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Ectopic Recombination between Ty Elements in *Saccharomyces cerevisiae* Is Not Induced by DNA Damage

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Mitotic recombination is increased when cells are treated with a variety of physical and chemical agents that cause damage to their DNA. We show here, using *Saccharomyces cerevisiae* strains that carry marked Ty elements, that recombination between members of this family of retrotransposons is not increased by UV irradiation or by treatment with the radiomimetic drug methyl methanesulfonate. Both ectopic recombination and mutation events were elevated by these agents for non-Ty sequences in the same strain. We discuss possible mechanisms that can prevent the induction of recombination between Ty elements.

Recombination between homologous sequences located at different positions in the genome (ectopic recombination) has been documented in *Saccharomyces cerevisiae* both in mitosis and in meiosis (reviewed in reference 27). Both reciprocal and nonreciprocal (gene conversion) events have been observed. When ectopic gene conversion between a pair of artificial repeats was selected for, an association with reciprocal exchange could be detected. This reciprocal exchange created reciprocal translocations or other chromosomal aberrations that could be easily monitored (16, 18, 19, 22).

Nonreciprocal recombination (gene conversion) events occur during vegetative growth at similar rates between sequences at different (ectopic) and identical (allelic) locations in the genome (19, 23). A sharp increase in the rate of both allelic and ectopic recombination is seen for artificial repeats upon entry to meiosis (16). It is possible that both types of recombination are under similar genetic control, since mutations known to affect allelic recombination seem to act in the same way for ectopic recombination (30, 40).

Mitotic recombination is increased by different kinds of radiation and chemicals that cause damage to the DNA (7, 13). It is believed that the induction of recombination is due to the creation of lesions in the DNA that are repaired by recombinational mechanisms. The dose-dependent induction usually seen reflects the increased level of lesions created (13).

Little is known about the rules that govern recombination between naturally occurring families of repeated sequences. These sequences represent a potential source of chromosomal instability, since reciprocal recombination events between members of a family will lead to chromosomal aberrations such as translocations, inversions, and deletions (16, 18).

The Ty elements are a family of dispersed retrotransposons that represent ~1 to 2% of the yeast genome. They are present in 30 to 40 copies per haploid genome and are related structurally and functionally to retroviruses (for a recent review, see reference 1). When ectopic meiotic recombination between Ty elements was monitored, the frequencies were found to be lower than those seen for other artificially constructed repeated sequences. In addition, only nonreciprocal events (gene conversions) were detected, in sharp contrast with the 10 to 50% association between crossing over and gene conversion seen for the artificial duplications (18).

Since Ty elements did not appear to respond in the same way as other sequences to the meiotic induction of recombination, we decided to check whether mitotic recombination between Tys can be induced by DNA damage. We present evidence showing that Ty recombination is not induced by UV light or methyl methanesulfonate (MMS) treatment.

MATERIALS AND METHODS

Media, growth conditions, and general procedures. Yeast cells were grown vegetatively at 32°C in either SD medium (0.67% yeast nitrogen base, 2% dextrose) with the appropriate nutrients added (35) or YPD (1% yeast extract, 2% Bacto Peptone, 2% dextrose). Bacto Agar (1.8%) was added for solid media. SD-Lys is SD with all nutrients added except for lysine. CAN medium is SD-complete without arginine, plus 40 μg of canavanine sulfate (Sigma) per ml. The adenine concentration in CAN plates was kept low (12 mg/liter) to allow proper red pigmentation of Ade^- colonies. Ura^- colonies were selected on SD-complete medium with 50 mg of uracil per liter and 0.85 mg of 5-fluoro-orotic acid per ml (4).

Standard molecular biology procedures such as cloning, restriction enzyme analysis, and Southern blot analysis were done as described by Sambrook et al. (34). Yeast molecular biology procedures (transformations, DNA preparations, etc.) were done as described in references 14 and 35.

Yeast strains. Strain AP1 was constructed by transforming strain CG731A ([MKPo]; [11]) *MATα ade2-1*-o *can1-100*-o *ura3-52 lys2-1 leu2-3,112 his3-Δ200 trp1-Δ901* (obtained from C. Giroux) with plasmid pM77. This plasmid carries a *URA3* marker and a *lys2*::Ty1Sup allele. The *lys2-1* allele was replaced by the *lys2*::Ty1Sup allele in a two-step procedure (45). AP2 is a *MATa* isogenic strain constructed by transforming strain AP1 with a *HO*-containing plasmid (YEpHO [15]). Strain AP5, carrying an ectopic copy of the *lys2*::*URA3* allele, was constructed by transforming AP1 with pAP5 to His^+ Ura^+. Strains MK95 and MK104 were

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created by transforming MK89 (*MAT***a** *ura3*-Nco$^-$ *his3-11,15 trp1*-Xba$^-$ *can1-101*) to Ura$^+$ Lys$^-$ with plasmids pM87 and pM115, respectively (24). These two strains differ at the 5′ long terminal repeat (LTR) of the Ty1Ura located at the *LYS2* locus. Strain MK49 (19) carries a marked Ty2Ura near the *LEU2* locus on chromosome III. After transformation, all the relevant chromosomal configurations were confirmed by Southern blot analysis.

Plasmids. pM77, carrying the *lys2*::Ty1Sup allele, was constructed by digesting plasmid FB103 (38) (a gift from F. Winston) with *Cla*I, filling in the ends with the Klenow fragment of DNA polymerase I, and inserting a 300-bp *Sal*I fragment from plasmid pJW221 (from R. Rothstein), which carries the *SUP4*-o gene (after filling in the ends). The *Cla*I site (position 3580 in the Ty; see reference 2) was destroyed, but the *Sal*I site was regenerated. The Ty1 present in FB103 was isolated from a spontaneous mutant of the *LYS2* gene (38). It had integrated between the *Bgl*II and *Eco*RI sites in the 5′ region of the *LYS2* gene. It is very similar in size and restriction map to the TyH3 element (2), except that it lacks the *Hin*dIII site and carries *Xho*I sites in both LTRs.

pAP5, carrying the *lys2*::*URA3* allele inserted next to the *HIS3* gene, was constructed in several steps. First, a 1.2-kb *Bam*HI fragment carrying the *URA3* gene (from pM21 [18]) was cloned in the unique *Hpa*I site of the *LYS2*-containing plasmid pDP6 (9) to give pM73. A *Sal*I fragment carrying the *lys2*::*URA3* construct was then inserted in the unique *Xho*I site close to the *HIS3* gene of plasmid pSR22, which contains the 1.7-kb *Bam*HI *HIS3*-containing fragment in pUC7 (from S. Jinks-Robertson).

pM115, carrying *lys2*::Ty1Ura, was constructed in several steps. First, the 1.2-kb *URA3* fragment was cloned into the 3′ *Bgl*II site of a GalTy (TyH3; [3]), thus creating plasmid pRJ2. The 5′ LTR was then reconstructed by replacing an *Xba*I-*Cla*I fragment containing the 5′ end of the Ty with the *Xba*I-*Cla*I fragment of another Ty (pGN821, from J. Boeke). Finally, a *Bam*HI fragment containing the whole Ty1Ura was inserted in the unique *Bgl*II site of the *LYS2* gene contained in plasmid pDP6 (9). The regenerated Ty1 element in pM115 is thus almost identical in sequence to TyH3 (2). For constructing plasmid pM87, the *Bam*HI fragment of pRJ2 containing the GalTy1Ura (see above) was inserted in the *Bgl*II site of pD6. Thus, in pM87, 238 bp at the 5′ LTR of the Ty1Ura are replaced by sequences from the *GAL1* promoter.

pM43 was used as a probe in Southern hybridization experiments. It carries a *Pvu*II fragment of the *LYS2* gene (18).

Measurement of Ty recombination. Recombination rates were measured by fluctuation test analysis. Briefly, 10 independent colonies of the same size grown on YPD were resuspended in sterile water and plated undiluted on CAN plates or on YPD plates after the appropriate dilutions were made. Colonies were scored after 3 days. Recombination rates were calculated by the method of the median (21).

UV irradiation. A fresh overnight culture was washed, resuspended in water at a concentration of 10^7 cells per ml, and irradiated with a germicidal UV lamp for different periods under constant stirring. The fluency used was 2 J/m^2/s. Aliquots were directly plated on SD-Lys or CAN plates or diluted and plated on YPD plates.

MMS treatment. A fresh overnight culture was washed and resuspended in water. MMS (Aldrich) was added to a final concentration of 0.3%, and the cells were incubated at room temperature with constant stirring. Aliquots were added to an equal volume of 10% sodium thiosulfate and plated on SD-Lys or CAN plates or diluted and plated on YPD plates.

Northern (RNA) blot analysis. RNA was extracted as described in reference 35, subjected to electrophoresis in formaldehyde-agarose (1%) gels, transferred to Hybond membranes (Amersham), and hybridized with random primed probes as recommended by the manufacturer. The 300-bp *Sal*I fragment containing the *SUP4*-o gene from plasmid pJW221, the 1.2-kb *Bam*HI fragment containing the *URA3* gene from pM21, and the 1.0-kb *Bgl*II fragment from pGN821 (from J. Boeke) internal to a Ty1 element were used as probes. For UV induction experiments, the cells were irradiated to give 50 to 70% survival and held for 1 h in liquid YPD in the dark before RNA was extracted.

After autoradiography, the intensities of the bands were determined with an LKB Ultroscan XL densitometer. At least two different exposures were measured for each blot.

RESULTS

Strain AP1 was constructed to monitor recombination events between Ty elements. This strain carries a Ty1 marked by the insertion of a 300-bp fragment containing the *SUP4*-o gene (designated Ty1Sup) at the *LYS2* locus. In addition, the strain carries the ochre-suppressible alleles *ade2-1*-o *can1-100*-o and thus is phenotypically Ade$^+$ Can^s (prototroph for adenine and sensitive to the arginine analog canavanine, respectively) because of the presence of Ty1Sup. A gene conversion event, in which Ty1Sup acts as a recipient of information, can replace Ty1Sup sequences by information from unmarked Tys, rendering the cell Ade$^-$ Can^r. This type of event can be selected for on canavanine-containing plates (Fig. 1). Canavanine-resistant cells can also be obtained as the result of a second mutation in the *CAN1* gene that cannot be suppressed by the *SUP4*-o gene. In this case, though, the strain will still be Ade$^+$ because of suppression of the *ade2-1*-o allele by *SUP4*-o at Ty1Sup. The Can^r Ade$^-$ yeast cells accumulate a red pigment and can be easily distinguished from white Can^r Ade$^+$ colonies (Fig. 1). Two other events can give rise to red Can^r cells, namely, a gene conversion event in which the *SUP4*-o allele in Ty1Sup is converted by the chromosomal *sup4* copy and a mutation in the *SUP4*-o gene. These events, though, are rare because of the small size of the *SUP4* gene and can be easily distinguished by Southern blot analysis.

The rate of spontaneous appearance of Can^r colonies was measured. Can^r Ade$^-$ colonies were formed at a rate of 3.4×10^{-6}, whereas Can^r Ade$^+$ colonies appeared at a rate of 3.9×10^{-7}. Thus, recombination events involving Ty1Sup in strain AP1 are 10-fold more frequent than mutations in the *CAN1* gene.

DNA from independent red Can^r colonies was subjected to Southern blot analysis (Fig. 2). Of 75 colonies analyzed, 45 (60%) consisted of events in which a solo delta element (LTR) replaced the whole Ty1Sup. This can be a result of an LTR-LTR reciprocal exchange, a gene conversion in which a solo LTR interacted with both LTRs of Ty1Sup, or an unequal sister chromatid event (33). Twenty-three of the Can^r Ade$^-$ colonies (30.7%) were the result of a Ty-Ty conversion event, and 7 colonies (9.3%) were the result of either a mutation or a conversion event in the *SUP4*-o fragment (we did not try to distinguish between these two possibilities).

The meiotic rate of recombination involving the marked Ty1Sup was measured in a diploid strain isogenic to AP1.

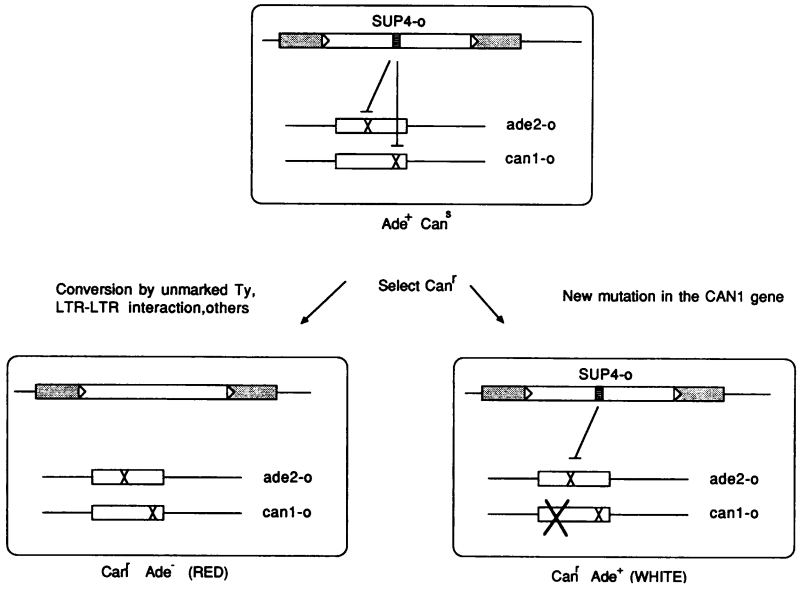

FIG. 1. Strategy for monitoring Ty ectopic recombination. A *SUP4*-o-marked Ty inserted at the *LYS2* locus (stippled box) suppresses ochre nonsense mutations at the *ADE2* and *CAN1* loci, rendering the cells Ade$^+$ (white) and Can^s. Two types of Can^r colonies can be selected for: those created by an event that removes the *SUP4*-o insert, giving rise to red Can^r colonies, and new mutations at the *CAN1* locus, which are still white (Ade$^+$) because of the presence of the suppressor.

Only a 10-fold increase in the rate of appearance of Can^r Ade$^-$ colonies was seen (4.8×10^{-5}). This value is lower than the 2 to 4 orders of magnitude increase usually seen in allelic or ectopic recombination of non-Ty sequences upon entry to meiosis (16, 18, 25, 36, 37). Southern blot analysis of 62 independent meiotic events showed that only 38.7% of the events were of the type that leaves a solo LTR, and 59% were created by conversion with unmarked Tys. Thus, as seen before (19), the proportion of recombination events involving the whole Ty element increased in meiosis, while that of solo LTR-producing events decreased. This may reflect a requirement for more homology in meiosis, as opposed to mitosis.

Throughout this report, we will refer to all the recombination events that create Can^r Ade$^-$ colonies as ectopic events, since all of them involve pairing between sequences located at different locations in the genome. They include Ty-to-Ty conversions, LTR-to-Ty conversions, and *sup4*-to-

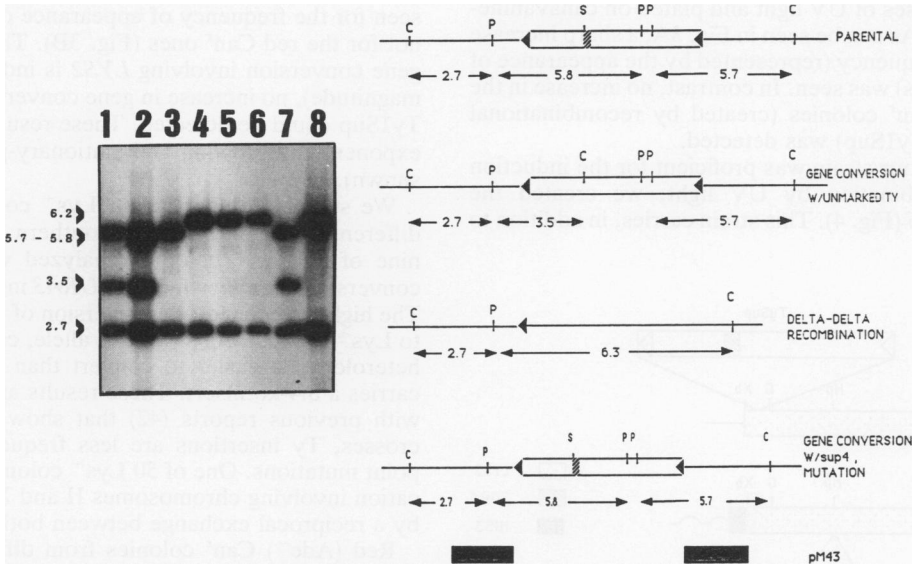

FIG. 2. Southern blot analysis of Can^r derivatives of strain AP1. DNA was extracted, cut with *Cla*I and *Pst*I, and, after electrophoresis and transfer, probed with *LYS2* sequences (pM43 [18]). Straight lines surrounding TySup are flanking *LYS2* sequences. *Cla*I (C), *Pst*I (P), and *Sal*I (S) sites are indicated. Lanes: 1, 2, and 7, conversion events by unmarked Ty elements; 3, mutation in the *SUP4* insert or conversion by the chromosomal *sup4* copy (parental pattern); 4, 5, 6, and 8, events that create a solo delta element (LTR). Numbers show size in kilobases.

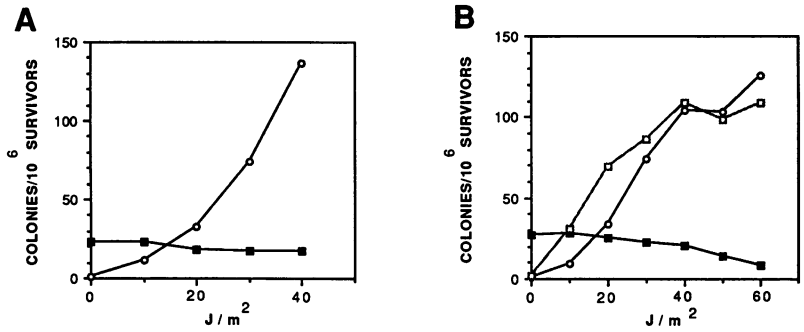

FIG. 3. (A) Induction of mutation (white Can^r colonies) and Ty1Sup recombination (red Can^r colonies) in strain AP1 by UV irradiation. Strain AP1 was irradiated for different periods and plated on CAN plates to score mutation and recombination or on YPD plates to score mutation and recombination or on YPD plates to score survival. Symbols: ○, white (Ade$^+$) Can^r colonies (mutations in the *CAN1* locus); ■, red (Ade$^-$) Can^r colonies (mainly recombination events involving Ty1Sup). The frequency of white Can^r and red Can^r colonies in the unirradiated sample was 0.519×10^{-6} and 22.8×10^{-6}, respectively. (B) Induction of mutation and recombination in strain AP5 by UV irradiation. Symbols: ○, white (Ade$^+$) Can^r colonies (mutations in the *CAN1* locus); ■, red (Ade$^-$) Can^r colonies (ectopic recombination events involving Ty1Sup); □, Lys$^+$ colonies (ectopic recombination events involving the *lys2::URA3* allele on chromosome XV and the *lys2*::Ty1Sup allele on chromosome II). Survival levels at 40 and 60 J/m^2 were 50 and 25%, respectively. No increase in the number of red Can^r cells per survivor was seen at higher doses (up to 0.1% survival). The frequency of white Can^r and red Can^r colonies in the unirradiated sample was 1.08×10^{-6} and 27.50×10^{-6}, respectively. The initial frequency of Lys$^+$ colonies was 1.97×10^{-6}.

SUP4-o conversions, as well as a minority of mutations in the *SUP4*-o locus.

Induction of recombination by UV light. Allelic mitotic recombination can be induced by a variety of treatments that cause damage to DNA (7, 10, 13). The induction can be as high as that seen in meiosis (25, 32, 44, 46). Experiments in our laboratory have shown that gene conversion between homologous sequences located at different positions in the genome (ectopic gene conversion) is induced by UV light in a similar way to allelic gene conversion (17a; also see below). Since recombination between Ty elements seems to follow different rules from those seen for other sequences (18, 24), we decided to check whether Ty-Ty recombination could be induced by UV irradiation.

Strain AP1, containing a marked Ty1Sup, was irradiated with increasing doses of UV light and plated on canavanine-containing plates. As can be seen in Fig. 3A, a sharp increase in the mutation frequency (represented by the appearance of white Can^r colonies) was seen. In contrast, no increase in the number of red Can^r colonies (created by recombinational events involving Ty1Sup) was detected.

To be sure that our strain was proficient for the induction of ectopic recombination by UV light, we created the isogenic strain AP5 (Fig. 4). This strain carries, in addition to the Ty1Sup at the *LYS2* locus, a second copy of the *LYS2* gene disrupted by the presence of a 1.2-kb fragment containing the *URA3* gene. This *lys2::URA3* element was inserted at the *HIS3* locus on chromosome XV. Since both copies of the *LYS2* gene present in strain AP5 are not functional, because of insertions (*lys2*::Ty1Sup on chromosome II and *lys2::URA3* on chromosome XV), the strain is phenotypically Lys$^-$. Ectopic recombination events that will restore a functional copy of the gene can be selected for on plates lacking lysine. Thus, by plating cells on CAN and SD-Lys plates after UV treatment, we can monitor both types of ectopic recombination events in the same cell population.

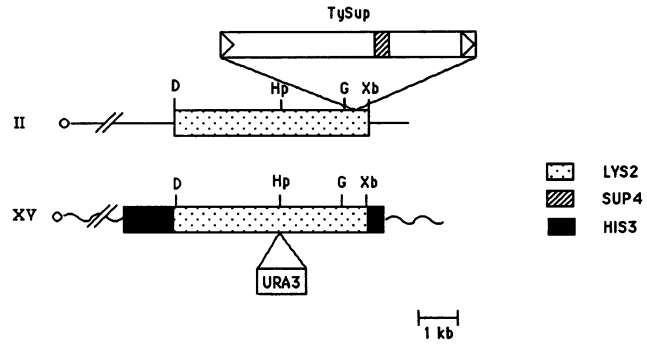

FIG. 4. Schematic representation of the relevant features of strain AP5. D, *Hin*dIII; Hp, *Hpa*I; 6, *Bgl*II; Xb, *Xba*I.

When strain AP5 was subjected to UV irradiation, an increase in the mutation frequency at the *CAN1* locus (white Can^r colonies) was seen, as with AP1. A similar increase was seen for the frequency of appearance of Lys$^+$ colonies, but not for the red Can^r ones (Fig. 3B). Thus, although ectopic gene conversion involving *LYS2* is induced (by 2 orders of magnitude), no increase in gene conversion events involving Ty1Sup could be detected. These results were seen in both exponentially growing and stationary-phase cells (data not shown).

We subjected independent Lys$^+$ colonies obtained after different radiation doses to Southern blot analysis. Forty-nine of 50 Lys$^+$ colonies analyzed were the result of a conversion event in which the *URA3* insertion was removed. The higher frequency of conversion of the *lys2::URA3* allele to Lys$^+$ is expected, since this allele, carrying only 1.2 kb of heterology, is easier to convert than *lys2*::Ty1Sup, which carries a 6.4-kb insert. These results are also in accordance with previous reports (42) that show that in heteroallelic crosses, Ty insertions are less frequently converted than point mutations. One of 50 Lys$^+$ colonies showed a translocation involving chromosomes II and XV, probably created by a reciprocal exchange between both *lys2* copies.

Red (Ade$^-$) Can^r colonies from different time points of irradiation were subjected to Southern blot analysis (Table 1). No difference was seen in the distribution of the two main classes of events in the different time points. Thus, neither the rate nor the type of recombinational event is changed after UV irradiation in strain AP5.

TABLE 1. Type of events that create red Can^r colonies in strain AP5

UV (J/m^2)	Event		
	Conversion by Ty	Solo LTR	Total
Expt 1			
No UV	6	10	16
50	3	9	12
100	2	5	7
Expt 2			
No UV	8	2	10
40	8	3	11
80	8	2	10

Induction of recombination by MMS. To test whether the lack of induction is particular to UV irradiation, we asked whether recombination and mutation in AP5 can be induced by the radiomimetic chemical MMS. MMS has been shown to cause an induction of both mutation and recombination rates (20, 29). Results can be seen in Fig. 5. MMS treatment has the same effect as UV treatment; namely, there is an induction of mutations in the *CAN1* locus and ectopic recombination between the *lys2* repeats, but not recombination between Ty1Sup and other Tys. Southern blot analysis of red Can^r colonies from different time points showed no difference in the distribution of the type of events detected (data not shown).

Experiments with Tys differently marked or differently located in the genome. To rule out the possibility that the lack of induction seen was related to the specific system used to monitor Ty recombination, we repeated the experiments described above with strains MK104 and MK95. These strains have the nonrevertible *ura3*-Nco$^-$ allele at the *URA3* locus on chromosome V, but are phenotypically Ura$^+$ because of the insertion of a Ty1Ura element at the *LYS2* locus. Ty1Ura is a Ty1 element marked by the insertion of a 1.2-kb fragment that carries the *URA3* gene. A conversion event between Ty1Ura and an unmarked Ty replaces the

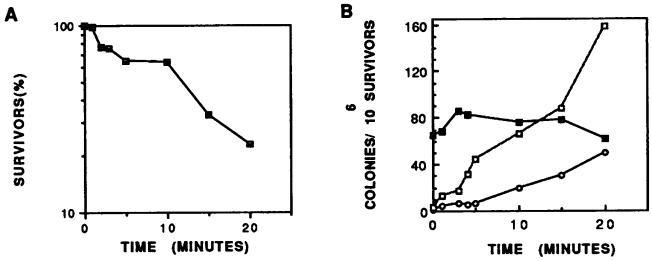

FIG. 5. Induction of mutation and recombination in strain AP5 by MMS. Strain AP5 was incubated in the presence of MMS for different periods and plated on CAN plates to score mutation and Ty recombination, on SD-Lys to score ectopic non-Ty recombination, or on YPD plates to score survival. (A) Survival curve. (B) Induction of mutation and recombination in strain AP5 by MMS. Symbols: ○, white (Ade$^+$) Can^r colonies (mutations in the *CAN1* locus); ■, red (Ade$^-$) Can^r colonies (ectopic recombination events involving Ty1Sup); □, Lys$^+$ colonies (ectopic recombination events involving the *lys2*::*URA3* allele on chromosome XV and the *lys2*::Ty1Sup allele on chromosome II). The frequency of white Can^r and red Can^r colonies in the untreated sample was 1.53×10^{-6} and 64.60×10^{-6}, respectively. The initial frequency of Lys$^+$ colonies was 2.01×10^{-6}.

TABLE 2. Type of events giving rise to Ura$^-$ colonies in strains carrying Ty1Ura elements at the *LYS2* locus

Strain and UV dose (J/m^2)	Event			
	Conversion by Ty	Solo LTR	Mutation at *URA3*	Total
MK104 (0)	1	16	0	17
MK104 (80)	0	15	0	15
MK95 (0)	14	7	4	25
MK95 (80)	8	2	4	14

URA3 information, giving rise to Ura$^-$ cells that can be selected for on 5-fluoro-orotic acid plates (18, 19). MK95 differs from MK104 in that part of the 5′ LTR has been replaced by a *GAL1* promoter. This has the effect of reducing homology with other LTR elements, which lowers the frequency of Ura$^-$ colonies because of interactions between the LTRs.

No increase in the frequency of Ura$^-$ colonies was detected upon treatment of MK104 or MK95 with either UV light or MMS (data not shown). The distribution of the different events leading to the Ura$^-$ phenotype in UV light-treated cells, as analyzed by Southern blots, is shown in Table 2. A contingency test showed no significant difference in the distribution of the different classes in irradiated versus unirradiated cells.

To ensure that Ty elements located in locations other than the *LYS2* locus behave similarly, strain MK49, which carries Ty2Ura on chromosome III, near the *LEU2* locus (19), was also subjected to UV treatment. No increase in the frequency of Ura$^-$ colonies could be seen (data not shown), also showing that Ty2 elements behave, with respect to UV irradiation, like Ty1 elements.

Since AP5, MK95, MK104, and MK49 have different genetic backgrounds and carry differently marked Ty elements located at different positions in the genome, we confirm our previous observations that mitotic recombination involving Ty elements is not induced upon treatment with DNA-damaging agents.

RNA analysis. UV irradiation has been shown to induce both the level of transcription of Tys (5, 31) and the frequency of transposition of these elements (5, 26). It has been assumed that the increase in transposition is the result of an increase in the level of Ty mRNA (and thus in the level of cDNA) after DNA damage. A direct relationship between transcription and recombination has been detected in both *S. cerevisiae* (17, 41, 43) and *Schizosaccharomyces pombe* (12). It could thus be possible that an increase in Ty transcription is a prerequisite for increased levels of recombination and that for some reason our marked Ty elements are not transcribed, or Ty transcription is not induced in our strains. Therefore, we decided to check the level of expression of the marked Tys used in these experiments, as well as that of the total Ty population.

Northern blot analysis was performed with total RNA isolated from untreated cells or from cells that had been irradiated with UV. As probes, we used a cloned Ty element (pGN821), which hybridizes to all the Ty RNA in the cell, and *SUP4*- or *URA3*-specific probes that enabled us to check the level of expression of the marked Ty elements. Results are shown in Fig. 6.

Two mRNA species hybridize to the *SUP4* probe with similar intensities (Fig. 6C; in addition, a very strong band

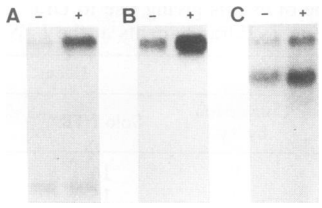

FIG. 6. Northern blot analysis. Equal amounts of RNA before (−) and after (+) UV irradiation were subjected to electrophoresis, blotting, and hybridization with the following probes: (A) total RNA of strain MK104 probed with *URA3*; (B) Total RNA of strain AP1 probed with a Ty-specific probe; (C) Total RNA of strain AP1 probed with *SUP4*-o.

the size of tRNA molecules was also seen, but is not shown here). The lower band has a size (~3.5 kb) that fits that of a premature termination of transcription at the *SUP4* insert, while the upper one correlates with a full-length Ty1Sup mRNA (~6 kb). The level of both transcripts increases three to four times upon UV treatment (Fig. 6C).

When the same blot was stripped of the *SUP4* probe and hybridized to a Ty probe, a sixfold induction in the level of total Ty mRNA was seen. The intensity of the Ty mRNA band is much stronger than that of the band specific for Ty1Sup, and that is the reason why the ~3.5-kb Ty1Sup transcript is not seen in Fig. 6B.

A similar result was obtained for Ty1Ura in strain MK104. A *URA3* probe hybridized with a small mRNA species (the *URA3* mRNA) and a large one, the size expected for the Ty1Ura message. While there is no increase in the level of the *URA3* mRNA after UV irradiation, a 14-fold increase in the level of Ty1Ura mRNA is seen (Fig. 6A). The increase in the steady-state Ty mRNA level seen is comparable or even higher than the one seen in previous experiments in which cells were exposed to DNA-damaging agents (5, 31). Thus, we can rule out the possibility that the level of induction of Ty recombination seen is due to improper transcription of the marked Ty elements.

DISCUSSION

We showed that recombination involving marked Ty elements cannot be induced by UV irradiation or MMS treatment. These treatments do induce ectopic recombination between two copies of the *LYS2* gene in the same strain. Thus, lack of inducibility is not a general property of ectopic recombination. In this respect, ectopic and allelic recombination are similar. We saw, after UV treatment, an induction of 2 to 3 orders of magnitude in the rates of ectopic recombination using different genes, different alleles, and different genomic locations (data not shown).

Lack of induction cannot be attributed to the type of event selected for (conversion of a small insertion), since in strain AP5 we selected for recombination events involving two alleles of *LYS2*, each with an insertion (*lys2*::*URA3* and *lys2*::Ty1Sup), and more than a 100-fold induction in the frequency of Lys^+ colonies was seen after UV irradiation or MMS treatment (Fig. 3 and 5). Similarly, it cannot be claimed that something is special about the location of the marked Tys (the *LYS2* gene) since the level of ectopic recombination involving the *LYS2* sequences was shown to be induced by DNA damage in the same strain, and Tys located on other chromosomes also fail to show induction. We conclude that Tys behave in a different way from other sequences with respect to induced mitotic recombination.

The level of total Ty mRNA has been shown to increase upon UV irradiation or chemical damage to DNA (5, 31). Ty transposition was also elevated by these treatments (5, 26). The simplest interpretation of these results is that after DNA damage there is an increase in Ty mRNA, leading to higher levels of Ty cDNA and Ty proteins, which in turn cause an increase in transposition. Recent experiments in our laboratory have shown that Ty cDNA is involved in gene conversion events with chromosomal copies of Tys (24). Ty1Ura-containing strains were transformed with plasmids carrying a differently marked Ty element (Ty1Neo) under the control of the *GAL* promoter. When these strains were grown in galactose, an increase in the rate of gene conversion was seen. This increase was due to an elevated level of recombination between Ty1Neo cDNA and the chromosomal Ty1Ura.

According to these results, we would expect that the increase in Ty mRNA seen upon DNA damage would cause an increase in recombination (similar to the increase in recombination seen upon expression of GalTy1Neo). This effect was not observed. The mRNA population present in the cell after DNA damage is derived from the total Ty population, which is mostly inactive in transposition and recombination (2, 5). In contrast, when a *GAL*-driven Ty is expressed, there is a large increase in the level of one specific mRNA species which is known to be competent for both processes. This difference may lead to an increase in recombination after induction of GalTy, but not after DNA-damaging treatments. Alternatively, the different results obtained by the two systems reflect a difference in the experimental conditions used: a short period of exposure to DNA-damaging agents versus several generations of growth under inducing conditions for GalTy1Neo.

Although we could not monitor transposition events in our system, an increase in transposition was seen by other investigators upon DNA damage under conditions similar to the ones used in the present study (5, 26). It is possible that this increase is not a direct consequence of the elevated level of Ty mRNA but is due to a different mechanism (6). It has recently been shown that in *rad6* strains the level of transposition is elevated, without a concomitant increase in Ty mRNA level (28). Thus, transposition induction is separable from Ty mRNA induction. It has also been pointed out (5) that DNA damage may stimulate transposition by altering one or more posttranscriptional steps, in addition to the increase in Ty mRNA levels. Although the mechanism is not known, our data suggest that the effect is different for transposition and for recombination.

Two basic types of models have been proposed to explain the induction of recombination seen after DNA damage. In one type of model, the damage causes lesions in the DNA, such as single- or double-strand breaks, that are similar to natural intermediates in the process of recombination. Thus, the damage bypasses the first steps of recombination, which seem to be the rate-limiting ones (13, 39). In the other type of model, the damage causes the appearance of some signal, which in turn causes the induction of the recombination activity. Evidence supporting both types of models has been obtained in yeasts (8, 39).

Recombination of Tys can take place with cDNA copies of these elements (24). It is possible that this process is not inducible by DNA damage, since it is driven by the presence of homologous double-stranded ends in the cDNA. Still, the lack of induction of recombination between the chromo-

somal Tys (in cells proficient for ectopic recombination of non-Ty sequences) remains to be explained. This lack of induction of recombination could be obtained if Ty DNA was protected from the physical damage, or if, after induction of the recombination enzymes in the cell, these enzymes could not act upon Ty sequences.

We noticed that the meiotic induction of ectopic recombination for Tys seems to be lower than that seen for other sequences (16, 18; this study). In studies in which meiotic allelic recombination between Ty elements was measured (19), it was found that the frequency of gene conversion of a marked Ty (0.74%) was lower than that of the adjacent *LEU2* gene (3.4%). The distance between the markers used was less than 1 kb. In the majority of the conversion events involving the marked Ty, a coconversion of the *LEU2* locus was seen. The level of ectopic recombination involving the marked Ty was 2 orders of magnitude lower than the allelic level.

These results are consistent with the possibility that initiation of the recombination events involving Tys occurs outside of the element. According to this hypothesis, only events initiated at the nearby *LEU2* gene that propagated toward the Ty could convert the markers at the Ty; the rate of allelic recombination between Tys is low because recombination is only rarely initiated inside the Ty sequences. The rate of ectopic recombination between Tys is even lower, because no homology in the sequences surrounding the Tys is usually found.

The absence of initiation at Tys could be explained if the enzymes participating in recombination could not act on Ty DNA. This could be due to special properties of the elements, such as sequence or chromatin configuration. It is also possible that Ty-specific proteins protect the elements from being reached. The same reasons may also explain the lack of induction of recombination after DNA damage. Ty DNA may be protected from damage by irradiation or chemicals and/or from the action of the repair enzymes of the cell. Alternatively, Ty sequences are damaged but either are not corrected or are corrected by a nonrecombinogenic mechanism. This last possibility would imply the existence of a Ty-specific repair mechanism in the cell. One possible mechanism of repair that would seem to be nonrecombinogenic is one in which the damage is always corrected by information present in the sister chromatid. According to this model, cells in the stationary stage, mostly in the G_1 stage of the cell cycle, could now be available for repair by ectopic templates, giving rise to red Can^r colonies. We were, however, unable to induce Ty recombination by UV irradiation in logarithmic- or stationary-phase cultures.

The data presented here show that Ty elements behave differently from other genes with respect to induced mitotic recombination and suggest the existence of Ty-specific mechanisms of recombination and repair. It is possible that these mechanisms have evolved to avoid reciprocal recombination events between members of families of repeated sequences that would create chromosomal rearrangements. Further studies are required to understand the rules governing these mechanisms.

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