Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12711–12715, December 1994
Genetics

Transcriptional induction of Ty recombination in yeast

(repeated sequences/ectopic gene conversion/DNA repair/*RAD* genes)

YAEL NEVO-CASPI AND MARTIN KUPIEC*

Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv 69978, Israel

Communicated by Roger D. Kornberg, September 6, 1994 (received for review February 18, 1994)

ABSTRACT **Families of repeated sequences are present in the genomes of all eukaryotes. Little is known about the mechanism(s) that prevents recombination between repeated sequences. In the yeast *Saccharomyces cerevisiae*, recombination between homologous sequences placed at nonhomologous locations in the genome (ectopic recombination) has been shown to occur at high frequencies for artificially created repeats, but at relatively low frequencies for a natural family of repeated sequences, the Ty family. We have previously shown that a high level of Ty cDNA in the cell causes an increase in the rate of nonreciprocal recombination (gene conversion) of a marked Ty element. In the present study, we show that it is also possible to elevate the rate of recombination of a marked Ty by increasing its transcription. This induction is different from, and acts synergistically to, the one seen upon increased levels of donor Ty cDNA. We show that the induction by transcription does not require the products of the *RAD50*, *RAD51*, and *RAD57* genes. In contrast, cDNA-mediated recombination is dependent on the product of the *RAD51* gene but not on products of the genes *RAD50* or *RAD57*.**

Ty retrotransposons constitute the main family of naturally occurring repeated sequences in the yeast genome, representing 1–2% of the genome (for review, see ref. 1). Ty elements are retrovirus-like transposons that code for an end-to-end transcript that is reverse-transcribed to cDNA by Ty-encoded proteins. They transpose at very low frequencies (10^{-9} to 10^{-7} per locus per cell division). The two main classes of Ty elements, Ty1 and Ty2, share extensive homology. There are ≈30–40 copies of Ty elements per haploid genome (1).

Recombination between homologous sequences located at nonhomologous positions in the genome (ectopic recombination) occurs readily between artificially duplicated genes, in mitotic and meiotic yeast cells. Both reciprocal and nonreciprocal recombination (gene conversion) have been observed. Ectopic reciprocal recombination causes chromosomal aberrations, such as translocations, deletions, and inversions (2). Since repetitive sequences are present in the genomes of all eukaryotes, the karyotypic stability might be maintained by (a) mechanism(s) that prevent(s) recombination between such repeats. We have been using the Ty family of retrotransposons as a model to study recombination between naturally occurring repeated sequences. The rate at which Ty elements engage in homologous ectopic recombination is relatively low and is mainly nonreciprocal in nature (3). Recent experiments in our laboratory have shown that Ty cDNA participates in gene conversion events involving a marked Ty element (4).

Transcription by DNA-dependent RNA polymerase I has been shown to cause an increase in recombination in yeast (5). Elevated levels of recombination between directly repeated *GAL10* genes have also been observed upon induction of transcription (6). Evidence for induced recombination upon transcription was also obtained in *Schizosaccharomyces pombe* (7) and in mammalian cells (8–11).

A close relationship between transcription and DNA repair has been found: Lesions in actively transcribed genes are repaired faster than those in inactive ones and the template strand of actively transcribed genes is preferentially repaired (12–15). In addition, it was also recently found that the DNA repair genes *RAD3*, *RAD25*, and *SSL1* encode proteins that play a role also in transcription (16, 17).

In this paper we present evidence that elevated transcription of a Ty element causes an increase in its rate of recombination, and we investigate the role of different *RAD* genes in this process.

MATERIALS AND METHODS

Yeast strains. All the *Saccharomyces cerevisiae* strains used in the present study are isogenic and were derived from strain MK89 (4) (*MAT***a** *ura3-Nco*$^-$ *his3-11,15 leu2-3,112 trp1-Xba*$^-$ *can1-101*) by transformation. They carry, at the *LYS2* locus, a Ty element marked by the insertion of a *URA3* gene (TyUra). Strains MK87 and MK104 have been described (4); MK87 carries a Ty2Ura, and MK104 carries a Ty1Ura. MK95, MK116, and YN2 were created by transforming MK89 with plasmids pM106, pM128, and pYN10, respectively. In MK95, the first 237 nt of the Ty1 have been replaced by 741 bp from the *GAL1* promoter (GalTy1Ura) (18); in MK116, a frameshift mutation was introduced in the GalTy1Ura by filling-in the *Asp*718 site of the Ty; in YN2, a linker containing amber stop mutations in all three frames was inserted at nt 475 of the Ty. YN3 carries a deletion of the Ty promoter and was created by transformation with plasmid pYN9. Strains MK172 (*rad50*::*TRP1*), UB1, (*rad51*::*LEU2*), and UB2 (*rad57*::*LEU2*) were created by a one-step replacement transformation (19) using plasmids pME305 (20), pAM28 (21), and pSM51 [a *Pvu* II–*Sal* I fragment of the *RAD57* gene replaced by the *LEU2* marker (22)], respectively. After transformation, all the relevant chromosomal configurations were confirmed by Southern blot analysis.

Plasmids. pM106, carrying the *lys2*::*GalTy1Ura* allele was constructed in several steps: First, the 1.2-kb *URA3* fragment was cloned into the 3′*Bgl* II site of a GalTy (18), thus creating plasmid pRJ2. Then the *Bam*HI fragment containing the whole GalTy1Ura was inserted in the unique *Bgl* II site of the *LYS2* gene in plasmid pDP6 (23). Plasmid pM128 was constructed by filling-in the *Asp*718 site in the Ty of pM106 with the Klenow fragment of *Escherichia coli* DNA polymerase I, followed by ligation; this process generates a *Sna*BI site in its place. pYN10 was constructed in several steps: First, a 6.2-kb *Bam*HI–*Cla* I fragment of pM106 was replaced by a *Bam*HI–*Cla* I 380-bp fragment of pBR322, thus creating pYN6. Then, a *Xho* I–*Asp*718 fragment of the Ty was replaced by a similar one from pGTy1-H3His3 (Tya-475Am)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: LTR, long terminal repeat.

*To whom reprint requests should be addressed.

(24) containing a triple amber linker insertion. Finally, the 6.2-kb *Bam*HI–*Cla* I fragment deleted in the first step was restored (instead of the pBR322 fragment), recreating a full-length Ty element. This plasmid was called pYN10. pYN9 was created by first deleting a *Nhe* I–*Xho* I fragment (containing the Ty promoter) from pYN6 and then reconstructing the whole Ty with the *Bam*HI–*Cla* I fragment, as described above. Plasmids pM97, pM102, and pM109 have been described (4). pYN8, carrying the same triple-amber linker as pYN10 was constructed by replacing the *Xho* I–*Cla* I fragment of pM97 with the corresponding fragment from plasmid BJC28 (24). pJef1678 (a gift from Jef Boeke, Johns Hopkins University, Baltimore) carries a GalTy1neo on a 2-μm plasmid-containing vector with a *HIS3* selectable marker. pM43 was used as a probe in Southern blot hybridization experiments; it carries a *Pvu* II fragment of the *LYS2* gene (25).

Media, Growth Conditions, and General Procedures. Standard molecular biology procedures such as cloning, restriction enzyme analysis, and Southern blot analysis were carried out as described in ref. 26. Yeast media and molecular biology procedures (transformations, DNA preparations, etc.) were carried out as in ref. 27. Ura$^-$ colonies were selected on SD complete medium with uracil (50 mg/liter) and 5-fluoroorotic acid (0.85 mg/ml) (28).

Measurement of Ty Recombination. Recombination rates were measured by fluctuation test analysis as described (4) and calculated by the method of the median (29). Twelve to 24 cultures grown in SD or SGal medium (4, 27) were used in each experiment. DNA was prepared from one Ura$^-$ Leu$^-$ colony (strains carrying pM97) or one Ura$^-$ His$^-$ colony (strains carrying pJef1678) from each culture and subjected to Southern blot analysis using *LYS2*, neomycin-resistance gene (neo), and *URA3* sequences as probes (4).

RESULTS

Increased transcription of a marked Ty (GalTy1neo), resulting in high levels of Ty cDNA, causes elevated rates of recombination of another differently marked Ty (TyUra), due to gene conversion events in which cDNA copies of the GalTy1neo act as donors of information (4). The level of conversion of the marked TyUra can be estimated by selecting for Ura$^-$ cells on 5-fluoroorotic acid medium (28).

To analyze the effect of transcription of the recipient molecule on the level of recombination, we constructed yeast strain MK95 (Fig. 1). This strain carries a Ty1Ura element on chromosome II in which the natural promoter has been replaced by the *GAL1* promoter (GalTy1Ura) (4). This Ty is not expressed in a glucose-containing medium, but high levels of transcription are seen in a medium containing galactose (results from Northern blot analysis not shown). As controls, isogenic strains with different TyUra elements were used: MK104, bearing a Ty1 with its natural promoter, and

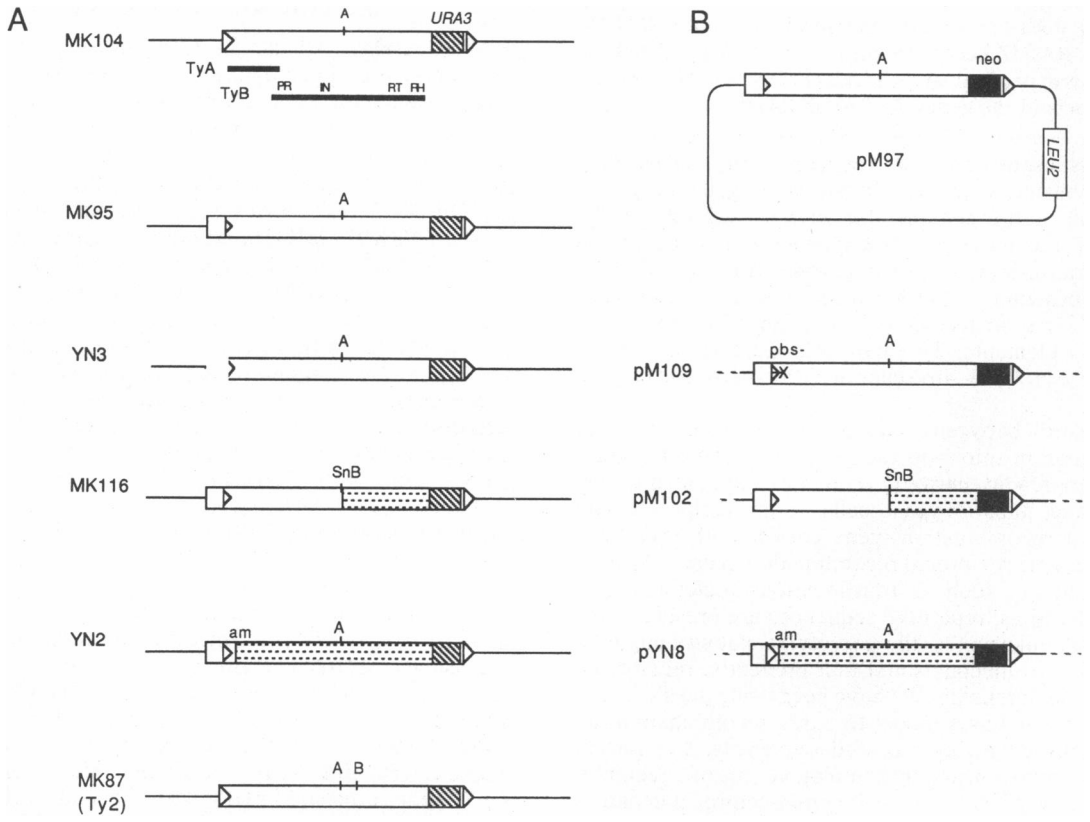

FIG. 1. Schematic representation of Ty elements used in the present study. Ty elements are depicted as open boxes bounded by open triangles (LTRs). (*A*) Chromosomal copies of recipient Ty elements. The two open reading frames TyA and TyB and the proteins produced from the Ty mRNA are shown. PR, protease; IN, integrase; RT, reverse transcriptase; RH, RNase H. The hatched box at the 3′ end of the Ty elements show the *URA3* insert. MK104 carries a wild-type Ty1Ura (4). In MK95, the first 237 nt of the Ty1 have been replaced by 741 bp from the *GAL1* promoter (18). In YN3, this fragment was deleted. The Ty1 in MK116 carries a frameshift mutation, created by filling-in the *Asp*718 site with the Klenow fragment of *Escherichia coli* DNA polymerase I; this process generates a *Sna*BI site in its place. In YN2, a 12-nt linker carrying amber stop codons (am) in all the three frames was inserted at nt 475 of the Ty (24). MK87 carries a Ty2 element (4). A, *Asp*718; B, *Bam*HI; SnB, *Sna*BI. Stippled boxes indicate inactive regions in the Ty. (*B*) Plasmid-borne donor Ty elements. pM97 (4) is a high copy number plasmid that carries a *GAL1*-promoted Ty1 and a *LEU2* marker. The solid box at the 3′ of the Ty represents the bacterial neomycin resistance (neo) gene. Three other plasmids were constructed, based on pM97. pM109 (4) carries substitutions in the primer binding site (pbs) of the Ty. pM102 (4) carries the same *Asp*718 filled-in site as MK116. pYN8 carries the same linker insertion as the Ty1Ura in strain YN2.

MK87, carrying a Ty2 (4) (Fig. 1). Strain MK104 produces high levels of Ura$^-$ colonies by a mechanism that replaces the whole Ty by a solo long terminal repeat (LTR) (4). There are several ways by which this result can be obtained: an LTR–LTR reciprocal exchange (intrachromatidal recombination), a conversion of the whole element by a solo LTR, an unequal sister chromatid exchange, or a single-strand annealing event between the direct repeats (30). To focus on ectopic conversion between Ty elements, we constructed strain YN3, in which part of the 5′ LTR is deleted (Fig. 1). This strain shows a lower basal level of Ura$^-$ colonies (Table 1), which are mainly the result of conversion events in which the TyUra information is replaced by information from another Ty element (3, 4). The level of appearance of Ura$^-$ colonies of YN3 and that of the other control strains is not changed when the cells are grown on a galactose-containing medium (Table 1). In contrast, the rate of appearance of Ura$^-$ colonies in MK95 cells grown on galactose is one order of magnitude higher than that of cells grown under transcription-repressing conditions (Table 1).

Independent Ura$^-$ colonies from induced and uninduced cultures of MK95 were subjected to Southern blot analysis to determine the type of event that had created the Ura$^-$ phenotype. *LYS2* sequences were used as a probe, since the Ty1Ura is located at the *LYS2* locus. In glucose-grown cells, 57% of the Ura$^-$ colonies (20 of 35 colonies) were the result of gene conversion of the GalTy1Ura by other Ty elements, and in 31% (11 of 35 colonies) the whole Ty had been replaced by a solo LTR. The remaining Ura$^-$ colonies (4 of 35 colonies) still carried the *URA3* insert at the Ty and were the result of mutations in the *URA3* gene of the Ty or ectopic conversion by the *ura3-Nco*$^-$ allele on chromosome V. Even though the rate of appearance of Ura$^-$ colonies was 20-fold higher in galactose-grown cells, the ratio between the different types of Ty recombination remained similar: 60% (15 of 25 colonies) conversions and 36% (9 of 25 colonies) solo LTR events.

When MK95 cells carrying a GalTy1neo on a high copy number plasmid (pM97) were grown on galactose, thus producing increased levels of Ty1neo cDNA, the rate of appearance of Ura$^-$ colonies increased by another order of magnitude, to a rate of 2.7×10^{-5} per cell per generation (Table 2). Thirty-one of 36 colonies analyzed had the recipient GalTy1Ura replaced by a GalTy1neo. These events are due to recombination with the abundant Ty1neo cDNA present in the cell (4). We note that while the presence of increased levels of Ty1neo cDNA causes a one order of magnitude increase in strains MK87 and YN3, MK95/pM97 grown in galactose shows a two order of magnitude increase. This can be explained by a synergistic effect between the two causes for elevated rates of recombination—namely, increased donor cDNA levels and transcription of the recipient Ty.

This hypothesis was tested by introducing changes in the donor and the recipient Ty elements. Three additional plasmids were used as donors: pM109 carries mutations in the primer-binding site (pbs) of the Ty, rendering it unable to sustain a reverse-transcription reaction (4); pM102 has a frameshift mutation in the Ty, so that no reverse transcriptase or RNase H is produced (4); pYN8 carries amber mutations in all three frames, so that no protein is produced from this Ty (24). When these plasmids were introduced into strains MK104 (Ty1Ura) or MK87 (Ty2Ura), no increase in the rate of appearance of Ura$^-$ colonies was seen in galactose, showing that high cDNA levels are essential for increased recombination between TyUra and GalTy1neo (ref. 4 and data not shown). Introducing these plasmids into MK95 resulted in a rate of Ura$^-$ colonies similar to the one obtained without any plasmid (Table 2). These results confirm that the additional increase in recombination seen in the presence of pM97 in galactose is cDNA-mediated and imply that the induction of transcription of the GalTy1Ura cannot complement the defect in the GalTy1neo on the plasmid.

When the frameshift mutation or the triple amber mutation were introduced into the recipient Ty in MK95, creating strains MK116 and YN2, respectively, the high rate of conversion in galactose was unaffected (Table 2). Thus, the increase in recombination caused by transcription of the recipient Ty, as opposed to that seen when the donor Ty, is highly transcribed, does not require reverse transcription, and is probably due to transcription *per se*. Furthermore, upon transformation of strains MK116 and YN2 with pM97, the level of Ura$^-$ colonies obtained was similar to the one seen in MK95/pM97 (Table 2), showing once more the synergistic nature of both types of induction.

To further characterize the genetic requirements of the cDNA-mediated and the transcription-induced recombination, we created three isogenic strains, each carrying a mutation in a different *RAD* gene. These repair genes have been shown to be required for homologous recombination in yeast (for review, see ref. 2). *rad51* and *rad57* mutants have been reported to be completely defective in meiotic and mitotic allelic recombination (2, 31); in contrast, the *RAD50* gene product seems to be needed only for meiotic recombination (2, 32). Table 3 shows the results obtained with the different *rad* strains that do or do not carry a GalTy1neo plasmid, in glucose- or galactose-containing medium. The *rad50* strain gave similar results to those obtained with the *Rad*$^+$ strain (MK95). Surprisingly, both *rad51* and *rad57* strains gave a very high rate of Ura$^-$ colonies. The basal rate of recombination in these mutants is two orders of magnitude higher than that of MK95. This increase is due to a very high rate of recombination between the LTRs of the recipient Ty: all the Ura$^-$ derivatives analyzed were the result of a LTR–LTR interaction. Table 3 also shows that both *rad51* and *rad57* strains still show an increase in the rate of appearance

Table 1. Rate of appearance of Ura$^-$ colonies

Strain	Rate	
	Glucose	Galactose
MK87 (Ty2Ura)	3.03 ± 0.63*	3.31 ± 0.66*
MK104 (Ty1Ura)	33.50 ± 7.41*	31.10 ± 2.79*
YN3 (Ty1Ura ΔLTR)	4.90 ± 0.86	2.36 ± 0.78
MK95 (GalTy1Ura)	1.43 ± 0.33	33.18 ± 4.41

Rate of appearance of Ura$^-$ colonies is expressed as number × 10^{-7} per cell per generation.
*Data are from ref. 4.

Table 2. Rate of appearance of Ura$^-$ colonies

Strain	Rate	
	Glucose	Galactose
MK87 (Ty2Ura)/pM97	3.66 ± 0.56	38.20 ± 5.29
YN3 (Ty1UraΔLTR)/pM97	8.00 ± 1.33	83.10 ± 9.88
MK95 (GalTy1Ura)	1.43 ± 0.33	33.18 ± 4.41
MK95 (GalTy1Ura)/pM97	3.79 ± 0.65	269.10 ± 59.01
MK95/pM109 (pbs$^-$)	3.69 ± 0.66	49.78 ± 5.85
MK95/pM102 (rt$^-$ rnh$^-$)	3.22 ± 0.71	31.38 ± 2.79
MK95/pYN8 (TyA amber)	3.26 ± 0.61	23.00 ± 3.00
MK116 (GalTy1Ura rt$^-$ rnh$^-$)	4.62 ± 0.89	18.58 ± 2.28
MK116 (GalTy1Ura rt$^-$ rnh$^-$)/pM97	3.22 ± 0.59	159.62 ± 1.0
YN2 (GalTy1Ura TyA amber)	3.00 ± 0.59	35.90 ± 4.68
YN2 (GalTy1Ura TyA amber)/pM97	3.20 ± 0.62	334.03 ± 38.74

Rate of appearance of Ura$^-$ colonies is expressed as number × 10^{-7} per cell per generation.

Table 3. Rate of appearance of Ura$^-$ colonies in various *rad* strains and distribution of the different types of events obtained

Strain	Rate	Events, no.				
		Tyneo*	Ty†	LTR‡	TyUra§	Total
MK95 (*Rad+*) Glu	1.43 ± 0.33	—	20	11	4	35
MK95 Gal	33.18 ± 4.41	—	15	9	1	25
MK95/pJef1678 Glu	2.47 ± 0.53	3	4	2	3	12
MK95/pJef1678 Gal	374.92 ± 53.43	11	0	2	0	13
MK172 (*rad50*) Glu	2.02 ± 0.48	—	7	3	1	11
MK172 Gal	33.20 ± 5.45	—	5	3	4	12
MK172/pJef1678 Glu	2.52 ± 0.50	2	4	2	3	11
MK172/pJef1678 Gal	182.33 ± 32.97	9	7	1	1	18
UB1 (*rad51*) Glu	238.37 ± 35.16	—	0	11	0	11
UB1 Gal	1040.00 ± 114.37	—	0	12	0	12
UB1/pJef1678 Glu	356.64 ± 56.24	0	0	18	0	18
UB1/pJef1678 Gal	1005.67 ± 140.09	0	0	12	0	12
UB2 (*rad57*) Glu	103.05 ± 13.27	—	0	12	0	12
UB2 Gal	379.65 ± 37.59	—	0	11	0	11
UB2/pJef1678 Glu	114.18 ± 22.23	0	0	16	0	16
UB2/pJef1678 Gal	507.93 ± 73.91	8	1	5	4	18

Rate of appearance of Ura$^-$ colonies is expressed as number × 10^{-7} per cell per generation.
*Replacement of the Ty1Ura by information from Ty1neo.
†Replacement of Ty1Ura by information from an unmarked Ty element.
‡Replacement of the Ty1Ura by a solo LTR.
§Mutation at the *URA3* gene in the Ty1Ura or conversion by the *ura3–Nco*$^-$ allele on chromosome V.

of Ura$^-$ colonies when grown in galactose, implying that the induction due to transcription is still present. When they were transformed with a plasmid carrying a GalTy1neo, no change in the rate of appearance of Ura$^-$ cells was seen in glucose-grown cells; on galactose-containing medium, however, these strains behaved differently from each other. Galactose-grown UB1 (*rad51*) carrying the GalTy1neo plasmid showed the same level of recombination as the strain without the plasmid; in addition, no conversion events were detected. Thus, the *RAD51* gene product is needed for Ty1neo cDNA-mediated recombination. When the *rad57* strain carrying the plasmid was grown in galactose, however, a modest but reproducible increase in the number of Ura$^-$ colonies was seen. Analysis of independent Ura$^-$ colonies showed that 8 of 16 carried a replacement of the Ty1Ura by a Ty1neo, implying that cDNA-mediated recombination can take place in *rad57* strains.

DISCUSSION

Nonreciprocal recombination (gene conversion) between repeated sequences constitutes an evolutionary mechanism that promotes homogeneity between members of the family [concerted evolution (33)] and can also generate new combinations with adaptive advantage. Reciprocal recombination between repeated sequences, on the other hand, can result in gross karyotypic changes, such as deletions, inversions, and translocations, which have immediate effects in fitness and may also contribute to speciation mechanisms (2, 33). Since all eukaryotic cells carry many repetitive sequences, the level of recombination between them is probably regulated. The Ty elements of yeast constitute a good example of a naturally occurring family of repeated sequences. The level of recombination between Ty elements is relatively low and is almost exclusively nonreciprocal in nature (3). Furthermore, this low recombination frequency is not increased by DNA damage (25). In the present paper, we show that a higher rate of ectopic recombination can be obtained upon induction of transcription of a marked Ty. This elevated level of recombination is not dependent on gene products encoded by the Ty itself (proteins, RNA, or cDNA); moreover, a synergistic increase in the rate of recombination can be observed when Ty1neo cDNA is overproduced in the cells together with the transcriptional induction of the recipient Ty. These results imply that the induction of recombination observed upon transcription of the recipient Ty is due to a different mechanism than the induction caused by overexpression of Ty cDNA. The synergistic effect observed implies that two different factors or steps are rate-limiting for Ty recombination. One of them is the availability of cDNA to act as donor of information; the second one limits recombination when the Ty is not expressed but is no longer limiting upon transcription from the *GAL* promoter.

What is the reason for this increased recombination level upon transcription? We note that the distribution of recombination events in repressed or induced conditions remains unchanged, implying that there is no induction of a specific type of event. More likely, a general mechanism increases all types of recombination. Moreover, even in *rad51* and *rad57* strains, in which an unusually high level of LTR–LTR interactions is seen, transcription of the Ty still causes a modest increase in the level of recombinants observed. Three nonexclusive models can be proposed to explain transcription-induced recombination: (*i*) Recombination may result from changes in chromatin caused by the passage of the RNA polymerase through the Ty. The transcription complex may remove factors that normally prevent recombination or may render the DNA more amenable to the recombination machinery by altering its topology or by allowing the interaction of unwound DNA with recombination proteins (8, 10, 11). (*ii*) Alternatively, the high levels of recombination could be due to an increase in repair activity (34, 35). Such an activity can result in secondary lesions in the DNA that may promote recombination. (*iii*) Finally, the binding of transcription factors to the *GAL1* promoter under transcription conditions may be directly or indirectly responsible for the increase in recombination. A dual role for proteins that act in transcriptional silencing and DNA replication has been found lately (36–38). Similarly, it is possible that protein–protein interactions exist between transcription and recombination factors, as has been suggested for the immunoglobulin genes in mammalian cells (10, 11, 39).

This laboratory has shown (25) that UV irradiation causes an increase in Ty RNA levels without a concomitant increase in recombination; this result seems to be in contradiction with model *ii*. Support for model *iii* comes from the finding (40)

that in the *HIS4* gene of yeast, binding of the RAP1 transcription factor to the promoter increases meiotic recombination in the region, showing that this protein can play a dual role in transcription and recombination. It is possible that the galactose-promoted transcription induction and the UV-promoted transcription induction are mediated by different sets of proteins and that in the latter case, no coupling to a recombinational mechanism (or, alternatively, coupling to other types of repair) exists. Further experiments are needed to distinguish between the different models.

We have also shown that the *RAD50* gene product is not needed for cDNA-mediated recombination or for transcription-dependent induction. The *RAD50* gene product does not seem to be needed for any kind of mitotic recombination (2, 41), although its absence confers sensitivity to ionizing radiation.

In the absence of the *RAD51* or the *RAD57* gene products, an unusually high level of LTR–LTR interactions is seen. Similarly, McDonald and Rothstein (42) have shown increased levels of recombination between direct repeats in *rad51* and *rad57* strains. Two possible explanations can account for this increase: (*i*) In the absence of the Rad51 or Rad57 proteins, lesions are created in the DNA, which are repaired by a mechanism, such as single-strand annealing (30), that leads to a solo LTR product. The net increase in Ura^- colonies would then be due to a net increase in initiating events. (*ii*) The absence of the *RAD51* or *RAD57* gene products does not create new lesions; the high level of Ura^- colonies observed reflects the normal level of initiating lesions. Their repair in the presence of the Rad51 and Rad57 proteins is such that the majority of the events are not detected, and only a minority are seen as Ty conversions. In the absence of any one of these proteins, however, all the lesions are repaired by an alternative mechanism and are thus fully detected. The high level of LTR–LTR interactions seen in these strains is still inducible by transcription, implying that this induction is carried out by a general mechanism, which is independent of the *RAD51* and *RAD57* gene products.

It is important to note that, although *RAD51* and *RAD57* share DNA sequence homology (and are also homologous to bacterial RecA proteins) (21, 43, 44), *rad57* strains are proficient in cDNA-mediated Ty conversion, whereas *rad51* strains are not (Table 3), implying that they have different functions. Since *rad57* strains are able to carry out cDNA-mediated Ty conversion, we could expect to see conversion events involving unmarked Ty cDNAs in *rad57* strains grown in the absence of pJef1678, as we see with MK95. It is possible that the high level of LTR–LTR interactions in the *rad57* strain (two orders of magnitude higher than normal) preclude us from detecting a normal level of conversion events. Alternatively, *RAD57* may still be required for spontaneous interchromosomal conversion events but not for cDNA-mediated events.

We have shown that active transcription of a Ty element, a member of a family of repeated sequences, affects its level of recombination. Further studies are required to elucidate the precise mechanism of this induction. These studies should contribute to the understanding of basic mechanisms by which eukaryotic cells control recombination between repeated sequences.

We thank Jef Boeke, Joan Curcio, David Garfinkel, Shirleen Roeder, and Lorraine Symington for plasmids; Cathy Melamed for her contribution in the early steps of the project; Udi Barki for help in strain constructions; and Rina Jaget and Rivka Steinlauf for excellent technical assistance. These studies were supported by a grant from the Basic Research Foundation of the Israel Academy of Sciences and Humanities.

1. Boeke, J. D. & Sandmeyer, S. B. (1991) in *Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Broach, J. R., Pringle, J. R. & Jones, E. W. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 193–291.
2. Petes, T. D., Malone, R. E. & Symington, L. S. (1991) in *Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Broach, J. R., Pringle, J. R. & Jones, E. W. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 407–521.
3. Kupiec, M. & Petes, T. D. (1988) *Mol. Cell Biol.* **8,** 2942–2954.
4. Melamed, C., Nevo, Y. & Kupiec, M. (1992) *Mol. Cell Biol.* **12,** 1613–1620.
5. Voelkel-Meiman, K. & Roeder, G. S. (1990) *Genetics* **124,** 561–572.
6. Thomas, B. J. & Rothstein, R. (1989) *Cell* **56,** 619–630.
7. Grimm, C., Schaer, P., Munz, P. & Kohli (1991) *Mol. Cell. Biol.* **11,** 289–298.
8. Nickoloff, J. A. (1992) *Mol. Cell. Biol.* **12,** 5311–5318.
9. Blackwell, T. K., Moore, M. W., Yancopoulos, G. D., Suh, H., Lutzker, S., Selsing, E. & Alt, F. W. (1986) *Nature (London)* **324,** 585–589.
10. Oltz, E. M., Alt, F. W., Lin, W., Chen, J., Taccioli, G., Desiderio, S. & Rathbun, G. (1993) *Mol. Cell. Biol.* **13,** 6223–6230.
11. Lauster, R., Reynaud, C., Martensonn, I., Peter, A., Bucchini, D., Jami, J. & Weill, J. C. (1993) *EMBO J.* **12,** 4615–4623.
12. Bohr, V. A., Smith, C. A., Okumoto, D. S. & Hanawalt, P. C. (1985) *Cell* **40,** 359–369.
13. Sweder, K. S. & Hanawalt, P. C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10696–10700.
14. Sweder, K. S. & Hanawalt, P. C. (1993) *Science* **262,** 439.
15. Leadon, S. A. & Lawrence, D. A. (1992) *J. Biol. Chem.* **267,** 23175–23182.
16. Qiu, H., Park, E., Prakash, L. & Prakash, S. (1993) *Genes Dev.* **7,** 2161–2171.
17. Feaver, W. J., Svejstrup, J. Q., Bardwell, L., Bardwell, A. J., Buratowski, S., Gulyas, K. D., Donahue, T. F., Friedberg, E. C. & Kornberg, R. D. (1993) *Cell* **75,** 1379–1387.
18. Boeke, J. D., Garfinkel, D. J., Styles, C. A. & Fink, G. R. (1985) *Cell* **40,** 491–500.
19. Rothstein, R. (1983) *Methods Enzymol.* **101,** 202–211.
20. Engebrecht, J. & Roeder, G. S. (1989) *Genetics* **121,** 237–247.
21. Basile, G., Aker, M. & Mortimer, R. K. (1992) *Mol. Cell. Biol.* **12,** 3235–3246.
22. Kans, J. A. & Mortimer, R. K. (1991) *Gene* **105,** 139–140.
23. Fleig, U. N., Pridmore, R. D. & Philipsen, P. (1986) *Gene* **46,** 237–245.
24. Curcio, M. J. & Garfinkel, D. J. (1992) *Mol. Cell. Biol.* **12,** 2813–2825.
25. Parket, A. & Kupiec, M. (1992) *Mol. Cell. Biol.* **12,** 4441–4448.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
27. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
28. Boeke, J. D., Lacroute, F. & Fink, G. R. (1984) *Mol. Gen. Genet.* **197,** 345–346.
29. Lea, D. E. & Coulson, C. A. (1948) *J. Genet.* **49,** 264–284.
30. Lin, F. L., Sperle, K. & Sternberg, N. (1984) *Mol. Cell. Biol.* **4,** 1020–1034.
31. Saeki, T., Machida, I. & Nakai, S. (1980) *Mutat. Res.* **73,** 251–265.
32. Malone, R. E., Ward, T., Lin, S. & Waring, J. (1990) *Curr. Genet.* **18,** 111–116.
33. Edelman, G. M. & Gally, J. A. (1970) in *The Neurosciences: Second Study Program*, ed. Schmitt, F. O. (Rockefeller Univ. Press, New York) pp. 962–972.
34. Selby, C. P. & Sancar, A. (1993) *Science* **260,** 53–57.
35. Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeulen, W., Hoeijmakers, J. H., Chambon, P. & Eagly, J. M. (1993) *Science* **260,** 58–63.
36. Foss, M., McNally, F. J., Laurenson, P. & Rine, J. (1993) *Science* **262,** 1838–1844.
37. Bell, S., Kobayashi, R. & Stillman, B. (1993) *Science* **262,** 1844–1849.
38. Micklem, G., Rowly, A., Hardwood, J., Nasmyth, K. & Diffley, J. F. X. (1993) *Nature (London)* **366,** 87–89.
39. Jenuwein, T., Forrester, W. C., Qiu, R. & Grosscheld, R. (1993) *Genes Dev.* **7,** 2016–2032.
40. White, M. A., Wierdl, M., Detloff, P. & Petes, T. D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9755–9759.
41. Steele, D. F., Morris, M. E. & Jinks-Robertson, S. (1991) *Genetics* **127,** 53–60.
42. McDonald, J. P. & Rothstein, R. (1994) *Genetics* **137,** 393–405.
43. Aboussekhra, A., Chanet, R., Adjiri, A. & Fabre, F. (1992) *Mol. Cell. Biol.* **12,** 3224–3234.
44. Shinohara, A., Ogawa, H. & Ogawa, T. (1992) *Cell* **69,** 457–470.