty element in the *lys2* gene retains the *Bgl*II, *Pst*I, and *Bam*HI sites characteristic of a Ty2 element.

In addition to the sequence differences between Ty1 and Ty2 elements, sequence heterogeneity has been observed within each of these two subclasses (40). We, therefore, digested the DNA of class 1 spores with other enzymes (*Cla*I, *Hin*dIII, *Hpa*I, *Eco*RI, *Pvu*II, *Sac*I, *Xho*I, and *Spe*I). Two of the spores had a variant restriction pattern with all enzymes, except *Pst*I, *Bgl*II, and *Bam*HI; the differences were consistent with the pattern expected for a conversion event involving TyUra and a variant Ty2 (data not shown). In addition, seven of the spores lacked a *Sac*I site present in the original TyUra element, although all other restriction sites were conserved. In summary, most of the class 1 events represent conversions between TyUra and similar Ty2 elements. An alternative possibility is that class 1 events involve TyUra and a random selection of other Ty elements, but coconversion of the *URA3* gene and variant sites seldom occurs.

Analysis of class 2 spores. The second class of Ura$^-$ spores (8 of 144; frequency of 0.2×10^{-6}) had the restriction pattern expected as the result of a gene conversion event between TyUra (deleting the *URA3* insertion) and TyCla (Fig. 3C). The diagnostic feature of class 2 spores is the loss of the *URA3* insertion without insertion of a *Bgl*II site; the presence of a *Cla*I site at this position was confirmed in *Cla*I digests of DNA from all class 2 spores. This result clearly demonstrates that meiotic conversion events occur between Ty elements on nonhomologous chromosomes, since TyUra is on chromosome II and TyCla is on chromosome III. The low frequency of class 2 spores indicates that the observed low levels of recombination between nonallelic Ty elements is not solely the result of heterogeneity in the DNA sequence of different Ty elements, since TyUra and TyCla are identical in sequence except for the insertion at the *Bgl*II site (and the delta elements, which are 80% identical).

Analysis of class 3 spores. Class 3 Ura$^-$ spores (28 of 144; frequency of 0.7×10^{-6}) had the same restriction pattern as the original Ura$^+$ strain (Fig. 3A and D). Two types of genetic mechanisms would be expected to produce this pattern, a new mutation within the *URA3* insertion in TyUra and a gene conversion event between the *URA3* insertion in TyUra and the *ura3-50* mutant allele on chromosome V. Since these mechanisms cannot be readily distinguished physically, we did a genetic test on all class 3 spores. We treated each spore culture with UV light after plating them on medium lacking uracil. If the Ura$^-$ phenotype is the result of a new mutation, the UV light should stimulate mitotic gene conversion between the new mutant *ura3* gene and the *ura3-50* gene on chromosome V, resulting in Ura$^+$ papillae.

TABLE 3. Restriction fragment sizes for different classes of Ura$^-$ spores

Class	Restriction fragment size (kb)		No. of spores
	*Pst*I	*Bgl*II	
Ura$^+$ control	4.9, 7.9	5.1, 9.7, 12.0	
Class 1	11.7	2.9, 5.1, 12.0	103
Class 2	11.7	5.1, 8.6, 12.0	8
Class 3	4.9, 7.9	5.1, 9.7, 12.0	28
Class 4			
MK42-U$^-$(F10)	8.5	12.0, 12.0	1
MK42-U$^-$(F14)	8.5, 12.0	2.9, 5.1, 12.0	1
MK42-U$^-$(H14, H15)	8.0	4.8, 5.1, 12.0	2
MK42-U$^-$(G23)	9.0	3.7, 5.1, 12.0	1

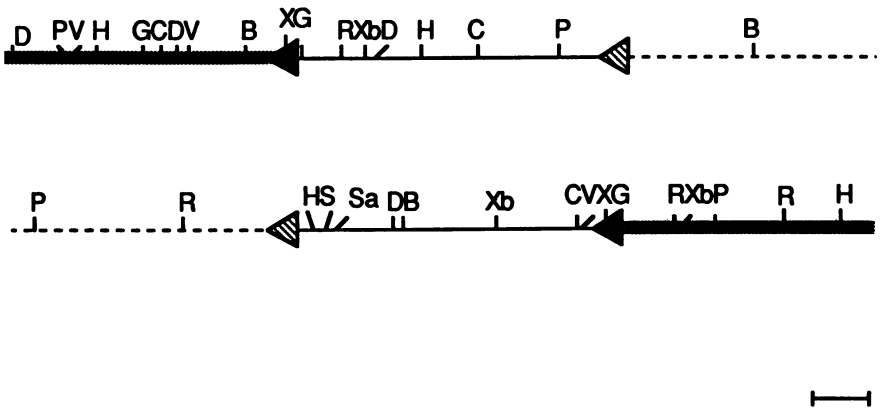

FIG. 4. Restriction map of the *LYS2* region of the Ura$^-$ strain MK42-U$^-$(F14). Sites for the different restriction endonucleases were deduced from Southern analysis, using the *LYS2* gene sequences (▬) as probe. Thus, only the first restriction site beyond the probe can be determined. The maps are consistent with the presence of Ty1 and Ty2 (——) elements adjacent to the *LYS2* sequences. Hypothetical delta elements (◀) and chromosomal DNA of unknown origin (-----) are indicated. The maps are consistent with the possibility of a reciprocal exchange event involving TyUra and a Ty1 element, in which the *URA3* gene was lost by gene conversion. Abbreviations: B, *Bam*HI; C, *Cla*I; D, *Hin*dIII; G, *Bgl*II; H, *Hpa*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Sa, *Sac*I; V, *Pvu*II; X, *Xho*I; Xb, *Xba*I.

Alternatively, if the Ura$^-$ phenotype is the result of meiotic gene conversion between the *URA3* insertion and the *ura3-50* allele (resulting in two *ura3-50* alleles), no papillae should be observed, since the *ura3-50* allele is not revertible to Ura$^+$. By this criterion, we found that 22 of the 28 class 3 spores were the result of a new mutation and 6 were presumably the result of gene conversion.

The frequency of gene conversion between *URA3* in TyUra and *ura3-50* in strain MK42 (1.5×10^{-7}) was much lower than that observed between *URA3* and *ura3-50* in strain MK60 (1.6×10^{-5}). Three possible explanations for this difference are (i) the size of the *URA3* repeat (1.1 kb in MK42 and 5.5 kb in MK60), (ii) differences in DNA sequences (the *URA3* gene in MK42 has about 10 base pair differences from the *ura3-50* gene [42], whereas the *URA3* gene in MK60 is likely to be identical to *ura3-50*, except at the position of the mutant substitution), and (iii) the *URA3* gene in MK42 is inserted within the Ty in the *lys2* gene, whereas the *URA3* gene in MK60 is located in the *lys2* gene without flanking Ty sequences. To distinguish among these alternatives, we constructed a diploid strain (MK54) that was isogenic with MK42 and MK60, except that the 1.1-kb *URA3* gene present in MK42 was inserted into the *lys2* gene instead of the 5.5-kb fragment (without any Ty sequences). We found that the frequency of Ura$^-$ spores derived from this strain was $4.3 \times 10^{-7} \pm 0.8 \times 10^{-7}$ (Table 2). A total of 51 of these Ura$^-$ strains were examined by the Ura$^+$ papillation test and 18 were found to contain a new *ura3* mutation. Thus, the frequency of meiotic gene conversion between the *ura3* genes in MK54 (2.8×10^{-7}) is similar to that observed in MK42. This result suggests that either the size of the repeat or the heterogeneity of the sequence of the interacting genes is responsible for the low frequency of recombination in MK42 and MK54 compared with MK60.

Analysis of class 4 spores. Class 4 spores (5 of 144; frequency of 0.1×10^{-6}) were those with DNA restriction patterns for *Pst*I and *Bgl*II that did not fit into the other three classes. Four different restriction patterns were represented among the five class 4 spores (Table 3). We expected that this class of spores might represent conversion events between Ty elements that were associated with reciprocal recombination resulting in either deletions, duplications, inversions, or translocations. Such recombination events should split the *lys2* gene into segments located in different chromosomal contexts, resulting in two genomic fragments hybridizing to a *LYS2*-specific probe for all enzymes analyzed. We found, however, that only one of the five class 4 spores [MK42-U$^-$(F14)] had this pattern of hybridization. The DNA of all other spores hybridized to a single fragment for some of the enzymes analyzed.

To confirm these results, we crossed all class 4 spores to a wild-type haploid strain and sporulated the resulting diploids. All such diploids had normal spore viability except the cross involving MK42-U$^-$(F14). In this cross, most tetrads contained either 4 live:0 dead, 2 live:2 dead, or 0 live:4 dead spores. This pattern is that expected for a heterozygous translocation or inversion (34, 36). Since we detected no alteration in the mobility of chromosome II (which contains the *lys2* gene) on orthogonal-field alternation electrophoretic gels in MK42-U$^-$(F14) and only chromosome II hybridized to the *LYS2* probe, it is most likely that the alteration in this strain is an inversion resulting from recombination between two Ty elements located on chromosome II in different orientations. The restriction maps derived from this strain (Fig. 4) indicate that the recombination event involved TyUra and a Ty1-class element. Previously, Chaleff and Fink (7) reported an inversion resulting from a mitotic interaction of Ty elements.

It is unclear what types of genetic events are responsible for the other four class 4 spores. Since preliminary analysis of their restriction patterns indicates that the *lys2* sequences flanking the Ty elements are not rearranged, these spores appear to result from recombination of TyUra with grossly altered Ty elements or internal substitutions of non-Ty DNA into TyUra.

We were surprised that none of the Ura$^-$ spores was the consequence of a reciprocal recombination between the delta elements flanking TyUra, since mitotic recombination between delta elements occurs readily (14, 32, 44, 54). There are two possible explanations for the lack of this recombinant class. First, it is possible that meiotic recombination requires more homology than that provided by the delta element (about 330 bp). Second, the sequence differences heterology existing between the delta elements of TyUra may have prevented recombination. The construction of TyUra (described in the Materials and Methods section)

TABLE 4. Analysis of Ura⁻ spores derived from independent cultures

Class	No. of spores in culture[a]:						Total (% of total)
	1	2	3	4	5	6	
1	8	18	27	23	24	3	103 (71.5)
2	0	0	1	0	7	0	8 (5.6)
3A[b]	2	2	5	3	10	0	22 (15.3)
3B[c]	1	1	1	2	0	1	6 (4.2)
4	0	0	2	0	1	2	5 (3.5)

[a] Each independent meiotic culture was started from a single colony.
[b] Mutation of *URA3* in TyUra.
[c] Conversion of the *URA3* gene in TyUra by *ura3-50* on chromosome V.

resulted in hybrid delta elements derived from two different Ty elements (Ty1-17 and TyD15) that share about 80% homology. Since we have detected meiotic recombination between delta elements at other chromosomal sites in which these elements share 100% homology (M. Kupiec and T. Petes, unpublished data), the latter explanation is more likely.

Frequency of mitotic recombination between Ty elements. Since the frequency of Ura⁻ spores derived from MK42 was so low, it is necessary to show that the Ura⁻ phenotype is the result of meiotic recombination, rather than a mitotic event that occurred in the culture prior to sporulation. One argument that most of the Ura⁻ spores are not the result of mitotic recombination is based on an analysis of the frequency of Ura⁻ spores derived from independent mitotic cultures of MK42. We found (Table 4) that a chi-square test of homogeneity indicated no significant difference among the different classes in six cultures examined (chi-square value of 19.7, with 12 degrees of freedom). Since a mitotic recombination event can occur at any time during growth of a culture, the number of Ura⁻ spores would be expected to differ significantly in independent cultures if these spores reflected a mitotic recombination event.

We could not directly measure the mitotic frequency of Ura⁻ cells in MK42, since this strain contains two wild-type copies of the *URA3* gene and inactivation of only one would not make the cells Ura⁻. We, therefore, constructed a diploid strain (MK44) that was isogenic to MK42 but contained only a single insertion of TyUra at the *lys2* locus. Mitotic events that produced Ura⁻ diploid derivatives of MK44 were selected by plating the cells on 5-FOA. We calculated (29) the mitotic rate of formation of Ura⁻ cells to be about $2.4 \times 10^{-5} \pm 0.7 \times 10^{-5}$ based on an analysis of 20 independent colonies.

In the strain MK44, the Ura⁻ phenotype could be produced by a number of different genetic mechanisms. Since the *LYS2* gene is not closely linked to the centromere of chromosome II, mitotic crossing over between the *lys2* gene containing the TyUra insertion and the centromere would be expected to generate Ura⁻ cells that were homozygous for the wild-type allele of *LYS2* (Fig. 5). Alternatively, Ura⁻ cells could result from mitotic gene conversion between the TyUra and other Ty elements in the genome or between the *URA3* gene in TyUra and *ura3-50* on chromosome V. Ura⁻ cells resulting from either of these two types of gene conversion would be expected to have one copy of the wild-type *LYS2* gene and one mutant *lys2* allele (Fig. 5). Diploid cells containing one copy of a wild-type *LYS2* allele can be distinguished from those which have two copies by plating the cells on medium containing α-aminoadipate. Cells that contain a wild-type *LYS2* allele do not grow in these conditions (8). Cells with a single wild-type allele, however, readily papillate on aminoadipate plates (whereas those cells with two wild-type alleles do not). Therefore, we examined about 150 Ura⁻ colonies from each of 10 independent cultures of MK44 for their ability to papillate on aminoadipate plates. Since none of these 1,500 colonies papillated, we conclude that most of the Ura⁻ cells formed by mitotic events were the result of mitotic crossing over, rather than Ty-Ty conversion. On the basis of these data, we estimate

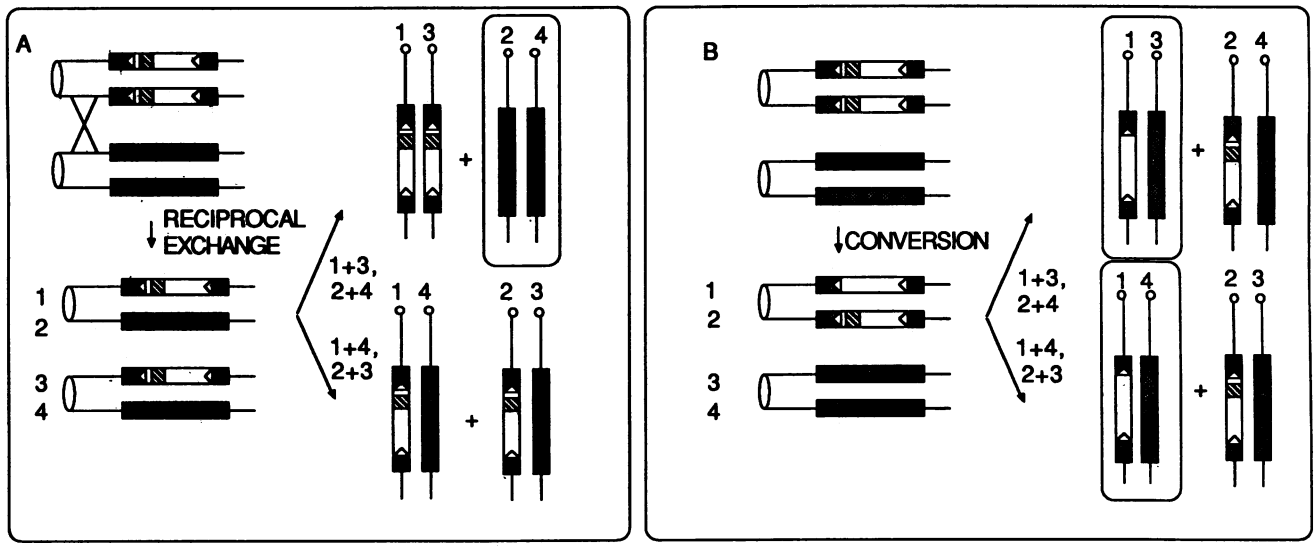

FIG. 5. Mitotic recombination in strain MK44 leading to Ura⁻ Lys⁺ cells. In the diploid strain MK44 (which is heterozygous for the TyUra insertion in *LYS2*), the two following types of mitotic exchange can result in Ura⁻ colonies. (A) A reciprocal exchange between *LYS2* and the centromere will produce, in half of the cases, a Ura⁻ cell (circled). These cells are Lys⁺ and will not papillate on α-aminoadipate plates, since they are homozygous for the wild-type *LYS2* gene and only cells that are homozygous for mutant *lys2* alleles can grow on such plates. (B) A mitotic gene conversion event that deletes or inactivates the *URA3* gene from TyUra (as illustrated in Fig. 3) will also produce Ura⁻ Lys⁺ cells (circled). Since these cells should be heterozygous for the mutant *lys2* gene, strains derived from this type of event can form papillations on α-aminoadipate plates; such papillations are the result of secondary mitotic recombination events that produce Lys⁻ cells.

(with 95% confidence limits calculated by a method developed by M. Stein [personal communication]) that the upper limit to the mitotic frequency of Ty-Ty conversion is about 1.25×10^{-7}. Since this estimate is about 1 order of magnitude less than the meiotic frequency of Ura$^-$ spores, we conclude that most of these spores reflect meiotic recombination events.

DISCUSSION

Most meiotic recombination studies involve heterozygous single-copy genes located at comparable positions on homologous chromosomes (alleles). In *S. cerevisiae*, it is clear that meiotic recombination events between regions of sequence homology at nonallelic positions also occur (ectopic events); ectopic events have also been detected in *Drosophila melanogaster* (10, 21). Most studies (for example, reference 24) have been done using yeast strains containing artificially duplicated genes. In this paper, we examined meiotic recombination between the naturally occurring repeated Ty elements in *S. cerevisiae*. Our conclusions can be summarized as follows. (i) Ty elements located at nonallelic chromosomal loci recombine in meiosis; these recombination events are usually nonreciprocal (gene conversions) and sometimes involve nonhomologous chromosomes. (ii) The frequency of gene conversion among Ty elements is less than expected on the basis of previous studies of recombination between artifically constructed repeats. (iii) These gene conversion events are only rarely associated with reciprocal exchange of flanking DNA. (iv) The conversion events involve either a small subset of the total Ty elements in the genome or the conversion tract is restricted to a small region of the Ty element. Each of these conclusions will be discussed in detail below.

Most previous studies of recombination among Ty elements have been done in mitotically dividing cells (4, 7, 32, 39, 41). In these analyses, both gene conversion events and reciprocal exchanges between Ty elements have been detected. Not much is known, however, about the level of recombination between Ty elements during meiosis. Roeder (38) found a high frequency of unequal meiotic crossing over between two different Ty elements on chromosome III. We find that meiotic gene conversion events involving nonallelic Ty elements also occur. At least some of these conversion events involve Ty elements that are located on nonhomologous chromosomes. Thus, the Ty elements within the genome represent a pool of sequences that recombine. These interactions are likely to be an important factor influencing the sequence of Ty elements in the genome, since repeated cycles of gene conversion within a family of repeated sequences would be expected to maintain sequence homogeneity among different family members (11).

In previous studies of meiotic conversion between duplications of selectable genes, we and others found that gene conversion between repeats on nonhomologous chromosomes occurred at about the same frequency as conversion between allelic sequences (23, 24, 31). In this study, the frequency of meiotic gene conversion involving TyUra and other Ty elements was about 2.7×10^{-6}, which is considerably less than the frequency of conversion for allelic loci (0.63 to 18.2%, Table 6 of reference 17). This frequency was also low relative to that observed between a single pair of nonallelic *ura3* or *lys2* genes in isogenic strains (1×10^{-5} to 1.6×10^{-5}), even though TyUra could potentially interact with 60 to 80 other Ty elements. Although we cannot exclude the possibility that sequence heterogeneity between nonallelic Ty elements lowers the frequency of meiotic recombination, this explanation is not sufficient to explain our results, since TyUra and TyCla (which share almost perfect homology) also convert infrequently. The *LYS2* fragment integrated at *HIS4* in chromosome III, present in only one copy, converted at a frequency of 10^{-5}, while TyCla, also in chromosome III, converted at a frequency of 2×10^{-7}.

There are a number of plausible explanations for the observed low levels of meiotic recombination between Ty elements. One possibility is that the yeast cell has a *trans*-acting system, analogous to that controlled by *EDR1* in mitotic cells (43), which specifically represses Ty-Ty recombination. It is likely that there would be a strong selective pressure for developing such a system, since in its absence many of the spores would contain haploid lethal chromosome aberrations. A higher rate of meiotic gene conversion of *LEU2* (a gene closely linked to Ty1-17) was found in diploid yeast strains heterozygous for a mutation in the *edr1* gene, compared with an isogenic wild-type strain (52). One interpretation of this result is that the wild-type *EDR1* gene represses meiotic recombination near delta elements.

If a *trans*-acting system repressing Ty-Ty meiotic exchange exists, there are two results that indicate the system may be specific for elements located on nonhomologous chromosomes or far apart within one chromosome. First, Roeder (38) found a high amount of unequal meiotic crossovers between two Ty elements on chromosome III. These crossovers occurred between Ty1-17 and a very similar element (Ty917) located about 21 kb away. Second, we have recently found that the frequency of allelic meiotic conversion between Ty elements, although somewhat lower than observed for most genes, is within the normal range (0.7%; Kupiec and Petes, unpublished observations).

A second possibility is that the Ty sequences (although not affected by a specific repression system) are not efficiently recognized by the enzymes that catalyze meiotic exchange; certain yeast sequences have been previously reported to be cold for exchange (28; L. Symington and T. Petes, unpublished observations); naturally occurring repeats (such as Ty elements) may have an altered sequence or structure that makes them less likely to recombine.

A third possibility is that the frequency of recombination of Ty elements is independent of the number of copies of the element in the genome. In our experiments, we measured the frequency of gene conversion events in which a single Ty element (TyUra) acted as a recipient of information derived from other Ty elements. If the event that initiates a gene conversion occurs within the recipient sequence and if this initiating event is always resolved as a recombination event, one might find that the frequency of recombination is independent of the number of copies of homologous sequences. Although we cannot exclude this possibility for meiotic recombination events, in experiments in which transforming DNA sequences mitotically recombine with genomic sequences, the frequency of such events is roughly proportional to the copy number of homologous genomic sequences (49).

A fourth explanation for the low frequency of Ty-Ty meiotic conversion is that this frequency varies with the chromosomal location of the element, and the particular location of TyUra on chromosome II gives low levels of recombination. Different frequencies of ectopic recombination at different positions in the genome have been previously observed (31). The results obtained with strain MK68,

however, show that the *LYS2* region is not cold for ectopic recombination.

Although it is unlikely that the low frequency of ectopic recombination observed in our experiments is the result of chromosomal location, the rate of ectopic recombination for Ty elements may be elevated by surrounding sequences, particularly, closely associated delta elements. For example, in experiments involving the insertion of a marked Ty on chromosome III, we found a higher level of ectopic meiotic recombination (about 10^{-5}); most of these ectopic events, however, were the result of intrachromosomal recombination between the numerous delta elements found in this location on the chromosome (Kupiec and Petes, unpublished observations). In addition, in these unpublished experiments, we found recombination events between the delta elements of the Ty. The lack of such events involving TyUra may reflect the lack of complete homology between the flanking delta elements.

Since approximately half of gene conversion events involving allelic loci are associated with reciprocal exchange of flanking DNA (17), it is surprising that only 1 of 112 conversion events involving Ty elements was associated with a reciprocal exchange. Part of this lack of associated exchange can be explained by the inviability of the spores receiving the expected products of a reciprocal recombination. If the interacting Ty elements are on the same chromosome and in the same orientation, a reciprocal exchange would produce a deletion, which could be haploid lethal. An intrachromosomal recombination event between Ty elements located in inverted orientation would result in an inversion, which would probably be haploid viable. If a reciprocal exchange occurs between two Ty elements that are on different chromosomes and are inverted in relation to their respective centromeres, one would expect to get either dicentric or acentric chromosomes, which are likely to be lost and lead to spore inviability. If a reciprocal exchange occurs between two Ty elements located in the same orientation on different chromosomes, a reciprocal translocation should occur. If the translocation products segregated randomly in meiosis, about one-quarter of the spores would receive the balanced translocation, and the remaining three-quarters would be inviable (24, 31); in an experiment involving meiotic recombination between duplicated genes on different chromosomes, of 94 spores that had a conversion, 15 contained the expected products of a reciprocal exchange (24). Similarly, 5 out of 30 conversion events analyzed between the *URA3* insertion at *LYS2* and the *LYS2* fragment in chromosome III in strain MK68 resulted in a translocation between chromosomes II and III. It is, therefore, unlikely that the low levels of reciprocal exchange seen in MK42 are due to the relative position or size of the *URA3* insertion in the Ty element, since the structures of the sequences involved in ectopic conversion events in strain MK68 are very similar to those in strain MK42. If we assume that most Ty-Ty interactions occur between nonhomologous chromosomes and half of these interactions occur between Ty elements located in the same orientation with respect to their centromeres, we calculate (using a contingency chi-square test, as well as Poisson 95% confidence intervals [35]) that gene conversion of Ty elements is at least twofold less often associated with reciprocal exchange than the conversion events between repeated genes used in our previous study (24). Alternatively, it is possible that the conversion events in our present study occur preferentially between a subset of Ty elements that are oriented such that the reciprocal recombination products are inviable (for example, directly oriented Ty elements within chromosome II). No translocations were associated with a conversion by TyCla (which is suitably oriented), but the numbers are too small to be conclusive.

The low frequency of Ty-Ty crossing over is consistent with the observed stability of the yeast karyotype. Although variants in chromosome sizes have been detected by orthogonal-field alternation electrophoretic gel analysis (5, 46), these changes do not usually involve chromosomal translocations. In addition, in many yeast strains, the spore viability is high (greater than 90%); this finding is not consistent with high rates of exchange between Ty elements. J. Boeke (personal communication) found that spore viability is high, even in yeast strains that have approximately twice the normal number of Ty elements.

The meiotic gene conversion events between Ty elements appeared nonrandom, since most of the conversion events involving TyUra (a Ty2 element) did not result in coconversion of restriction sites characteristic of Ty1 elements. The simplest interpretation of these results is that Ty2 elements preferentially recombine with other Ty2 elements. This result could indicate that nearly perfect sequence homology is required for high-frequency meiotic conversion. We cannot, however, rule out the alternative possibility that Ty elements recombine randomly but that coconversion of the adjacent sites is prevented by the sequence differences existing between the two classes of elements.

In summary, we found that Ty elements in the genome can recombine in meiosis with other Ty elements. Almost all of these recombination events represent gene conversions that are not associated with reciprocal exchange of flanking DNA. These conversion events usually do not generate hybrid Ty1-Ty2 elements. The frequency of Ty-Ty recombination is low relative to that expected from previous studies involving other repeated sequences.

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