Induction of Ty Recombination in Yeast by cDNA and Transcription: Role of the *RAD1* and *RAD52* Genes

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ABSTRACT

In the yeast *Saccharomyces cerevisiae* 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. Little is known about the mechanism(s) that prevent recombination between repeated sequences. We have previously shown that nonreciprocal recombination (gene conversion) of a genetically marked Ty can be induced either by the presence of high levels of Ty cDNA or by transcription of the marked Ty from a *GAL1* promoter. These two kinds of induction act in a synergistic manner. To further characterize these two kinds of Ty recombination, we have investigated the role played by the *RAD52* and *RAD1* genes. We have found that the *RAD52* and *RAD1* gene products are essential to carry out transcription-induced Ty conversion whereas cDNA-mediated conversion can take place in their absence.

TY elements are the main family of natural repeated sequences in *Saccharomyces cerevisiae,* comprising ~1–2% of the yeast genome (for review, see BOEKE and SANDMEYER 1991). They are retrovirus-like transposons (retrotransposons) that consist of a ~5-kb central region flanked by two direct repeats (LTRs) ~330 bp long. Ty elements code for a mRNA that is reverse-transcribed to cDNA by Ty-encoded proteins. There are 30–40 copies of Ty elements dispersed throughout a haploid genome.

Ectopic recombination (recombination between sequences located at different positions in the genome) occurs readily between artificially duplicated genes, in mitotic and meiotic yeast cells. Both reciprocal and nonreciprocal recombination (gene conversion) events have been observed. Ectopic reciprocal recombination can cause chromosomal aberrations such as translocations, deletions, duplications and inversions (for review, see PETES *et al.* 1991). Despite their large number, the rate at which Ty elements engage in ectopic recombination is relatively low and the vast majority of the conversion events detected is not associated with crossing over (KUPIEC and PETES 1988a,b). Since repetitive sequences are present in the genomes of all eukaryotes, the karyotypic stability might be maintained by a mechanism(s) that prevents recombination between such sequences. The Ty family is a good model to study recombination between naturally occurring repeated sequences. We have previously shown that Ty cDNA can participate as a donor of information in gene conversion events between Tys (MELAMED *et al.* 1992) and that high levels of transcription of a Ty element induce its participation as a recipient of information in conversion events (NEVO-CASPI and KUPIEC 1994). In addition, we have shown that the induction observed upon transcription of the recipient Ty and the induction seen due to the presence of large amounts of Ty cDNA are due to separate mechanisms that act synergistically to each other (NEVO-CASPI and KUPIEC 1994).

The *RAD52* gene product plays a central role in mitotic recombination in *S. cerevisiae. rad52* mutants are defective in mating-type switching, mitotic and meiotic recombination, and in the repair of double-strand breaks caused by ionizing radiation and chemicals (reviewed in PETES *et al.* 1991). The *RAD1* gene participates in nucleotide excision repair and it is probably homologous to the mammalian *ERCC4* gene (VAN VUUREN *et al.* 1993). *RAD1* has been also shown to be involved in recombination between direct repeats (KLEIN 1988; SCHIESTL and PRAKASH 1988; LIEFSHITZ *et al.* 1995). *RAD52* and *RAD1* participate in alternative mechanisms of direct-repeat recombination (MCDONALD and ROTHSTEIN 1994; LIEFSHITZ *et al.* 1995).

In this study, we have investigated the role of the *RAD52* and *RAD1* genes in cDNA-mediated and transcription-induced Ty conversion. We present further evidence that these two processes occur by different mechanisms: cDNA-mediated Ty recombination is only partially dependent on the *RAD52* gene product and completely independent of the *RAD1* gene product, while transcription-induced Ty recombination is abolished in *rad52* and in *rad1* strains.

MATERIALS AND METHODS

Yeast strains: All *S. cerevisiae* strains used in the present study are isogenic and were derived from strain MK89 (MELAMED *et*

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al. 1992) (*MATa ura3-Nco⁻ his3-11,15 leu2-3,112 trp1-Xba⁻ can1-101 ade2-1*). YN3 and MK95 have been previously described (NEVO-CASPI and KUPIEC 1994); YN3 carries a TyUra in which the first 237 nucleotides of Ty*1* have been deleted. This TyUra was inserted at the *LYS2* locus. MK95 carries a GalTyUra (a TyUra in which the first 237 nucleotides of Ty*1* have been replaced by 753 nucleotides from the *GAL1* promoter) inserted at the *LYS2* locus. YNC13 and MK98 (both *rad52::TRP1*) were constructed by transforming YN3 and MK95, respectively, with plasmid pSM21, which carries the *RAD52* gene disrupted by the *TRP1* gene (KUNZ *et al.* 1989). YNC12 and MK168 (both *rev3::LEU2*) were constructed by transforming YN3 and MK95, respectively, with plasmid pAM56, which carries the *REV3* gene disrupted by the *LEU2* gene (MORRISON *et al.* 1989). YNC15 and MK169 (both *rad52::TRP1 rev3::LEU2*) were created by transforming YNC12 and MK168, respectively, with plasmid pSM21. YNC18 and MK167 (both *rad1::LEU2*) were constructed by transforming YN3 and MK95 with plasmid pRR46, which carries the *RAD1* gene disrupted by the *LEU2* gene (SCHIESTL and PRAKASH 1989). After transformation, all the relevant chromosomal configurations were confirmed by Southern blot analysis.

Plasmids: pJEF1678 (a gift from JEF BOEKE, Johns Hopkins University, Baltimore) carries a GalTyNeo on a 2μ plasmid with a *HIS3* selectable marker. As probes in Southern blot analysis the following plasmids were used: pM43, which carries a fragment of the *LYS2* gene (PARKET and KUPIEC 1992); pM21, which carries the *URA3* gene (MELAMED *et al.* 1992); and pGH54, which carries the bacterial *Neo* gene (MELAMED *et al.* 1992).

Media, growth conditions and general procedures: Standard molecular biology procedures such as restriction enzyme analysis and Southern blot analysis were carried out as described in SAMBROOK *et al.* (1989). Yeast media and molecular biology procedures (transformations, DNA preparations, etc.) were carried out as in SHERMAN *et al.* (1986). Ura⁻ colonies were selected on SD complete medium with uracil (50 mg/l) and 5-fluoroorotic acid (5-FOA) (0.8 g/l) (BOEKE *et al.* 1984).

PCR analysis: PCR reactions were carried out starting with a small lump of cells in a tube containing the reaction mix consisting of the following: 40 mM dNTPs, 40 ng of primers, 1× buffer (Fermentas) and 1 mM $MgCl_2$ in a total volume of 50 μl. Following cell lysis (3 min at 97°), 1 unit of Taq DNA polymerase (Fermentas) was added and the cells were subjected to 30 cycles as follows: 60 sec at 94°, 60 sec at 54° and 90 sec at 72°. Three different primers were used: MO2, MO6 and MO9. MO2 (5′AACTGAGGGGTCCTTTCC3′) is near the *Bgl*II site of the *LYS2* gene, while MO6 (5′GTGATGACAAAACCTCTTCCG3′) is internal to the Ty at position 5502 (BOEKE *et al.* 1988). MO2 and MO6 flank the *URA3* gene in the Ty and therefore products derived from these two primers can distinguish between the different possible events occurring at the 3′ end of the Ty. MO9 (5′ACGCCCACAACAAGAACC3′) is near the *Bgl*II site in the *LYS2* gene and together with MO2 flanks the point of insertion of the Ty in the *LYS2* gene. These two primers enable detection of events in which the whole TyUra was replaced by a solo LTR.

Measurement of Ty recombination: Recombination rates were measured by fluctuation analysis as described (MELAMED *et al.* 1992) and were calculated by the method of the median (LEA and COULSON 1948). Calculated standard deviations were always lower than 30%. Statistical analysis was carried out by a Confidence Limits Test (CARLSON 1973). At least 36 cultures were grown in SD or SGal complete medium (cultures bearing pJEF1678 were grown in SD −His or SGal −His medium). Ura⁻ colonies were selected on 5-FOA and one Ura⁻ His⁻ colony from each culture was subjected to PCR analysis. Colonies for which PCR analysis failed were subjected to Southern blot analysis using *LYS2, URA3* and *Neo* probes.

Papillation test: Ura⁻ cells that showed, by molecular analysis, that they still bear the *URA3* gene in the Ty could have acquired their Ura⁻ phenotype in two ways: either by gene conversion with the *ura3-Nco* allele from chromosome *V*, resulting in a cell with two copies of the allele, or by a new mutation in the *URA3* gene in the Ty. To distinguish between these two possibilities, a papillation test was carried out as described in KUPIEC and PETES (1988a). Since *rad52* cells are defective in recombination, such cells were mated, before UV treatment, to strain LS4B (*RAD52 ura3-Nco*) to obtain a diploid cell capable of recombination.

RESULTS

Experimental design: The strains used in the present study are isogenic and carry at the *LYS2* locus a Ty element in which a copy of the *URA3* gene has been inserted, conferring to the cell a Ura⁺ phenotype. Recombination events involving the marked Ty give rise to Ura⁻ colonies that can be selected on 5-FOA medium (BOEKE *et al.* 1984). To study the effect of transcription on Ty recombination, some strains carry a marked Ty in which the natural promoter has been replaced by the *GAL1* promoter (GalTyUra). This Ty element is not expressed in a glucose-containing medium but is highly transcribed in a medium containing galactose. Hence, we can monitor transcription-induced recombination involving GalTyUra by growing the cells in galactose. Growth of the same cultures in glucose serves as a control for noninducing conditions. As an additional control we used isogenic strains that carry promoterless Ty elements marked with the *URA3* gene (referred to as TyUra).

Four different events involving GalTyUra can generate Ura⁻ cells: (1) the occurrence of a new mutation at the *URA3* gene of the Ty, (2) a conversion event in which information from the *ura3-Nco* allele on chromosome *V* is transferred to the *URA3* of the Ty, (3) participation of GalTyUra as a recipient of information in a conversion event with an unmarked Ty (of chromosomal origin or Ty cDNA), and (4) replacement of the whole GalTyUra element by a solo long terminal repeat (LTR).

In addition to transcription-induced events, we also scored cDNA-mediated recombination. This type of event can be monitored in our system by introducing into the cells a multicopy plasmid carrying a GalTyNeo (a GalTy marked with the insertion of the bacterial *Neo* gene). When the transformed cells are grown in galactose, large amounts of TyNeo cDNA are produced. We have previously shown that in wild-type strains grown under such conditions, the majority of the Ura⁻ cells acquire their phenotype by a gene conversion event between the genomic TyUra and TyNeo cDNA (MELAMED *et al.* 1992). Thus, Ura⁻ colonies obtained from such cells can be the result of one of the events described above, or of cDNA-mediated recombination,

TABLE 1

Rate of appearance and distribution of the different types of Ura⁻ events obtained ($\times 10^{-7}$ per cell per generation)

Strain	Relevant genotype	Medium	Total rate of Ura⁻[a]	Rate of Ura⁻ products					Total Ura⁻ analyzed[g]
				TyNeo[b]	Ty[c]	LTR[d]	Deletion[e]	Mutation[f]	
MK95	GalTyUra	Glu	1.5	—	1.0	0.45	<0.04	<0.04	35
MK95		Gal	33.2	—	20.8	11.6	<0.04	0.8	83
MK95/pJEF1678		Glu	2.5	1.1	0.82	0.44	<0.06	0.12	40
MK95/pJEF1678		Gal	375	262	50	50	12.5	<12.5	30
YN3	TyUra	Glu	2.7	—	1.6	0.77	0.11	0.16	49
YN3		Gal	2.4	—	1.2	1.1	0.05	0.05	49
YN3/pJEF1678		Glu	4.8	1.7	1.9	0.84	<0.12	0.36	40
YN3/pJEF1678		Gal	133	98	31	3.9	<3.9	<3.9	34
MK98	GalTyUra *rad52*	Glu	7.8	—	<0.03	0.07	<0.03	7.7	210
MK98		Gal	14.8	—	<0.08	1.0	0.17	13.6	173
MK98/pJEF1678		Glu	4.7	<0.05	<0.05	0.16	0.71	3.8	86
MK98/pJEF1678		Gal	12	2.8	0.52	0.7	1.0	7.0	69
YNC13	TyUra *rad52*	Glu	13.1	—	<0.08	0.17	<0.08	12.9	156
YNC13		Gal	2.3	—	0.02	0.11	0.01	2.1	187
YNC13/pJEF1678		Glu	7.1	<0.08	<0.08	<0.08	0.08	7.0	85
YNC13/pJEF1678		Gal	6.3	0.53	<0.10	0.21	0.32	5.3	60
MK168	GalTyUra *rev3*	Glu	2.3	—	1.1	1.1	0.11	0.06	41
MK168		Gal	27.2	—	12.4	13.0	1.2	0.62	44
MK168/pJEF1678		Glu	5.3	2.6	0.7	1.4	0.13	0.38	42
MK168/pJEF1678		Gal	478	340	85	42	10.6	<10.6	45
YNC12	TyUra *rev3*	Glu	3.7	—	1.8	1.5	0.25	0.12	30
YNC12		Gal	2.5	—	1.4	0.85	0.06	0.12	41
YNC12/pJEF1678		Glu	7.7	3.4	2.0	2.0	0.39	<0.20	39
YNC12/pJEF1678		Gal	135	111	16	8.2	<4.1	<4.1	33
MK169	GalTyUra *rad52 rev3*	Glu	3.2	—	<0.02	0.05	0.46	2.7	132
MK169		Gal	2.3	—	0.02	0.28	0.25	1.8	131
MK169/pJEF1678		Glu	3.4	0.35	0.27	0.54	0.98	1.2	38
MK169/pJEF1678		Gal	12	5.7	0.86	2.3	2.8	0.28	42
YNC15	TyUra *rad52 rev3*	Glu	6.5	—	<0.19	0.38	0.38	5.7	34
YNC15		Gal	1.7	—	<0.04	0.18	0.41	1.1	46
YNC15/pJEF1678		Glu	2.5	<0.04	<0.04	0.49	0.45	1.6	56
YNC15/pJEF1678		Gal	0.62	0.24	0.05	0.06	0.13	0.13	38
MK167	GalTyUra *rad1*	Glu	2.7	—	1.4	0.15	0.07	1.1	37
MK167		Gal	19.8	—	2.8	2.8	1.1	13	35
MK167/pJEF1678		Glu	2.2	0.66	0.29	0.29	0.07	0.88	30
MK167/pJEF1678		Gal	54	22.5	20	4.3	<1.42	7.1	38
YNC18	TyUra *rad1*	Glu	3.2	—	1.4	0.09	0.09	1.6	34
YNC18		Gal	3.3	—	1.4	0.43	<0.11	1.5	31
YNC18/pJEF1678		Glu	4.1	1.1	0.9	0.45	0.11	1.5	36
YNC18/pJEF1678		Gal	71	55	11.8	2.0	<2.0	2.0	36

[a] Rate of appearance of Ura⁻ colonies.
[b] Replacement of TyUra with information from TyNeo.
[c] Replacement of TyUra with information from an unmarked Ty or conversion of the *URA3* gene in the Ty by the *ura3-Nco* allele on chromosome *V*.
[d] Replacement of TyUra by a solo LTR.
[e] A deletion involving the *URA3* gene in the Ty.
[f] A new mutation acquired by the *URA3* in the Ty.
[g] Total number of independent Ura⁻ colonies analyzed.

in which case they carry a TyNeo replacing the original TyUra.

After growing the different strains in galactose or in glucose, Ura⁻ cells were selected by plating on 5-FOA medium (BOEKE *et al.* 1984) and their rate of appearance was calculated (LEA and COULSON 1948). Independent Ura⁻ colonies were subjected to PCR or Southern blot analysis to determine the nature of the events leading to the Ura⁻ phenotype. Table 1 shows the rate of appearance of Ura⁻ colonies, as well as the calculated rates of each of the different categories obtained: (1) conversion by TyNeo cDNA, (2) conversion by an unmarked Ty, (3) recombination between the LTRs, (4) deletions that include the *URA3* marker, and (5) mutations at the *URA3* marker. Table 2 shows the level of induction of the gene conversion class of events (TyNeo + unmarked Tys) in glucose and galactose.

The role of the *RAD52* gene in transcription-induced and cDNA-mediated Ty recombination: MK95 (wild type) and MK98 (*rad52::TRP1*) are isogenic strains carrying GalTyUra constructs. The rate of appearance of Ura⁻ cells in glucose-grown cultures was higher in the

TABLE 2

Rate of conversion of GalTyUra and TyUra

Strain	Relevant genotype	Glucose[a]	Galactose[a]	Induction
MK95	GalTyUra	1.0	20.8	×21
MK95/pJEF1678		2.0	312	×156
YN3	TyUra	1.6	1.2	×0.8
YN3/pJEF1678		3.6	129	×36
MK98	GalTyUra *rad52*	<0.03	<0.08	—
MK98/pJEF1678		<0.05	3.3	>66
YNC13	TyUra *rad52*	<0.08	0.02	—
YNC13/pJEF1678		<0.08	0.53	>6.6
MK168	GalTyUra *rev3*	1.1	12.4	×11
MK168/pJEF1678		3.3	425	×129
YNC12	TyUra *rev3*	1.8	1.4	×0.8
YNC12/pJEF1678		5.4	127	×24
MK169	GalTyUra *rad52 rev3*	<0.02	0.02	>1.0
MK169/pJEF1678		0.62	6.6	×11
YNC15	TyUra *rad52 rev3*	<0.19	<0.04	—
YNC15/pJEF1678		<0.04	0.29	>7.3
MK167	GalTyUra *rad1*	1.4	2.8	×2
MK167/pJEF1678		0.95	42	×44
YNC18	TyUra *rad1*	1.4	1.4	×1
YNC18/pJEF1678		2.0	67	×34

[a] Rate of conversion events involving TyUra expressed as number × 10^{-7} per cell per generation. It was calculated by multiplying the rate of appearance of Ura$^-$ colonies × the fraction of TyNeo and Ty conversions among the events analyzed.

rad52 strain (MK98) than the rate seen in the isogenic wild-type strain (MK95) (Table 1). In addition, after growing the cells in galactose, only a twofold increase in the rate of appearance of Ura$^-$ cells was seen in MK98 as opposed to the 20-fold increase seen for MK95. Southern blot or PCR were used to determine the nature of the events leading to the Ura$^-$ phenotype in MK95 and MK98. This analysis (Table 1) revealed that in glucose or galactose-grown MK95 (Rad$^+$), the majority of the Ura$^-$ colonies were due to conversion of the TyUra by an unmarked Ty. In contrast, in MK98 (*rad52*) the vast majority of the cells (over 90%) had become Ura$^-$ due to new mutations acquired by the *URA3* gene in the Ty. The *rad52* control strain YNC13 is isogenic to MK98 and carries a TyUra (instead of the GalTyUra in strain MK98). A high rate of mutations was also observed when YNC13 (TyUra *rad52*) was grown in glucose or in galactose (Table 1). The high rate of appearance of mutations in the *URA3* gene in *rad52* strains is in accordance with previous reports of a mutator phenotype in *rad52* cells (VON BORSTEL *et al.* 1971; KUNZ *et al.* 1989).

To examine the role of *RAD52* in cDNA-mediated gene conversion, the *rad52* strains MK98 and YNC13 were transformed with pJEF1678 bearing a GalTyNeo, and Ura$^-$ cells were selected after growth in glucose or in galactose. Again, the vast majority of Ura$^-$ cells selected after growth in both media were cells that acquired their Ura$^-$ phenotype due to new mutations in the *URA3* gene (Table 1). However, despite the high mutation rate in these strains, gene conversion events involving TyNeo could be detected when the cells were grown in the presence of large amounts of TyNeo cDNA.

Due to the high mutation rates seen in the *rad52* strains, it was difficult to accurately quantify the induction by transcription and by cDNA of Ty conversion in these cells. To reduce mutation rates, we deleted the *REV3* gene in MK98 and YNC13, thus creating the isogenic strains MK169 and YNC15, respectively, both of which are *rad52 rev3*. *REV3* has been shown to play a role in spontaneous and induced mutation formation (LEMONTT 1971; QUAH *et al.* 1980). Moreover, the antimutator effect conferred by *rev3* has been shown to be epistatic over the mutator phenotype of several *rad* mutants (ROCHE *et al.* 1995). Rad$^+$*rev3* control strains (MK168 and YNC12) were also analyzed and showed wild-type levels of transcription-induced and cDNA-mediated conversion (Tables 1 and 2). The influence of the *rev3* allele can be most clearly seen in the *rad52 rev3* double mutants (Table 1); mutation rates are less than half of those seen in the *rad52* strains. This decrease enabled us to monitor gene conversion events occurring in uninduced conditions (MK169/pJEF1678), and thus to estimate the rate of induction obtained when the cells were grown in galactose (Table 2).

Only a 11-fold increase in the rate of Ty conversion was seen in the galactose-grown *rad52 rev3* strain MK169/pJEF1678 compared to a 156-fold increase in the wild-type MK95/pJEF1678 under similar conditions (Table 2). Analysis of independent Ura$^-$ cells revealed that this induction was due to the higher rate of conver-

sion events between TyNeo cDNA and TyUra (Table 1). This 11-fold level of induction of cDNA-mediated conversion is comparable to the one observed in strains that do not carry out transcription-induced recombination but can undergo cDNA-mediated induced recombination (such as YN3/pJEF1678 or YNC12/pJEF1678). No gene conversion events among 132 Ura$^-$ independent colonies (rate $< 2 \times 10^{-9}$) were detected when MK169 (GalTyUra *rad52 rev3*) was grown in glucose without a plasmid. This result indicates that in a *rad52 rev3* strain, the basal rate of conversion events involving TyUra is lower, by at least 50-fold, than in the wild-type strain. No induction in the rate of conversion could be observed when MK169 cells were grown in galactose (Table 2). We conclude that although transcription-induced conversion is not detected, cDNA-mediated recombination can occur in *rad52 rev3* strains.

Our system enables us to monitor also direct repeat recombination involving the LTR elements of the Ty-Ura. Direct repeat recombination can occur by several mechanisms, including unequal crossing over between sister chromatids in G2, single-strand annealing (Lin *et al.* 1990) and one-end-invasion crossover (Belmaaza and Chartrand 1994; Prado and Aguilera 1995). It has been proposed (Prado and Aguilera 1995) that one-end-invasion is *RAD52* dependent, while single-strand annealing is *RAD52* independent. The spontaneous level of LTR interactions in the glucose-grown *rad52* strains was only about fivefold lower than in *RAD52* strains ($0.07–0.17 \times 10^{-7}$ *vs.* $0.45–0.77 \times 10^{-7}$, respectively); this result is in agreement with other studies that showed that *RAD52* plays a greater role in conversion than in direct-repeat recombination (Jackson and Fink 1981; Liefshitz *et al.* 1995). In contrast to conversion events, however, LTR recombination *is* induced by transcription 10- to 20-fold in both *rad52* and *RAD52* strains (Table 1, compare GalTyUra results in glucose and galactose). Thus, the *RAD52* gene product is not needed for the transcriptional induction of LTR recombination, but seems to be required for that of conversion events.

Southern blot analysis showed that many of the Ura$^-$ colonies obtained in *rad52* strains carried deletions involving the *URA3* gene in the Ty. The few deletions observed in the wild-type strains were smaller than 3 kb in size. In contrast, in the *rad52* strains, in 55 of the 88 cases analyzed, the deletion included, in addition to the Ty sequence, also parts of the adjacent *LYS2* sequence. Due to the limits of the probes used, we cannot determine the exact boundaries of these deletions, but they involve $\geq$7–20 kb. Thus, in the absence of the *RAD52* gene, lesions in the Ty are repaired by a mechanism that involves extensive degradation of the chromosome. In double-strand break-initiated recombination, extensive single-stranded degradation has been reported to occur in *rad52* strains (White and Haber 1990). It is possible that nicks in the intact strand lead to deletions that are repaired by *RAD52*-independent recombination events using regions of micro-homology (Kramer *et al.* 1994).

The role of the *RAD1* gene in transcription-induced and cDNA-mediated Ty recombination: Ty conversion was examined in strains MK167 (GalTyUra *rad1*) and YNC18 (TyUra *rad1*) grown in glucose and galactose. As expected from the mutator phenotype of *rad1* cells (Kunz *et al.* 1990), mutation rates in the *URA3* gene in these strains were higher than those seen in the wild-type isogenic strains (Table 1). The rate of conversion of the marked Ty in both strains, when grown in glucose, was similar to the rate obtained for the isogenic *RAD* strains (Table 2). When the *rad1* strain (MK167) bearing a GalTyUra was grown in galactose, however, only a twofold increase in rate was observed; the rates of conversion in glucose and galactose are not statistically different from each other [$0.1 > P > 0.05$ in a Confidence Limit Test (Carlson 1973)]. The lack of induction in the *rad1* strain in galactose is clearly different from the 20-fold increase seen in the wild-type isogenic strain, and implies that transcription-induced Ty conversion is dependent on the *RAD1* gene product. LTR recombination, in contrast, was still induced in MK167 by transcription 19-fold (from 0.15×10^{-7} to 2.8×10^{-7}; Table 1), as in the wild-type strain. Thus, the *RAD1* gene product is dispensable for the induction of LTR recombination.

When MK167 was grown in the presence of pJEF1678 in galactose, only a 44-fold increase in the rate of conversion events involving GalTyUra was seen (Table 2). This induction is the one expected from a strain that does not carry out transcription-induced recombination but can undergo cDNA-mediated induced recombination. The level of induction seen is comparable to the results obtained for YNC18 (TyUra *rad1*) and YN3 (TyUra *RAD*) in the presence of high levels of Ty cDNA (Table 2). We therefore conclude that the *RAD1* gene product is essential for transcription-induced Ty conversion but not for cDNA-mediated Ty conversion.

DISCUSSION

Ty elements are the main family of dispersed repeated sequences in yeast. It is still unclear what mechanism prevents the karyotypic instability that high levels of recombination between these sequences would generate. We have previously shown that gene conversion of a marked Ty is elevated in the presence of large amounts of cDNA molecules derived from a differently marked Ty that can serve as donors of information (Melamed *et al.* 1992). In addition, we have shown that transcription of a marked Ty induces its participation in recombination events as a recipient of information. In this study, we have investigated the role of the *RAD52* and *RAD1* gene products in the two kinds of induction of Ty conversion.

The role of *RAD52*: *RAD52* has been found to be essential for meiotic recombination and for almost all kinds of mitotic recombination, although its role still remains unclear (for review, see PETES *et al.* 1991). In our system a mutation in *RAD52* decreased the basal level of TyUra conversion by at least 50-fold; in addition, we could not detect an increase in the level of conversion following transcription in *rad52* strains. Spontaneous LTR recombination was about fivefold lower in the *rad52* strain, as compared to the wild type, and its induction by transcription was not affected.

Since LTR recombination is not completely abolished in *rad52* strains, and can be induced by transcription, we conclude that these events are carried out, in wild-type strains, via at least two different mechanisms, one dependent and the other independent of *RAD52*. It has been proposed, for example, that the *RAD52*-independent pathway of direct-repeat recombination is carried out by single-strand annealing, while one-end invasion mechanisms require the presence of the *RAD52* gene product (PRADO and AGUILERA 1995). In an analysis of recombination between direct repeats at the *GAL10* locus (THOMAS and ROTHSTEIN 1989), mutations in the *RAD52* gene lowered the basal level of recombination by only twofold, but abolished the transcriptional induction. It is therefore possible that the induction of recombination between the Tys' 300-bp-long LTRs takes place by a different mechanism from that of recombination between the much bigger (2.6 kb) *GAL10* direct repeats.

The rate of cDNA-mediated Ty conversions can be induced by the presence of large amounts of cDNA of a donor Ty in *rad52* and *RAD52* strains, despite the lower spontaneous levels seen in *rad52* strains. ORR-WEAVER *et al.* (1981) and MEZARD and NICOLAS (1994) investigated intermolecular recombination involving linear DNA molecules and concluded that the *RAD52* gene product was required for this kind of event. The linear Ty cDNA molecule, therefore, seems to undergo recombination via a different, *RAD52*-independent mechanism, which remains to be elucidated.

The role of *RAD1*: The *RAD1* gene plays a central role in nucleotide excision repair, and has been also shown to participate in direct-repeat recombination, where it is believed to act by removing overhanging strands left after annealing of the repeats (FISHMAN-LOBELL and HABER 1992). Our results imply that this gene may play a third role, in the induction of gene conversion following transcription. Although a mutation in *RAD1* did not affect the basal level of Ty recombination, transcription-induced conversion was almost completely abolished (Table 2). In a previous study of *Pol*II-dependent transcriptional induction of recombination between direct repeats, the rate of conversion was not affected by mutations in *RAD1* (ZEHFUS *et al.* 1990). The different results may imply a role for the RNA polymerase (or its associated factors) in the recombinational induction. For example, an interaction between the *RAD1* gene product and RNA *Pol*II may be required for the induction, whereas *Pol*II may be able to exert its effect without Rad1p. Alternatively, the differences may be due to special properties of Ty chromatin or DNA topology, which require the action of the *RAD1* gene for the transcriptional induction. Finally, it is possible that the *RAD1* gene product is dispensable when the interacting genes are located in close proximity on the chromosome (ZEHFUS *et al.* 1990) but is required for ectopic interactions, as in our system.

The *RAD1* gene product has been shown to be involved in recombination between several types of direct repeats (KLEIN 1988; SCHIESTL and PRAKASH 1989), including the LTRs of Ty elements (LIEFSHITZ *et al.* 1995). In our system, the basal rate of recombination between the LTR elements of TyUra was, as expected, slightly lowered in the *rad1* mutant. This type of recombination, however, showed an induction similar to the one obtained in the wild-type strains following transcription. Similar results were obtained by THOMAS and ROTHSTEIN (1989) in a study of direct-repeat recombination at the *GAL10* locus.

This study shows that the *RAD1* gene product is not essential for Ty cDNA recombination. Since a linear molecule (as Ty cDNA) may not require any further processing before recombination, interactions between this molecule, as a donor of information, and the chromosome would not be expected to be impaired in *rad1* mutant strains. Interestingly, conversion events of chromosomal sequences by linear molecules has been shown to be increased in *rad1* strains (SAFFRAN *et al.* 1994).

Mutations in *rad52* and *rad1* backgrounds: Our system enabled the detection of mutations occurring in the *URA3* gene in the Ty. The rate of appearance of such mutations was increased as a result of high levels of transcription, by at least 20-fold in a wild-type strain and 10-fold in *rev3* strains (Table 1). *rad52* strains showed a very high basal rate of mutations but did not show any increase following transcription. DATTA and JINKS-ROBERTSON (1995) have also reported an increase in mutations associated with high levels of transcription. In their system, however, the high basal mutation rate seen in a *rad52* strain was further induced upon transcription. The different results may be due to the different systems used, to some special property of repeated sequences when compared to unique sequences, or to the fact that Tys have additional possibilities of repair (such as LTR-LTR recombination). We have found that 65% of the mutations seen in the *rad52* strain are eliminated in the *rad52 rev3* double mutant strain, and thus are *REV3* dependent. Surprisingly, mutation rates in the *rad52 rev3* double mutant, when grown in the presence of large amounts of TyNeo cDNA (MK169/pJEF1678 in galactose, Table 1), are lower than those obtained when the same strain is grown in the absence

of such molecules (0.28×10^{-7} *vs.* 1.2×10^{-7}). This result may indicate that transcription-induced lesions created in the Ty can be repaired either by recombination or by a mutagenic repair mechanism, and that these two mechanisms compete with each other for the repair of the same lesions. Since a *rad52 rev3* strain preferentially carries out cDNA-mediated recombination when large amounts of Ty cDNA are present in the cell, the recombination pathway appears to be preferred.

What mechanism is responsible for the induction by transcription of mutation and Ty recombination, and what are the roles of the *RAD52* and *RAD1* genes in this induction? We propose two different general hypotheses: (1) these genes are needed *before* lesions are created in the DNA, or (2) they act *after* transcription has, directly or indirectly, created these lesions.

(1) Transcription is associated with changes in the DNA topology or chromatin that render the DNA more accessible to damaging agents and/or to the repair machinery. For example, in V(D)J recombination, both transcription and recombination are dependent on a chromatin configuration that makes the locus accessible to the enzymatic machinery (for review, see GANGLOFF *et al.* 1994). The *RAD52* and *RAD1* gene products may play a role in conferring a more accessible configuration to the Ty DNA. Alternatively, these genes may participate in the cross-talk between transcription factors and the recombination machinery; precedent for such an intimate relationship between transcription and excision repair has been well established (FEAVER *et al.* 1993; QIU *et al.* 1993; DRAPKIN *et al.* 1994). It is possible that in the absence of *RAD1* and *RAD52*, transcription can occur without a concomitant increase in recombination. Whatever their mechanism of action may be, by this hypothesis *RAD1* and *RAD52* are needed to facilitate the access of the recombination enzymes to the transcribed DNA.

(2) *RAD1* and *RAD52* may play a role in repairing lesions created by transcription. The passage of the transcriptional machinery may cause a relaxation of the chromatin structure or changes in the torsional stress of the DNA that may lead to lesions in the DNA. These can be repaired via several pathways, in which *RAD52* and *RAD1* participate. Thus, by this second hypothesis, *RAD1* and *RAD52* play a strictly repair role and are not involved in promoting changes in chromatin configuration.

We prefer the second proposed mechanism because it agrees better with the different phenotypes seen in the mutant strains tested. Both *RAD1* and *RAD52* are needed for the induction of conversion, but not for that of LTR recombination; on the other hand, mutation induction depends on *RAD52* but not on *RAD1*. If the *RAD52* and *RAD1* gene products were to play a role *before* the creation of lesions, we would expect that mutations in these genes would abolish all the different types of events seen following transcription. The different phenotypes of the mutants lead us to believe that the induction is due to the lesions in the DNA caused by transcription, which can be repaired via different mechanisms, some involving *RAD52* and others involving *RAD1*. Our previous results (NEVO-CASPI and KUPIEC 1994) show that *RAD51* and *RAD57* may also participate in the repair of transcription-induced lesions; each *rad* mutant investigated so far has shown a specific phenotype with respect to the different types of events induced by transcription.

A clear link between transcription and direct-repeat recombination in yeast has been established lately. Yeast cells defective for the *HPR1* gene show a high level of recombination between direct repeats (AGUILERA and KLEIN 1989). This gene seems to act as a positive regulator of transcription (ZHU *et al.* 1995). Suppressors of *hpr1* include several transcription factors and components of the transcriptional machinery (FAN and KLEIN 1994; FAN *et al.* 1996; SANTOS-ROSA *et al.* 1996). Transcription probably also affects the level of allelic and ectopic gene conversion (GANGLOFF *et al.* 1994; NEVO-CASPI and KUPIEC 1994; this study); we propose that the increase in recombination and mutation is due to the repair of lesions created in the process of transcription, either directly by the action of the transcriptional machinery and its associated factors, or indirectly, by exposing single-stranded DNA to potential damage. Once damaged, the DNA is repaired in a way that depends on the availability of nearby repeats or (for Tys) the presence of cDNA molecules; different *RAD* genes control these processes (LIEFSHITZ *et al.* 1995).

Repeated sequences in the genome are subjected to special evolutionary constraints; their level of recombination must be kept low to avoid karyotypic instability. On the other hand, the observed coevolution of the family members requires constant conversion between ectopically located repeats (EDELMAN and GALLY 1970; BALTIMORE 1981). Retroelements may use cDNA-mediated conversion to achieve this goal. The mechanism by which it is carried out still remains to be elucidated. It is not clear, for example, if a double-strand break in the recipient molecule is needed for the initiation of this type of event. During the life cycle of Ty elements the end-to-end mRNA molecule is reverse-transcribed to cDNA by Ty-encoded proteins. Several intermediate stages, including partial single- or double-stranded copies of the element could, in principle, participate as donors in a conversion event. We have shown that this type of recombination can be carried out in the absence of the main homologous recombination pathway in yeast (*i.e.*, in the absence of the *RAD50, RAD52, RAD57,* and *RAD1* gene products) (NEVO-CASPI and KUPIEC 1994; this study). The fact that families of retroelements can carry out cDNA-mediated recombination allows them to evolve independently from the rest of the genome.

A high level of ectopic recombination between dispersed elements would create chromosomal aberrations. Transcription may play a role in regulating this process. The level of Ty recombination is naturally low. Ty mRNA has been estimated to represent 10–50% of the polyA RNA in the cells (ELDER *et al.* 1983; CURCIO *et al.* 1990). It is not clear, however, whether all Tys are equally transcribed. In our studies, the level of transcription of a GalTyUra in galactose is about five- to 10-fold higher than that of a Ty under its natural promoter (Y. NEVO-CASPI, unpublished results). It is possible that the low level of Ty recombination is not correlated with the level of expression, but with a special feature of the normal transcription of these elements. For example, transcription from the natural Ty promoter may use a different enzymatic machinery that causes fewer lesions; alternatively, if lesions *are* caused by this complex, they may be repaired in a nonrecombinogenic manner. In support of this view, we have previously shown (PARKET and KUPIEC 1992) that DNA damage causes an elevation of Ty transcription from its natural promoter, without a concomitant increase in recombination. Further studies are needed to understand the complex interactions between basic cellular processes such as transcription, repair and recombination, and the role they play in the maintenance of genome stability.

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