The *Saccharomyces cerevisiae* DNA Recombination and Repair Functions of the *RAD52* Epistasis Group Inhibit Ty1 Transposition

Alison J. Rattray, Brenda K. Shafer and David J. Garfinkel

Gene Regulation and Chromosome Biology Laboratory, ABL-Basic Research Program, NCI-FCRDC, Frederick, Maryland 21702

Manuscript received June 18, 1999
Accepted for publication October 1, 1999

ABSTRACT

RNA transcribed from the *Saccharomyces cerevisiae* retrotransposon Ty1 accumulates to a high level in mitotically growing haploid cells, yet transposition occurs at very low frequencies. The product of reverse transcription is a linear double-stranded DNA molecule that reenters the genome by either Ty1-integrase-mediated insertion or homologous recombination with one of the preexisting genomic Ty1 (or δ) elements. Here we examine the role of the cellular homologous recombination functions on Ty1 transposition. We find that transposition is elevated in cells mutated for genes in the *RAD52* recombinational repair pathway, such as *RAD50*, *RAD51*, *RAD52*, *RAD54*, or *RAD57*, or in the DNA ligase I gene *CDC9*, but is not elevated in cells mutated in the DNA repair functions encoded by the *RAD1*, *RAD2*, or *MSH2* genes. The increase in Ty1 transposition observed when genes in the *RAD52* recombinational pathway are mutated is not associated with a significant increase in Ty1 RNA or proteins. However, unincorporated Ty1 cDNA levels are markedly elevated. These results suggest that members of the *RAD52* recombinational repair pathway inhibit Ty1 post-translationally by influencing the fate of Ty1 cDNA.

TY elements of yeast are members of a widely disseminated class of eukaryotic repetitive sequences functionally and structurally related to retroviruses (for reviews see Temin 1985; Boeke and Sandmeyer 1991; Flavell 1995). Ty1 elements and their solo long-terminal-repeat (LTR) derivatives (δ elements) are the most abundant dispersed repetitive sequence in *Saccharomyces cerevisiae*, representing ~4% of the genome (see Goffeau *et al.* 1996). Ty elements can destabilize the genome in two ways: *de novo* transposition can result in insertional mutagenesis of cellular genes, and homologous recombination between the dispersed copies can lead to gross chromosomal rearrangements such as translocations, inversions, and large insertions or deletions (Boeke and Sandmeyer 1991). A majority of the full-length endogenous Ty1 elements appears to be competent for transposition (Curcio *et al.* 1988; Curcio and Garfinkel 1994; Jordan and McDonald 1998; Kim *et al.* 1998) and Ty1 RNA is very abundant, comprising 0.1–1% of the total RNA in haploid cells (Elder *et al.* 1983; Curcio *et al.* 1990). However, Ty1 proteins are not abundant (Garfinkel *et al.* 1985; Curcio and Garfinkel 1992) and transposition occurs at a low rate (10^{-5} to 10^{-7} events/cell/generation; Curcio and Garfinkel 1991). Ectopic expression of Ty1 from the inducible *GAL1* promoter overcomes "transpositional dormancy" by a mechanism that is largely post-translational (Curcio and Garfinkel 1992, 1999; Conte *et al.* 1998; Lee *et al.* 1998). *De novo* Ty1 insertions occur preferentially upstream of genes transcribed by RNA polymerase III, and therefore they appear to target "nondeleterious" regions of the genome (Ji *et al.* 1993; Devine and Boeke 1996). Homologous recombination between genomic Ty1 elements occurs at rates much lower than at most artificially introduced ectopic sequences and are rarely associated with crossovers (Kupiec and Petes 1988a,b). Furthermore, recombination between the LTRs of a single element, leaving a solo δ, has been shown to remove the deleterious effects of some Ty insertions (Roeder and Fink 1980; Fink *et al.* 1986; Jordan and McDonald 1999). Taken together, these data suggest that Ty1 elements and yeast cells have a commensal relationship in which new insertions are rare, and those that do occur are targeted to "safe" sites (Boeke and Devine 1998). Since most of the Ty1 elements in the genome appear to be functional, cDNA conversion of preexisting Ty1 elements should be of little consequence, and the unusually low level of crossovers between endogenous Ty1 elements should minimize rearrangements by ectopic recombination.

The relationship between Ty1 transpositional integration and *RAD52*-mediated recombination has been examined in two ways. First, the role of Ty1 integrase (IN) has been analyzed in *spt3* cells that are defective in expression from Ty1 promoters (Winston *et al.* 1984), but not defective for expression from *GAL1*-promoted Ty1 elements (Boeke *et al.* 1986). Transposition rates from *GAL1*-promoted Ty1 elements carrying the *in-2600* mutation, which has no *in vitro* integrase activity

Corresponding author: Alison J. Rattray, Gene Regulation and Chromosome Biology Laboratory, ABL-Basic Research Program, NCI-FCRDC, P.O. Box B, Bldg. 539, Rm. 151, Frederick, MD 21702.
E-mail: rattray@mail.ncifcrf.gov

(Eichenger and Boeke 1988), are not significantly different from wild type (Sharon *et al.* 1994). However, a majority of Ty1 insertion events observed in an *in-2600* mutant are *RAD52* dependent. Interestingly, His^+ events from an endogenous Ty1*his3-AI* element are markedly elevated in a *rad52* mutant (Curcio and Garfinkel 1994; Conte *et al.* 1998; Lee *et al.* 1998), suggesting that the *RAD52* gene product normally inhibits transpositional events. Here, we expand this observation by determining the consequences of defects in other DNA recombination and repair genes and by examining what stage of the transposition process they affect.

The *RAD50–RAD57* members of the *RAD52* recombinational repair pathway were initially identified by their sensitivity to ionizing radiation and were subsequently shown to be required for most homologous recombination events. A *rad52* mutant exhibits the strongest defects (for reviews see Petes *et al.* 1991; Game 1993; Paques and Haber 1999), including a defect in cDNA-mediated homologous recombination (Derr and Strathern 1993; Sharon *et al.* 1994; Liefshitz *et al.* 1995). The Rad52p is known to bind to the ends of DNA molecules (Dyck *et al.* 1999), to promote DNA strand annealing between single-stranded oligonucleotides (Mortensen *et al.* 1996; Sugiyama *et al.* 1998), and to potentiate the strand-exchange activity of Rad51p (Sung 1997a; New *et al.* 1998; Shinohara and Ogawa 1998). The Rad51 protein is homologous to the *Escherichia coli* RecA protein and, like RecA, it forms an extended helical filament on DNA (Shinohara *et al.* 1992; Ogawa *et al.* 1993) and can promote DNA strand exchange *in vitro* (Sung 1994; Sung and Robberson 1995). The addition of one of several different factors including Rad52p, Rad54p, or the heterodimeric Rad55p/Rad57p complex stimulates strand exchange (Sung 1997a; New *et al.* 1998; Petukhova *et al.* 1998; Shinohara and Ogawa 1998). Rad55p and Rad57p are two additional yeast RecA homologs that have not been shown to possess strand-exchange activity by themselves or together (Sung 1997b), but appear to function as a heterodimeric complex facilitating Rad51p function (Johnson and Symington 1995; Sugawara *et al.* 1995; Sung 1997b). The Rad54p is a member of the *SWI2/SNF2* family of DNA-dependent ATPases (Emery *et al.* 1991) and may function by opening up the DNA for repair and recombination. *RAD50* was initially included in the *RAD52* epistasis group on the basis of its severe sensitivity to ionizing radiation (Game 1993); however Rad50p is a member of a different protein complex that also includes Mre11p and Xrs2p (Johzuka and Ogawa 1995; Usui *et al.* 1998). Rad50p and Mre11p share homology with the *E. coli* SbcCD proteins, respectively (Sharples and Leach 1995), and have both single-stranded (ss) and double-stranded (ds) DNA exonuclease and ssDNA endonuclease activity *in vitro* (Furuse *et al.* 1998; Moreau *et al.* 1999). In addition to its role in DNA damage repair and meiotic double-strand break (DSB) repair (Game 1993; Johzuka and Ogawa 1995), the Rad50p, Mre11p, and Xrs2p complex is also involved in telomere maintenance (Nugent *et al.* 1998) and nonhomologous end-joining (Schiestl *et al.* 1994; Nairz and Klein 1997; Tsukamoto *et al.* 1996), but is not required for mitotic heteroallelic recombination (Malone *et al.* 1990).

Nucleotide-excision repair (NER) is the process by which distortions of the helical structure of DNA are repaired. This complex involves the products of ~30 genes including the *RAD1* and *RAD2* genes, which participate in the recognition and incision of the damaged DNA (Gudzer *et al.* 1995). Mutations in *RAD1* or *RAD2* result in extreme sensitivity to ultraviolet light (UV) (Friedberg *et al.* 1995; Bhatia *et al.* 1996). Rad1p forms a complex with Rad10p that specifies a ssDNA endonuclease (Sung *et al.* 1993; Tomkinson *et al.* 1993; Davies *et al.* 1995). A *rad1* mutant is also deficient in a subset of mitotic recombination events that require removal of nonhomologies from the ends of the recombining molecules (Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989; Fishman-Lobell and Haber 1992; Ivanov and Haber 1995; Paques and Haber 1997). *RAD2* encodes a flap-endonuclease (Habraken *et al.* 1995) and is a subunit of the TFIIH repairosome essential for NER (Svejstrup *et al.* 1995). However, *rad2* mutants have only minor defects in mitotic recombination (Ivanov and Haber 1995).

Mismatch-directed repair (MMR) is required for the correction of mismatched bases that are generated by replication errors or by heteroduplex formation during homologous recombination (Modrich and Lahue 1996; Jiricny 1998; Prolla 1998). A *msh2* null mutant has a strong spontaneous mutator phenotype and is defective in the removal of nonhomologies generated during recombination when these are between 30 bp and ~1 kb in length (Saparbaev *et al.* 1996; Paques and Haber 1997; Sugawara *et al.* 1997). Although *MSH2* does not appear to affect the level of recombination between identical sequences, it normally inhibits recombination between slightly diverged (homeologous) sequences (Alani *et al.* 1994; Datta *et al.* 1996, 1997; Chen and Jinks-Robertson 1998). The major replicative DNA ligase is encoded by the essential *CDC9* gene (Tomkinson *et al.* 1993). Mutants show increased mitotic recombination, even when grown at the permissive temperature (Game *et al.* 1979), suggesting that these conditions do not restore wild-type levels of activity.

Our results indicate that mutations in *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD57*, and *CDC9* genes lead to an increase in Ty1 transposition, whereas mutations in the *RAD1*, *RAD2*, or *MSH2* genes do not. In all cases, the increased transposition rate is due to a post-translational mechanism because Ty1 RNA and protein levels remain at wild-type levels, but the amount of unincorporated Ty1 cDNA is significantly increased.

TABLE 1

Plasmids

Name	Description	Source
pSLH149	*msh2::hisG-URA3-hisG* disruption plasmid	S. Holbeck
pL962	*rad1::LEU2* disruption plasmid	R. Keil
pKM155	*rad2Δ::URA3* disruption plasmid	D. Higgins and K. Madula
pNKY83	*rad50::hisG-URA3-hisG* disruption plasmid	Alani *et al.* (1989)
pBDG542	*rad52::hisG-URA3-hisG* disruption plasmid	Curcio and Garfinkel (1994)
pAM28	*rad51::LEU2* disruption plasmid	M. Aker
pSM31	*rad54::LEU2* disruption plasmid	D. Schild
pSTL11	*rad55::LEU2* disruption plasmid	Lovett and Mortimer (1987)
pSM51	*rad57::LEU2* disruption plasmid	D. Schild
pMB38::9mer-WT	+1 frameshifting between TyA and TyB	Belcourt and Farabaugh (1990)
pMB38::9mer-Fusion	TyA1 and TyB1 fused in frame	Belcourt and Farabaugh (1990)
pBDG689-*ACT1*	3.5-kb *Bam*HI-*Eco*RI fragment of *ACT1* in pBS	Lee *et al.* (1998)
pBDG456-*Ty1*	1.4-kb *Hpa*I-*Sal*I fragment of *Ty1-H3* in pSP71	Curcio *et al.* (1990)
pBJC42-*his3AI*	*his3-AI* gene in pCLA12HIS3	Curcio and Garfinkel (1991)

MATERIALS AND METHODS

Media, growth conditions, and genetic methods: Rich medium (YEPD) and synthetic complete (SC) medium lacking the appropriate amino acid or nucleic acid base were prepared as described (Rose *et al.* 1990). Cells were grown at 30° unless otherwise indicated. Transformations were performed by the lithium acetate method (Ito *et al.* 1983), and tetrad dissection was performed as described previously (Rose *et al.* 1990). Methyl methane thiosulfonate (MMS; Sigma, St. Louis) was used to assay the sensitivity of strains by replica plating test strains to YEPD medium containing 0.05% MMS. The UV sensitivity of strains was scored by replica plating test strains to YEPD medium and irradiating with 150 J/m^2 at 254 nm. Forward mutation rates were measured by plating on SC − Arg + canavanine media (Rose *et al.* 1990). Genomic DNAs were isolated by the glass bead disruption method (Hoffman and Winston 1987), unless otherwise noted.

Plasmids: The plasmids utilized in this study are described in Table 1. The disruption plasmids were digested with the appropriate restriction enzymes and were used for one-step transplacement (Rothstein 1983).

Yeast strains: The strains used in this study are listed in Table 2. Mutant phenotypes of the different disruptions were scored phenotypically by measuring sensitivity to 0.05% MMS for the *rad50*, *rad51*, *rad52*, *rad54*, and *rad57* disruptions; sensitivity to UV (150 J/m^2) for the *rad1* and *rad2* disruptions; and an increase in spontaneous *CAN1* mutations for the *msh2* disruption. Gene disruptions were also confirmed by Southern blot analysis. A congenic *cdc9-1* strain, yAR314, was constructed by crossing strain JC364 with strain GRY645 and selecting spores that were temperature sensitive and papillated to His$^+$. Strain yAR314 was then backcrossed twice with strain JC364 to yield the *cdc9-1* strain yAR348, which was used in these studies.

Transposition assays: Qualitative assays were performed by spreading cells onto YEPD agar in 1.5 × 1-cm patches followed by incubation at 20° for 5 days. Cells were then replica plated to SC-histidine and incubated at 30° for 2–3 days prior to photographing. Due to the temperature sensitivity of *cdc9* strains, plates were incubated at 26° instead of at 30°, along with a wild-type control. Transposition rates were determined as described previously (Curcio and Garfinkel 1991). Briefly, 9 single colonies grown on solid YEPD media at 30° (or 26° for *cdc9* strains and a wild-type control) were resuspended in distilled water and ~1000 cells were inoculated into 3 ml YEPD broth. At this time an aliquot was removed to determine colony-forming units (cfu/ml) inoculated. Cells were then grown for 3 days at 20° with aeration and aliquots were plated to determine cfu and the proportion of His$^+$ cells. Rates were calculated from the median frequency by the Drake equation (Drake 1970). To examine whether transposition events were producting multimeric arrays, or whether there were any obvious gross changes in the distribution of transposition events, 104 independent His$^+$ colonies were isolated for each strain, purified, and DNA isolated in pools of eight. DNA was digested with *Pvu*II and separated by agarose gel electrophoresis and Southern blots were probed with a ^{32}P-labeled *his3-AI* probe, which hybridizes to the genomic junction fragment containing *his3-AI* or *HIS3* sequences.

Northern blot analysis: Total RNA was extracted by resuspending pelleted cells in 0.2 ml 0.1 m NaCl, 10 mm EDTA, 5% SDS, 50 mm Tris-HCl, pH 7.5, 0.2 ml phenol:chloroform:isoamyl alcohol 24:24:1 (PCI), and 0.3 ml glass beads. Samples were vortexed for 5 min and centrifuged. The supernatant was reextracted with PCI and precipitated with absolute ethanol. Samples were resuspended and treated with RNase-free DNase (Promega, Madison, WI), reprecipitated, and stored at −20°. Approximately 10 μg of RNA was separated electrophoretically on formaldehyde gels (Ausubel *et al.* 1994) and was blotted to Hybond N (Amersham, Piscataway, NJ) nylon membranes. Strand-specific probes were synthesized from plasmids pBDG689-*ACT1*, pBDG456-*Ty1*, and pBJC-*his3-AI* by *in vitro* transcription according to manufacturer's instructions (Promega). Filters were hybridized with either the Ty1 and actin probes or the his-*AI* and actin probes in formamide at 42° (Ausubel *et al.* 1994) and washed to a final stringency of 0.1× SSPE at 65°. Quantitation was performed by analysis on a STORM 8600 phosphorimager and analyzed with ImageQuant software. The data were normalized by dividing the total counts per minute for the Ty1 or his3-AI probe by the total counts per minute for the actin probe from the same blot and were divided by the corresponding ratio for the wild-type strain.

Protein analysis: Total protein extracts were prepared from 10^8 mid-to-late log phase cells by disrupting washed cells in 0.4 ml buffer (0.15 mm KCl, 10 mm HEPES-KOH, pH 7.8, 5 mm EDTA, 30 mm DTT, 2 mm PMSF, 1× complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany), and 0.2 ml acid-washed glass beads, followed by vortexing for

TABLE 2

Yeast strains

Name	Relevant genotype or description	Source
GRF167	*MATα his3-Δ200 ura3-167*	Boeke *et al.* (1985)
JC364	GRF167 *leu2::hisG* Ty1-*270mhis3-AI* Ty1-588*neo* Ty1-146[*tyb1::lacZ*]	Lee *et al.* (1998)
DG1141	GRF167 Ty1-242*mhis3-AI*	Curcio and Garfinkel (1991)
DG1181	DG1141 *rad2Δ::URA3*	This work
DG1520	JC364 *rad52::hisG-URA3-hisG*	Lee *et al.* (1998)
DG1644	JC364 *rad1::LEU2*	This work
DG1670	JC364 *msh2::hisG-URA3-hisG*	This work
yAR291	JC364 *rad50::hisG-URA3-hisG*	This work
yAR292	JC364 *rad54::LEU2*	This work
yAR294	JC364 *rad57::LEU2*	This work
yAR306	JC364 *rad51::LEU2*	This work
yAR314	Temperature-sensitive, His^+ papillating spores from cross between JC364 and GRY645	This work
yAR348	Temperature-sensitive spores from two successive backcrosses of yAR314 and JC364	This work
GRY645	*MATα can1 his3-Δ200 leu2-Δ1 trp1-Δ1 tyr7-1 ura3-52 cdc9*ts	J. Strathern

15 min at 4°. Supernatants were centrifuged for 5 min, and protein concentration was determined with the Bio-Rad colorimeter assay reagent (Bio-Rad, Richmond, CA). Protein (12 μg) was separated by SDS-PAGE and transferred to Immobilon P (Millipore, Bedford, MA) by a semi-dry Millipore apparatus. Immunodetection was performed with polyclonal antiserum to Ty1 virus-like particles (VLPs; Youngren *et al.* 1988) and the ECF immunodetection kit (Amersham). The blots were stripped according to manufacturer's instructions (Amersham), reprobed with antiserum to the heat-shock protein histidyl tRNA synthetase (a gift from T. Mason), and analyzed by ECF immunofluorescence. ECF immunofluorescence was visualized using a STORM 8600 phosphorimager and quantitated with ImageQuant software. Endogenous VLPs were partially purified from 1 liter of mid-to-late log phase cells grown in YEPD at 20° by sedimentation in sucrose step gradients as described previously (Eichinger and Boeke 1988). Protein (25 μg) was separated on 8% SDS-PAGE gels and transferred to Immobilon P. Western blot analysis was performed as described above by successively probing with antiserum against Ty1-IN (b2), Ty1-reverse transcriptase (RT) (b8), and Ty1-VLPs (Garfinkel *et al.* 1991), except that ECL (Amersham) and autoradiography were used for immunodetection. β-Galactosidase assays were performed on total protein glass bead extracts from 1 ml of the appropriate cells grown at 20° to mid-log phase in YEPD as described (Rose *et al.* 1990).

cDNA analysis: Approximately 500 cells from single colonies were resuspended in 10 ml YEPD broth and grown for 2 days at 20°. About 10^8 washed cells were embedded in 0.5 ml 1% LMP agarose (SeaKem) in 150 mm EDTA and digested for 10 hr with zymolyase (Seikagaku America). Cells were lysed by incubating agarose plugs in a solution containing 2 mg/ml proteinase K (Sigma) and 1% Sarkosyl (Sigma) overnight at 50° (Gerring *et al.* 1991). Approximately 50 μl of the plugs was subjected to 0.6% agarose gel electrophoresis and blotted to Hybond N^+. The resulting filters were first hybridized with randomly primed ^{32}P-labeled fragments from pBJC42-*his3-AI* or pBDG456-*Ty1*. After autoradiography and phosphorimage analysis, the blots were stripped and hybridized with a randomly primed ^{32}P-labeled 1.9-kb *Spe*I fragment from YEp24, containing sequences from the yeast 2-μm plasmid. Quantitation was performed by phosphorimage analysis using ImageQuant software. The relative increase in Ty1 cDNA is defined as the ratio of the mutant cDNA to 2-μm hybridization signal, divided by the ratio of the wild-type cDNA to 2-μm hybridization signal.

RESULTS

Recombinational-repair-deficient mutants lead to increased levels of Ty1 transposition: We used the *his3-AI* indicator gene (Curcio and Garfinkel 1991) to follow the transposition of a single genomic Ty1 element under control of its native promoter. Reverse transcription of a spliced transcript and insertion into the genome result in the formation of His^+ prototrophs, providing a phenotypic selection for reverse-transcription-mediated events. Previous results indicate that His^+ events are elevated in *rad52* mutant strains (Curcio and Garfinkel 1994; Conte *et al.* 1998; Lee *et al.* 1998), suggesting that Rad52p normally inhibits transposition. Although *rad52* mutants show the most severe defects in homologous recombination, certain recombination events are only dependent upon a subset in the *RAD52* epistasis group. Therefore, we examined the effect of mutations in other members of this repair pathway on Ty1 transposition. Isogenic strains bearing disruptions in the various *rad* genes were constructed by transplacement of the disrupted gene into the wild-type strain JC364, which has a chromosomally marked Ty1-270*his3*-AI element (Table 2). The presence of each mutation was confirmed by both phenotypic and physical analyses (see materials and methods).

Qualitative transposition assays with strains bearing the different disruptions are shown in Figure 1, and quantitative analyses are shown in Table 3. The rate of His^+ formation was 7.8×10^{-7} (events/cell/generation) in the wild-type strain JC364. We found a 24-fold increase in His^+ events in a *rad52* mutant, to a rate of

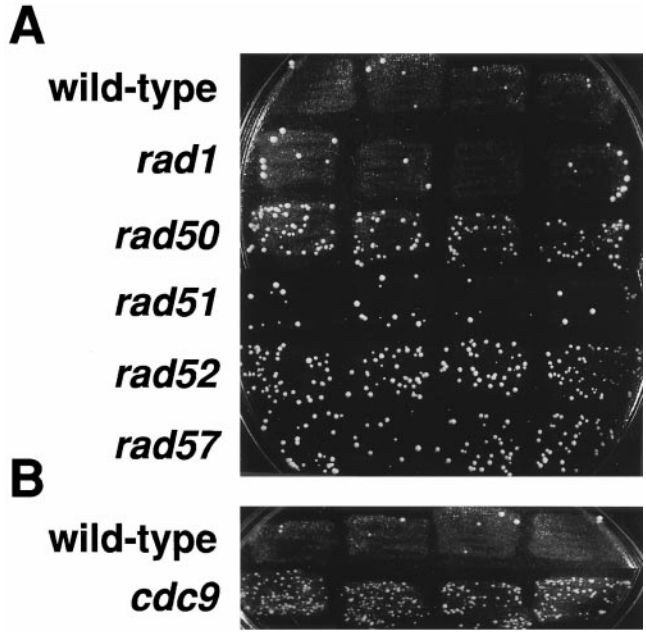

Figure 1.—Qualitative measurement of His^+ transposition events in wild-type and DNA-repair-deficient strains. Four individual colonies were patched to YEPD and were grown at 20° for 5 days, after which they were replica plated to SC-His and incubated at the indicated temperature for an additional 3 days prior to photographing. (A) Strains JC364, DG1644, yAR291, yAR306, DG1520, and yAR294. Only genotype of specific repair function disrupted in each strain is indicated. SC-His plates were incubated at 30°. (B) Strains JC364 and yAR348. SC-His plates were incubated at 26°.

1.9×10^{-5}. Although the actual level of stimulation varied when different members of the *RAD52* epistasis group were analyzed, all mutants had increased levels of transposition.

Both *RAD51* and *RAD57* encode RecA homologs that are not functionally redundant (Game 1993; Rattray and Symington 1995; Benson *et al.* 1998). Rad51p is necessary for cDNA conversion of a chromosomal Ty1 element, whereas Rad57p is not (Nevo-Caspi and Kupiec 1994; Liefshitz *et al.* 1998). We observed an 11-fold stimulation of transposition events in *rad51* mutants (Table 3). *rad57* mutants were similar to *rad52* mutants, with a 21-fold stimulation in transposition (Table 3). The recombination defect of *rad54* null mutants is most similar to that of *rad51* mutants (Game and Mortimer 1974; Saeki *et al.* 1980; Clever *et al.* 1997), and *RAD54* is also required for cDNA conversion of a chromosomal Ty1 element (Liefshitz *et al.* 1998). We found that transposition is elevated 5-fold in a *rad54* mutant (Table 3). We also observed a 12-fold stimulation of transposition in a *rad50* mutant (Table 3).

Mutants deficient in DNA ligase show increased levels of Ty1 transposition: Because of the results obtained with mutants in the members of the *RAD52* epistasis group, we hypothesized that unligated 3′ ends adjacent to a newly transposed Ty1 element might initiate recombination with a sister chromatid or by single-strand annealing that would result in removal of the element or leave a solo δ, respectively. Therefore, increased transposition in cells deficient for homologous recombination could be due to the inability to remove nascent

TABLE 3

Effect of recombination repair mutants on Ty1 transposition

	Strain[a]	Genotype[b]	*n*[c]	Median frequency His^+ (10^{-7})[d]	Fold induction
A	JC364		4	7.8 ± 1.4	1
	DG1520	*rad52*	5	190 ± 74	24
	yAR291	*rad50*	2	97 ± 14	12
	yAR306	*rad51*	3	83 ± 34	11
	yAR292	*rad54*	3	39 ± 14	5
	yAR294	*rad57*	3	160 ± 87	21
	DG1644	*rad1*	1	10	1
	DG1670	*msh2*	1	9	1
B[e]	JC364		2	7.4 ± 1.5	1
	yAR348	*cdc9*	2	300 ± 25	38
C[f]	DG1141		1	3.2	1
	DG1181	*rad2*	1	2.4	0.8

[a] Strains are described in Table 1.
[b] Only relevant genotype is indicated.
[c] *n*, number of different fluctuation tests performed.
[d] Average ± standard deviation of median frequencies from different fluctuation tests.
[e] SC-His plates incubated at 26° instead of 30°.
[f] Wild-type and *rad2* strains have different Ty1*his3-AI* elements than other strains in table.

transposition events. On the basis of the hyper-recombination phenotype of a cdc9 mutant (Game *et al.* 1979), unligated 3′ ends may survive longer in the absence of DNA ligase and may result in an even greater decrease in transposition in recombination-proficient cells. To test these ideas, we determined the level of Ty1*his3-AI* transposition in congenic strains with either the *CDC9* wild-type or the *cdc9-1* temperature-sensitive allele (see materials and methods). Surprisingly, Ty1 transposition increased 38-fold in a *cdc9-1* mutant (Table 3). This result suggests that nascent Ty1 transposition events are not removed by recombination.

***rad1*, *rad2*, and *msh2* mutants do not show increased levels of transposition:** The stimulation of transposition by mutations in DNA repair functions of the *RAD52* epistasis group, by a DNA ligase mutant, and by components of TFIIH, including alleles of the NER genes *SSL2* (*RAD25*) and *RAD3* (Lee *et al.* 1998), led us to ask whether increasing transposition was a general phenotype of repair-deficient mutants. Therefore, we mutated the NER genes *RAD1* and *RAD2*, as well as the MMR gene *MSH2*, and determined the level of Ty1*his3-AI* transposition. *RAD1* is not required for cDNA conversion of a Ty1 element (Nevo-Caspi and Kupiec 1996), although it is required for cDNA conversion of a chromosomal *his3* gene (Derr 1998). We observed wild-type levels of Ty1 transposition in a *rad1* null mutant (Figure 1; Table 3). *RAD2* is required for the NER incision step (Gudzer *et al.* 1995), and Rad2p coimmunoprecipitates with Msh2p (Bertrand *et al.* 1998). However, *rad2* null mutants only exhibit minor defects in mitotic recombination (Ivanov and Haber 1995). A mutation in *rad2* did not alter transposition levels from the wild-type strain (Table 3).

Because the presence of DNA sequence heterogeneity among the different chromosomal Ty1 elements suggests that cDNA recombination may be sensitive to the MMR system, we reasoned that mutants in MMR might be more permissive for cDNA recombination and could result in elevated levels of Ty1 transposition or cDNA recombination. However, we observed wild-type levels of His^+ formation in a *msh2* null mutant (Table 3). In the same experiment, we measured spontaneous resistance to the antimetabolite canavanine and observed a 90-fold increase in the *msh2* mutant. These results suggest that MMR does not affect the rate of Ty1 transposition, that the *msh2* mutant strain does not contain a secondary *msh2* suppressor, and that *his3-AI* does not revert even in a strain possessing a strong mutator phenotype.

In summary, we find that various DNA-repair-deficient mutants lead to elevated levels of Ty1*his3-AI* transposition. Three of the mutants are particularly interesting: a *rad52* mutant is extremely deficient in mitotic recombination and repair and has a 24-fold increase in Ty1 transposition; a *rad57* mutant is only moderately deficient in mitotic recombination and is increased for transposition 21-fold; and finally, a DNA-ligase-deficient *cdc9* mutant, which has an elevated level of mitotic recombination, also increases the rate of transposition 38-fold.

Pattern of Ty1*HIS3* insertions in a *rad52* mutant: Most *Ty1HIS3* insertions occur at multiple sites and reflect simple insertions. However, certain Ty1 insertions are composed of multimeric elements, and their formation was shown to be *RAD52* independent (Weinstock *et al.* 1990; Sharon *et al.* 1994). To determine whether we were seeing a similar phenomenon or, alternatively, insertion into only one or a few sites, we examined ~100 independent His^+ events by Southern blot analysis (in pools of 8) from the wild-type strain JC364 and the *rad52* mutant strain DG1520. DNA was digested with *Pvu*II, and Southern hybridizations were performed with a His3-specific probe, such that the hybridization represents the 3′ junction of the Ty1*His3* insertion (Figure 2). Multimeric arrays would result in a constant product of ~3 kb due to the junction fragment with a tandem Ty1 element. Although a band consistent with multimeric arrays was present in pools of the wild-type strain, this did not represent the majority of events, and there appeared to be even fewer bands migrating at the position expected for a multimeric array in the *rad52* mutant (Figure 2). Furthermore, it was possible to count ≥85 novel bands for each strain examined, indicating no extreme preference for an intergration or recombination sequence. As expected from the pool size, the genomic Ty1*his3-AI* element present in all samples hybridized with an approximately eightfold greater intensity than any single new transposition event (Figure 2). Similar results were obtained when ~100 His^+ events were examined from *rad51* or *rad57* null mutant strains (data not shown).

Ty1 mRNA levels in repair-deficient mutants: To determine whether Ty1 RNA levels were increased in the repair-deficient mutants, the steady-state levels of Ty1 RNA were examined by Northern blot analysis (see materials and methods). Duplicate Northern blots were hybridized with ^{32}P-labeled Ty1 or *HIS3* probes. Both of these probes hybridize with Ty1 RNA, except that the former will hybridize with all Ty1 transcripts, whereas the latter is specific for Ty1*his3-AI* and Ty1*HIS3* transcripts (Figure 3). Both blots were also hybridized with an actin probe as a loading control. We observed a 2.7-fold increase in the Ty1/actin RNA ratio in some of the strains. A smaller difference was noted among the different strains for the His3/actin ratio, which in all cases was <2-fold greater than that observed in the wild-type strain.

Ty1 gene expression: Because Ty1 RNA may be packaged within VLPs and therefore inaccessible to both RNA degradation and translation, a small increase in *de novo* transcription could lead to more efficient translation. We addressed this question by determining the level of Ty1 gene expression utilizing *TyA1/TyB1::lacZ*

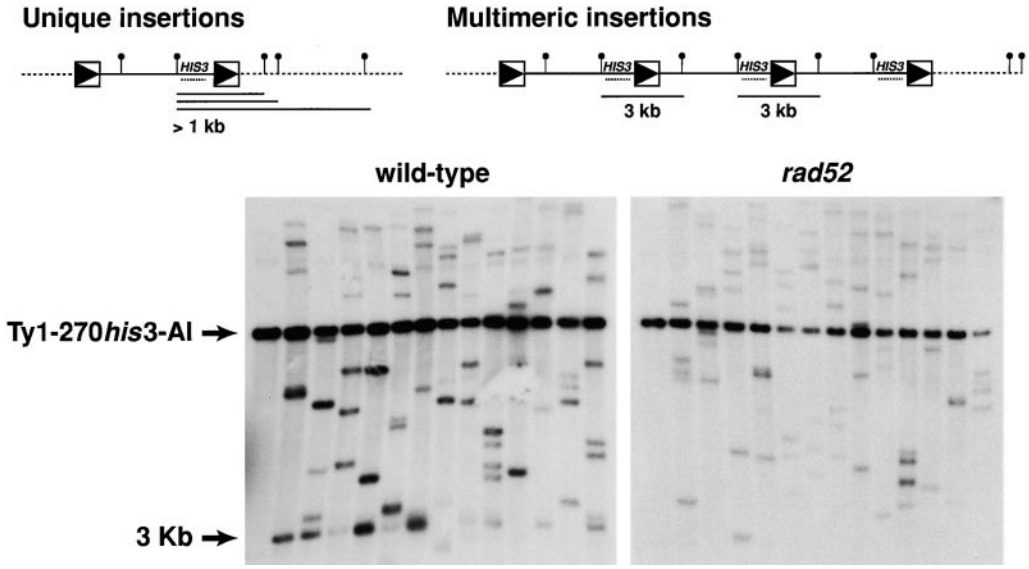

Figure 2.—Southern blot analysis of independent His^+ transpositional events in wild-type strain JC364 or in a *rad52* mutant strain DG1520. DNA was isolated from pools of eight independent His^+ colonies, digested with *Pvu*II, separated by agarose gel electrophoresis, and probed with *HIS3*. The strong band marked *his3-AI* represents the endogenous Ty1*his3AI*-270. The arrow points to the expected position for multimeric arrays of Ty1 insertions.

fusion plasmids and by determining the amount of TyA1 protein present in the repair-deficient mutants. β-Galactosidase levels were measured in whole cell extracts from two different *TyB1::lacZ* fusion plasmids. All of the strains also harbored a genomic Ty1 element with a *TyB1::lacZ* fusion construct in the genome (Ty1-146, Table 2). The strains were also transformed with either plasmid pMB38-9mer (wild type), where the *TyA1* and *TyB1* coding sequences are in their natural configuration, and *lacZ* translation requires a +1 frameshift near the end of the TyA1 open reading frame (Belcourt and Farabaugh 1990), or with plasmid pMB38-9mer (fusion), where the *TyA1*/*TyB1::lacZ* is expressed as a single open reading frame (Belcourt and Farabaugh 1990). The genomic Ty1-146 element was expressed at extremely low levels (no plasmid, Table 4) and was measured to ensure that it was not contributing significantly to the β-galactosidase activity derived from the plasmid-borne constructs. No significant differences were observed in any of the recombination repair mutants harboring plasmid pMB38-9mer (fusion). We conclude that the small differences detected in our Northern blot analysis are unlikely to contribute significantly to the translation efficiency of Ty1 RNA in the repair-deficient mutants. We also did not detect a significant difference in β-galactosidase for the pMB-9mer (wild-type) plasmid. As expected, β-galactosidase activity observed in pMB-9mer (wild-type) is ~10-fold lower than that observed in pMB-9mer (fusion), due to the programmed frameshifting event required for translation of *lacZ* (Clare *et al.* 1988; Belcourt and Farabaugh 1990). Therefore, Ty1 frameshifting does not appear to be affected in the mutant strains. Taken together, these results suggest that the rates of protein synthesis in the repair-deficient strains and in the wild-type strain are similar.

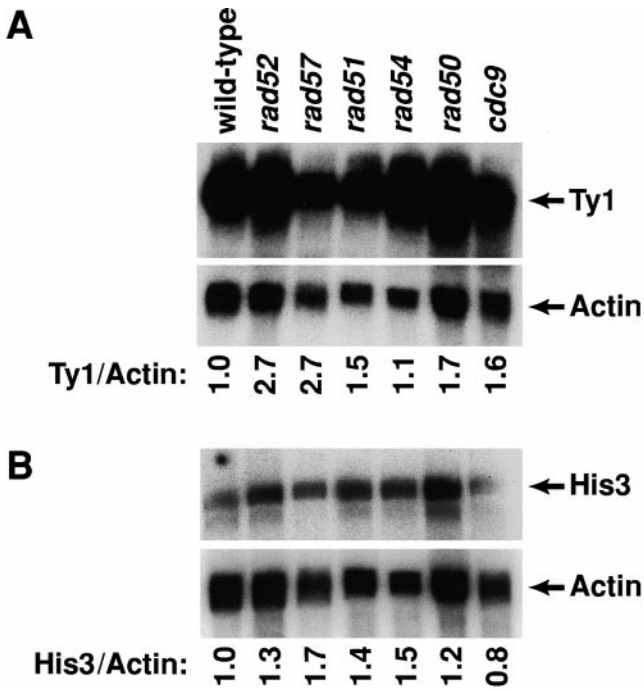

Figure 3.—Analysis of Ty1 RNA in DNA-repair-deficient strains. Northern blots were probed with either antisense Ty1 and antisense actin ssRNA probes (A) or with sense His3 and antisense actin ssRNA probes (B). Strains JC364, DG1520, yAR294, yAR306, yAR292, and yAR348. Only the relevant differences in genotype are shown. Hybridization signals were quantitated by phosphorimage analysis, and the Ty1/actin or His3/actin ratios were normalized to the wild-type control, which is considered to be 1×.

Ty proteins in repair-deficient mutants: Total protein was isolated from both wild-type cells and from repair-deficient cells, separated by SDS-PAGE electrophoresis, and transferred to membranes for Western blot analysis. The blots were first probed with a polyclonal antiserum directed against total Ty1-VLPs, which primarily recognizes TyA1 protein (Garfinkel *et al.* 1991). After immu-

TABLE 4

β-Galactosidase assays with Tyb::LacZ fusions

Strain[a]	Genotype[b]	Units of β-galactosidase[c]		
		No plasmid[d]	pMB38-9mer (WT)[e]	pMB38-9mer (fusion)[e]
JC364		1.8 ± 0.3	23 ± 5	243 ± 46
DG1520	*rad52*	2.3 ± 0.9	14 ± 6	184 ± 43
yAR291	*rad50*	2.3 ± 0.4	25 ± 0.4	172 ± 78
yAR306	*rad51*	1.6 ± 0.1	23 ± 1	220 ± 78
yAR292	*rad54*	2.1 ± 0.2	17 ± 7	324 ± 108
yAR294	*rad57*	2.3 ± 0.3	15 ± 4	173 ± 18
yAR348	*cdc9*	3.5 ± 0.4	28 ± 5	237 ± 55

[a] Strains are described in Table 1.
[b] Only relevant genotype is indicated.
[c] Units are nmol of ONPG converted/mg protein/minute.
[d] Average of three experiments.
[e] Average of two experiments.

nodetection and phosphorimage analysis, the blots were stripped and reprobed with antiserum directed against the histidyl tRNA synthetase (Hts1p) protein, which is abundantly expressed in yeast cells and is not expected to vary among the repair-deficient strains. We calculated the ratio of TyA1 to Hts1p for each of the strains, and divided this by the ratio in the wild-type strain. As a control, we also included an *spt3* mutant, which, as expected, has undetectable levels of TyA1 protein, but shows equivalent levels of Hts1p (Figure 4A). Phosphorimage analysis indicates that the level of TyA1 protein remains at the wild-type level in the different repair-deficient strains. Because of the low abundance of TyB1 proteins, we were unable to detect these proteins from total protein extracts.

Endogenous Ty1-VLPs were partially purified from total protein extracts from each of the repair-deficient mutants by sucrose sedimentation. Ty1 proteins do not constitute a major component of the sucrose-enriched fractions when purified from uninduced cells; however, the enrichment was sufficient to detect TyB1 proteins. A Western blot of the partially purified VLPs was prepared and was probed with antiserum directed against IN (b2). After immunodetection by ECL, the blot was stripped and was probed with antiserum directed against RT (b8), and immunodetection was also performed using ECL. The blots were then stripped a second time and were probed with anti-TyA1 antiserum, followed by ECL immunodetection. The presence of IN, RT, and TyA1 in VLPs prepared from wild-type, *rad50*, *rad52*, *rad57*, and *cdc9* strains is shown in Figure 4B. Note that although the same amount of total protein was loaded for each strain, the relatively minor contribution from VLPs in these partially purified preparations resulted in significant variation of Ty1 proteins from one preparation to another (perhaps dependent upon the efficiency of breakage with glass beads; Conte *et al.* 1998). We also measured the reverse transcriptase activity

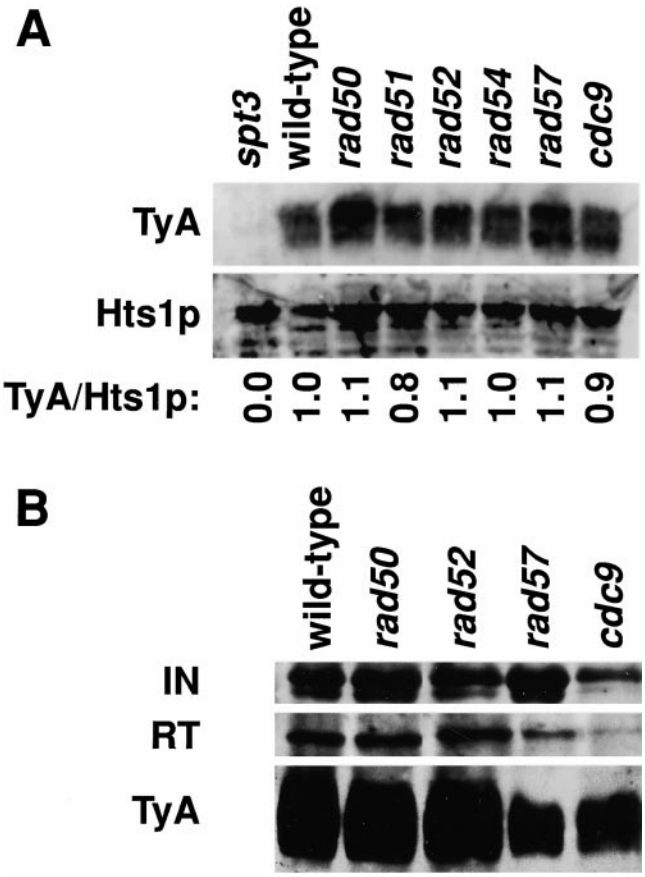

Figure 4.—Western blots of Ty1 proteins in DNA-repair-deficient strains. (A) Electrophoretically separated and transferred aliquots of total protein extracts were probed with antiserum generated against VLPs, which mainly recognizes the TyA p54 and p58 proteins. The blots were then reprobed with antiserum against the abundant heat-shock protein Hts1p. The relative ratio of the signals for anti-Ty to anti-Htsp1 quantitated by ECF and phosphorimage analysis was normalized to 1× for the wild-type strain (JC364). (B) VLP preparations from repair-deficient strains. Genotype is shown only for the relevant DNA repair gene mutated in each strain.

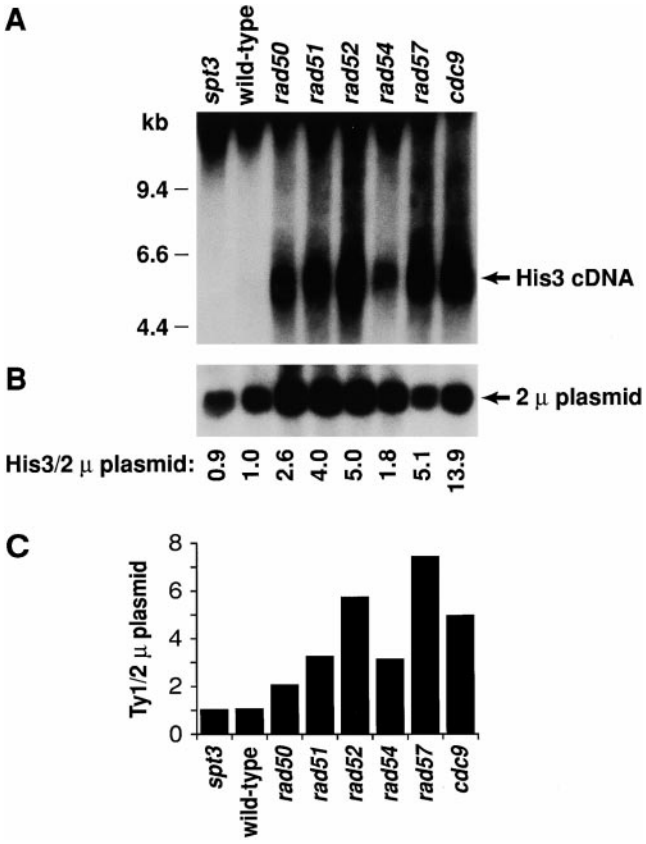

Figure 5.—cDNA quantitation from repair-deficient strains. Southern blot of total undigested DNA isolated from agarose plugs. (A) *his3-AI* probe. (B) 2-μm plasmid probe. Numbers below B indicate the quantitation of cDNA calculated by dividing the ratio of *his3* counts per minute to 2-μm cpm for each strain by the same ratio for the wild-type strain (such that the wild-type ratio is equal to 1×). (C) Quantitation from similar blots, where Ty1 internal sequences were used as the probe. Genotype is shown only for the relevant DNA repair gene mutated in each strain.

across the sucrose gradient, and found that the amount of activity, although low, accurately reflected the relative amount of VLP proteins present in each of the preparations (data not shown).

Analysis of cDNA levels in repair-deficient mutants: To determine whether the increased level of transposition in the repair-deficient mutants was due to increased levels of Ty1 cDNA, cells were grown to mid-to-late log phase, and DNA was isolated by a gentle procedure to minimize shearing of the genomic DNA (see materials and methods). Unincorporated linear Ty1 cDNA is ~6 kb in size and should migrate below the bulk genomic DNA. Undigested DNA was transferred to nylon filters following electrophoresis and the resulting blots were hybridized with ^{32}P-labeled probes for Ty1 or His3 sequences (Figure 5A). After autoradiography and phosphorimage analysis, the blots were stripped and rehybridized with a ^{32}P-labeled 2-μm plasmid probe (Figure 5B). We used 2-μm DNA as an internal standard because it migrates separately from the total genomic DNA, as does Ty1 cDNA. As a control, we also included an *spt3* strain, which should contain very little Ty1 cDNA. Our relative proportions of cDNA are calculated as the ratio of His3 to 2-μm signal from each strain, divided by this ratio from the wild-type strain (such that the wild-type strain is equal to 1), and these numbers are shown below each lane (Figure 5B). A similar blot was probed with a ^{32}P-labeled Ty1 probe, stripped, and rehybridized with the 2-μm plasmid probe. The ratio of the Ty1 to 2-μm signal (relative to the wild type) is shown in Figure 5C. Whereas the cDNA levels in the wild-type strain were not significantly above the *spt3* negative control, there was a significant amount of cDNA detectable in all of the repair-deficient mutants examined. Therefore, our quantitation probably is a minimum estimate for the fold increase of cDNA detected in the repair-deficient mutants over wild type. We conclude that all of these mutants have significantly increased amounts of Ty1 cDNA.

DISCUSSION

The results presented here permit two conclusions and raise several important issues concerning how Ty1 elements and their yeast host coexist. First, all of the members of the *RAD52* epistasis group examined inhibit Ty1 transposition post-translationally by limiting the accumulation of unincorporated cDNA. Second, our results suggest that the MMR pathway does not inhibit Ty1 transposition and reinforce earlier work indicating that the NER pathway also is not inhibitory (Lee *et al.* 1998). The *S. cerevisiae* genome contains ~30 dispersed copies of Ty1, most of which are competent for transposition (Curcio *et al.* 1988; Curcio and Garfinkel 1994; Jordan and McDonald 1998; Kim *et al.* 1998). Paradoxically, Ty1 RNA is very abundant (Elder *et al.* 1983; Curcio *et al.* 1990), yet Ty1 transposition is a rare event (Curcio and Garfinkel 1991). There are multiple steps during retrotransposition that could be modulated by host functions. In general terms, Ty1 transposition involves transcription of the element, synthesis of Ty1 proteins, packaging of Ty1 RNA and met-tRNA into VLPs, maturation of VLP proteins, reverse transcription to produce a double-stranded linear cDNA, transport of the cDNA into the nucleus, and finally, incorporation of the cDNA into the genome by either Ty1 IN or cellular homologous recombination functions (for reviews see Boeke and Sandmeyer 1991; Sandmeyer 1992). Host functions that destabilize Ty1 proteins (Conte *et al.* 1998), minimize insertional mutagenesis of genes transcribed by RNA polymerase II (Liebman and Newnam 1993; Huang *et al.* 1999), and prevent the accumulation of Ty1 cDNA (Lee *et al.* 1998) have been identified.

Given that DNA-repair-deficient mutants are likely to accumulate higher levels of endogenous unrepaired DNA lesions than repair-proficient cells and that Ty1

transcript levels are increased in cells that have been treated with DNA-damaging agents (McClanahan and McEntee 1984, 1986; Rolfe 1985; Ruby and Szostak 1985; Bradshaw and McEntee 1989), it is formally possible that repair-deficient cells are induced for Ty1 gene expression. For example, *rad52* mutant cells constitutively transcribe the DNA damage response gene, *DDR2*, whose message is normally undetectable in the absence of DNA damage (McClanahan and McEntee 1986). Also, temperature-sensitive mutants of DNA ligase I, *cdc9-1*, grow slowly (Tomkinson *et al.* 1993) and have increased mitotic recombination (Game *et al.* 1979) even at the permissive temperature. Both of these phenotypes may result from an increase in nicked DNA created during DNA replication. However, we do not observe a significant change in the RNA transcript levels of either the *his3-AI*-marked Ty1 or of Ty1 elements in any of the repair-deficient strains examined.

We have examined the accumulation of Ty1 proteins to determine whether members of the *RAD52* epistasis group affect protein accumulation. Mutants of the haploid-specific mitogen-activated protein (MAP) kinase *fus3* also have elevated rates of transposition (Conte *et al.* 1998), and Ty1 proteins are greatly stabilized in the absence of *FUS3*, suggesting that Fus3 phosphorylation leads either directly or indirectly to degradation of Ty1 proteins. A similar mechanism is probably not operating in the DNA-repair-deficient mutants, because the total amount of Ty1 proteins and the ratio of TyA1/TyB1 proteins remain unchanged in the mutants (Figure 4). However, we have not measured the rates of synthesis of the various proteins, or their half-lives, so we cannot rule out subtle changes in Ty1 protein synthesis or stability.

The finding that Ty1 transposition increases in a *cdc9* mutant is surprising and has led us to consider the possibility that Ty1 might transpose to sites of DNA damage. Interestingly, Ty sequences are occasionally found at the sites of DSBs when homologous recombination is blocked either by a *rad52* mutation or by lack of a homologous donor sequence (Moore and Haber 1996; Teng *et al.* 1996; Garfinkel 1997), and mutants in the *rad52* epistasis group could have elevated levels of spontaneous unrepaired DSBs. However, the capture of Ty sequences at the sites of unrepairable DSBs is rare (Moore and Haber 1996; Teng *et al.* 1996), does not include intact Ty elements, and requires the *RAD50* gene (Teng *et al.* 1996). Because we find that a null *rad50* mutant also results in an elevated level of Ty1 transposition, it is unlikely that our observed increase in His^+ frequency is due to "rescue" of DNA damage by Ty1 elements.

A striking correlation exists between increased transposition in DNA-repair-deficient mutants and increased levels of unincorporated Ty1 cDNA. A similar phenotype is observed in certain mutants of the TFIIH RNA polymerase II complex, which is involved in both RNA polymerase II transcription inititation and in NER (Orphanides *et al.* 1996; Svejstrup *et al.* 1996). One allele of the essential gene *SSL2/RAD25* has been isolated in a screen for hypertransposition mutants (Lee *et al.* 1998). Additional mutant alleles of this gene as well as in another component of TFIIH, *RAD3*, also cause increased Ty1 transposition and Ty1 cDNA accumulation, but do not affect Ty1 RNA or proteins. Because other alleles of *SSL2* and *RAD3* that are defective for NER do not result in hypertransposition or increased cDNA levels, the NER functions of TFIIH are not required to inhibit Ty1 transposition (Lee *et al.* 1998). Here, we provide additional evidence that NER functions are not important for inhibiting Ty1 transposition, because null mutants of *RAD1* and *RAD2*, which are required for NER but are not part of the core TFIIH complex, do not cause high levels of Ty1 transposition.

To gain insight into the mechanism by which homologous recombination and DNA repair functions might be affecting the accumulation of Ty1 cDNA, we consider several possible common similarities between a Ty1 cDNA molecule and a recombination or repair substrate. A DNA DSB, produced for example by endonucleases or ionizing radiation, is a potent recombination initiator (Game and Mortimer 1974; Strathern *et al.* 1982; Rudin *et al.* 1989; McGill *et al.* 1993), whose repair is strongly dependent upon the genes of the *RAD52* epistasis group (Game 1993; Sugawara *et al.* 1995). One feature that a Ty1 cDNA molecule shares with a DSB is the presence of (nontelomeric) DNA ends. Physical analyses of a DNA molecule after the introduction of a DSB *in vivo* show degradation of the 5′ ends of the DNA from the site of the break (Strathern *et al.* 1982; Sun *et al.* 1989; Cao *et al.* 1990; Haber 1995). Normal early degradation of the 5′ ends from the site of a DSB is still seen in mutants of the *rad52* epistasis group, but is generally far more extensive, and recombinant products are formed more slowly (Sugawara *et al.* 1995; Ivanov *et al.* 1996).

Perhaps there is a competition for cDNA ends between the DNA repair machinery and Ty1 IN. Limited IN DNA-binding activity might be insufficient to protect many of the cDNA ends, and degradation of the ends by the DNA repair machinery would remove sequences required for IN recognition. We propose that even if most cDNA molecules are acted upon by the DNA homologous recombination machinery, most do not result in His^+ cells via homologous recombination even though Ty1 cDNA can act as a donor in recombination (Melamed *et al.* 1992; Derr and Strathern 1993; Sharon *et al.* 1994; Nevo-Caspi and Kupiec 1996). Although a broken molecule can act as a donor in a homologous recombination event, such as in one-step gene replacement (Orr-Weaver *et al.* 1981; Rothstein 1983), it is the recipient of information >90% of the time (Strathern *et al.* 1982; McGill *et al.* 1993).

cDNA conversion of a chromosomal Ty1 element is

dependent on *RAD52*, *RAD51*, and *RAD54*, but not on *RAD57* (Liefshitz *et al.* 1995). If we assume that the ability to convert a chromosomal Ty1 element is related to the ability of a cDNA to function as a donor of His^+ events, then we might expect that in *rad52* mutants this pathway would be unable to lead to His^+ events, whereas in *rad57* mutants His^+ events should only be marginally affected. However, we see similar increases in unincorporated cDNA and Ty1*his3AI* transposition in both *rad52* and *rad57* mutants. Therefore, cDNA conversion of chromosomal Ty1 elements is probably not contributing to His^+ events in our experiments. Furthermore, events resulting in His^+ cells in a *rad52* mutant are most likely transpositional insertions mediated by IN (Sharon *et al.* 1994; Liefshitz *et al.* 1995; Nevo-Caspi and Kupiec 1997). Although Rad52p and Rad57p may both be components of the same recombination complex, we cannot rule out the possibility that the increased level of cDNA in the absence of Rad52p or Rad57p is caused by different mechanisms. Interestingly, a *rad57* null mutant is reasonably competent for homologous recombination and DNA repair at 30°, but is impaired for both recombination and repair at 20° (Lovett and Mortimer 1987; Kans and Mortimer 1991; Rattray and Symington 1995). Ty1 transposition is also a temperature-sensitive process, with a temperature optimum between 15° and 20° (Paquin and Williamson 1984). Perhaps the recombination complex forms inefficiently at 20°, and Rad57p acts by stabilizing the complex. It would be interesting to determine whether the level of Ty1 cDNA is different at 20° and at 30° in a *rad57* mutant strain.

We next consider the possibility that many of the cDNA molecules do serve as donors for conversion of chromosomal Ty1 elements, but that encountering the nonhomology provided by the *HIS3* gene aborts recombination, leading to a reversal of the initiation event (thus preserving the cDNA molecule). We consider this "recombination inititation/abortion" model unlikely because the increase in unincorporated cDNAs observed in the repair-deficient mutants is not specific for the *his3-AI*-marked element, but is also observed for unmarked Ty1 cDNAs. Furthermore, Msh2p and Msh3p have been shown to play a role in aborting or resolving certain recombination events (Paques and Haber 1997; Sugawara *et al.* 1997). However, a *msh2* null mutation does not stimulate Ty1*his3-AI* transposition. Finally, the *RAD1* gene is required for cDNA-mediated conversion of a chromosomal target (Derr 1998) and for some cDNA-mediated conversion events of genomic Ty1 elements (Nevo-Caspi and Kupiec 1996). If we assume that cDNA conversion of a genomic Ty1 element in our experiments also requires *RAD1*, then we would expect a large decrease in His^+ events in a *rad1* mutant. However, we observe that a *rad1* null mutant exhibits wild-type levels of Ty1 transposition. Therefore, unproductive or productive cDNA conversion of chromosomal Ty1 elements does not appear to contribute significantly to the His^+ events measured here.

We have not directly determined whether the increase in cDNA levels is due to increased cDNA synthesis or increased cDNA stability. Above, we have argued in favor of increased cDNA stability, in keeping with our knowledge about the process of DNA repair. Furthermore, we saw no stimulation in reverse transcriptase protein or activity in VLPs from the different mutants.

Now that the complete sequence of an *S. cerevisiae* laboratory strain is known, we can understand further the natural history of Ty elements and how transpositional dormancy is established and maintained. Most of the Ty1 elements appear to be functional, and codon bias analysis suggests that they are under selection (Jordan and McDonald 1998, 1999; Curcio and Garfinkel 1999). In addition, Ty1 elements have a narrow distribution and/or copy number within the genus Saccharomyces (Fink *et al.* 1981; K. G. Weinstock, D. J. Garfinkel and J. Strathern, unpublished results), and within wild isolates of *S. cerevisiae* (Eibel *et al.* 1981; Wilke *et al.* 1992). Together, these results suggest that Saccharomyces has efficient mechanisms to keep Ty1 elements out of the yeast genome and to largely inhibit its transpositional activity once the element is established (Curcio and Garfinkel 1999). Perhaps one reason that Saccharomyces has evolved such an efficient DNA repair system is to prevent both horizontal transmission and subsequent uncontrolled retrotransposition of Ty elements.

The authors thank Joan Curcio, Dwight Nissley, Jeffrey Strathern, and Bum Soo Lee for discussions, ideas, and for critical review of the manuscript. We also thank Sharon Moore, Lori Rinckel, and Shirong Zhang for sharing reagents and providing technical guidance, and Richard Fredrickson for the artwork. This research was sponsored by the National Cancer Institute, Department of Health and Human Services (DHHS), under contract with ABL. The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does any mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

LITERATURE CITED

Alani, E., S. Subbiah and N. Kleckner, 1989 The yeast *RAD50* gene encodes a predicted 153-kd protein containing a purine nucleotide binding domain and two large heptad-repeat regions. Genetics **122:** 47–57.

Alani, E., R. A. Reenan and R. D. Kolodner, 1994 Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. Genetics **137:** 19–39.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.* (Editors), 1994 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Belcourt, M. F., and P. J. Farabaugh, 1990 Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell **62:** 339–352.

Benson, F. E., P. Baumann and S. C. West, 1998 Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. Nature **391:** 401–404.

Bertrand, P., D. Tishkoff, N. Filosi, R. Dasgupta and R. Kolodner, 1998 Physical interaction between components of

DNA mismatch repair and nucleotide excision repair. Proc. Natl. Acad. Sci. USA **95:** 14278–14283.

Bhatia, P., Z. Wang and E. Friedberg, 1996 DNA repair and transcription. Curr. Opin. Genet. Dev. **6:** 146–150.

Boeke, J. D., and S. Devine, 1998 Yeast retrotransposons: finding a nice quiet neighborhood. Cell **93:** 1087–1089.

Boeke, J. D., and S. B. Sandmeyer (Editors), 1991 *Yeast Transposable Elements.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Boeke, J. D., D. J. Garfinkel, C. A. Styles and G. R. Fink, 1985 Ty elements transpose through an RNA intermediate. Cell **40:** 491–500.

Boeke, J. D., C. A. Styles and G. R. Fink, 1986 *Saccharomyces cerevisiae SPT3* gene is required for transposition and transpositional recombination of chromosomal Ty elements. Mol. Cell. Biol. **6:** 3575–3581.

Bradshaw, V. A., and K. McEntee, 1989 DNA damage activates transcription and transposition of yeast Ty retrotransposons. Mol. Gen. Genet. **218:** 465–474.

Cao, L., E. Alani and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

Chen, W., and S. Jinks-Robertson, 1998 Mismatch repair proteins regulate heteroduplex formation during mitotic recombination in yeast. Mol. Cell. Biol. **18:** 6525–6537.

Clare, J., M. Belcourt and P. Farabaugh, 1988 Efficient translational frameshifting occurs within a conserved sequence of the overlap between two genes of a yeast Ty1 transposon. Proc. Natl. Acad. Sci. USA **82:** 2829–2833.

Clever, B., H. Interthal, J. Schmuckli-Maurer, J. King, M. Sigrist *et al.*, 1997 Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. EMBO J. **16:** 2535–2544.

Conte, D., Jr., E. Barber, M. Banerjee, D. J. Garfinkel and M. J. Curcio, 1998 Post-translational regulation of Ty1 retrotransposition by mitogen-activated protein kinase Fus3. Mol. Cell. Biol. **18:** 2502–2513.

Curcio, M., and D. Garfinkel, 1999 New lines of host defense: inhibition of Ty1 retrotransposition by Fus3p and NER/TFIIH. Trends Genet. **15:** 43–45.

Curcio, M. J., and D. J. Garfinkel, 1991 Single-step selection for Ty1 element retrotransposition. Proc. Natl. Acad. Sci. USA **88:** 936–940.

Curcio, M. J., and D. J. Garfinkel, 1992 Posttranslational control of Ty1 retrotransposition occurs at the level of protein processing. Mol. Cell. Biol. **12:** 2813–2825.

Curcio, M. J., and D. J. Garfinkel, 1994 Heterogeneous functional Ty1 elements are abundant in the *Saccharomyces cerevisiae* genome. Genetics **136:** 1245–1259.

Curcio, M. J., N. J. Sanders and D. J. Garfinkel, 1988 Transpositional competence and transcription of endogenous Ty elements in *Saccharomyces cerevisiae*: implications for regulation of transposition. Mol. Cell. Biol. **8:** 3571–3581.

Curcio, M. J., A. M. Hedge, J. D. Boeke and D. J. Garfinkel, 1990 Ty RNA levels determine the spectrum of retrotransposition events that activate gene expression in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **220:** 213–221.

Datta, A., A. Adjiri, L. New, G. F. Crouse and S. Jinks Robertson, 1996 Mitotic crossovers between diverged sequences are regulated by mismatch repair proteins in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:** 1085–1093.

Datta, A., M. Hendrix, M. Lipsitch and S. Jinks-Robertson, 1997 Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc. Natl. Acad. Sci. USA **94:** 9757–9762.

Davies, A. A., E. C. Friedberg, A. E. Tomkinson, R. D. Wood and S. C. West, 1995 Role of the Rad1 and Rad10 proteins in nucleotide excision repair and recombination. J. Biol. Chem. **270:** 24638–24641.

Derr, L., and J. Strathern, 1993 A role for reverse transcripts in gene conversion. Nature **361:** 170–173.

Derr, L. K., 1998 The involvement of cellular recombination and repair genes in RNA-mediated recombination in *Saccharomyces cerevisiae*. Genetics **148:** 937–945.

Devine, S., and J. Boeke, 1996 Integration of the yeast retrotransposon Ty1 is targeted to regions upstream of genes transcribed by RNA polymerase III. Genes Dev. **4:** 324–330.

Drake, J. W., 1970 *The Molecular Basis of Mutation*. Holden Day, Oakland, CA.

Dyck, E. V., A. Stasiak, A. Stasiak and S. West, 1999 Binding of double-strand breaks in DNA by human Rad52 protein. Nature **398:** 728–731.

Eibel, H., J. Gafner, A. Stotz and P. Philippsen, 1981 Characterization of the yeast mobile element Ty1. Cold Spring Harbor Symp. Quant. Biol. **45:** 609–617.

Eichinger, D. J., and J. D. Boeke, 1988 The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54:** 955–966.

Elder, R. T., E. Y. Loh and R. W. Davis, 1983 RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80:** 2432–2436.

Emery, H. S., D. Schild, D. E. Kellogg and R. K. Mortimer, 1991 Sequence of *RAD54*, a *Saccharomyces cerevisiae* gene involved in recombination and repair. Gene **104:** 103–106.

Fink, G., P. Farabaugh, G. Roeder and D. Chaleff, 1981 Transposable elements (Ty) in yeast. Cold Spring Harbor Symp. Quant. Biol. **45:** 575–580.

Fink, G., J. Boeke and D. Garfinkel, 1986 The mechanism and consequences of retrotransposition. Trends Genet. **2:** 118–123.

Fishman-Lobell, J., and J. E. Haber, 1992 Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. Science **258:** 480–484.

Flavell, A. J., 1995 Retroelements, reverse transcriptase and evolution. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **110:** 3–15.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.

Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata *et al.*, 1998 Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J. **17:** 6412–6425.

Game, J., and R. K. Mortimer, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24:** 281–292.

Game, J. C., 1993 DNA double-strand breaks and the *RAD50-RAD57* genes in *Saccharomyces.* Semin. Cancer Biol. **4:** 73–83.

Game, J. C., L. H. Johnston and R. C. von Borstel, 1979 Enhanced mitotic recombination in a ligase-defective mutant of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **76:** 4589–4592.

Garfinkel, D., 1997 Genetic loose change: how retroelements and reverse transcriptase heal broken chromosomes. Trends Microbiol. **5:** 173–175.

Garfinkel, D. J., J. D. Boeke and G. R. Fink, 1985 Ty element transposition: reverse transcriptase and virus-like particles. Cell **42:** 507–517.

Garfinkel, D. J., A. M. Hedge, S. D. Youngren and T. D. Copeland, 1991 Proteolytic processing of pol-TYB proteins from the yeast retrotransposon Ty1. J. Virol. **65:** 4573–4581.

Gerring, S., C. Conneley and P. Hieter, 1991 Positional mapping of genes by chromosome blotting and chromosome fragmentation, pp. 57–76 in *Guide to Yeast Genetics and Molecular Biology*, edited by C. Guthrie and G. Fink. Academic Press, San Diego.

Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon *et al.*, 1996 Life with 6000 genes. Science **274:** 563–567.

Gudzer, S., Y. Habraken, P. Sung, L. Prakash and S. Prakash, 1995 Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. J. Biol. Chem. **270:** 12973–12976.

Haber, J. E., 1995 In vivo biochemistry: physical monitoring of recombination induced by site-specific endonucleases. Bioessays **17:** 609–620.

Habraken, Y., P. Sung, L. Prakash and S. Prakash, 1995 Structure-specific nuclease activity in yeast nucleotide excision repair protein Rad2. J. Biol. Chem. **270:** 30194–30198.

Hoffman, C., and F. Winston, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:** 262–272.

Huang, H., J. Hong, C. Burck and S. Liebman, 1999 Host genes that affect target-site distribution of the yeast retrotransposon Ty1. Genetics **151:** 1393–1407.

Ito, H., Y. Fukada, K. Murata and A. Kimura, 1983 Transformation

of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

Ivanov, E. L., and J. E. Haber, 1995 *RAD1* and *RAD10*, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:** 2245–2251.

Ivanov, E. L., N. Sugawara, J. Fishman-Lobell and J. E. Haber, 1996 Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. Genetics **142:** 693–704.

Ji, H., D. P. Moore, M. A. Blomberg, L. T. Braiterman, D. F. Voytas *et al.*, 1993 Hotspots for unselected Ty1 transposition events on yeast chromosome III are near tRNA genes and LTR sequences. Cell **73:** 1007–1018.

Jiricny, J., 1998 Replication errors: cha(lle)nging the genome. EMBO J. **17:** 6427–6436.

Johnson, R. D., and L. S. Symington, 1995 Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. Mol. Cell. Biol. **15:** 4843–4850.

Johzuka, K., and H. Ogawa, 1995 Interaction of Mre11 and Rad50 two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. Genetics **139:** 1521–1532.

Jordan, I., and J. McDonald, 1999 Tempo and mode of Ty element evolution in *Saccharomyces cerevisiae*. Genetics **151:** 1341–1351.

Jordan, I. K., and J. F. McDonald, 1998 Evidence for the role of recombination in the regulatory evolution of *Saccharomyces cerevisiae* Ty elements. J. Mol. Evol. **47:** 14–20.

Kans, J. A., and R. K. Mortimer, 1991 Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae*. Gene **105:** 139–140.

Kim, J. M., S. Vanguri, J. D. Boeke, A. Gabriel and D. F. Voytas, 1998 Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. Genome Res. **8:** 464–478.

Klein, H. L., 1988 Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. Genetics **120:** 367–377.

Kupiec, M., and T. D. Petes, 1988a Allelic and ectopic recombination between Ty elements in yeast. Genetics **119:** 549–559.

Kupiec, M., and T. D. Petes, 1988b Meiotic recombination between repeated transposable elements in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 2942–2954.

Lee, B. S., C. P. Lichtenstein, B. Faiola, L. A. Rinckel, W. Wysock *et al.*, 1998 Posttranslational inhibition of Ty1 retrotransposition by nucleotide excision repair / transcription factor TFIIH subunits Ssl2p and Rad3p. Genetics **148:** 1743–1761.

Liebman, S., and G. Newnam, 1993 A ubiquitin-conjugating enzyme, *RAD6*, affects the distribution of Ty1 retrotransposon integration positions. Genetics **133:** 499–508.

Liefshitz, B., A. Parket, R. Maya and M. Kupiec, 1995 The role of DNA repair genes in recombination between repeated sequences in yeast. Genetics **140:** 1199–1211.

Liefshitz, B., R. Steinlauf, A. Friedl, F. Eckardt-Schupp and M. Kupiec, 1998 Genetic interactions between mutants of the 'error-prone' repair group of *Saccharomyces cerevisiae* and their effect on recombination and mutagenesis. Mutat. Res. **407:** 135–145.

Lovett, S. T., and R. K. Mortimer, 1987 Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength and mating type. Genetics **116:** 547–553.

Malone, R. E., T. Ward, S. Lin and J. Waring, 1990 The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr. Genet. **18:** 111–116.

McClanahan, T., and K. McEntee, 1984 Specific transcripts are elevated in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **4:** 2356–2363.

McClanahan, T., and K. McEntee, 1986 DNA damage and heat shock dually regulate genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 90–96.

McGill, C., B. Shafer, L. Derr and J. Strathern, 1993 Recombination initiated by double-strand breaks. Curr. Genet. **23:** 305–314.

Melamed, C., Y. Nevo and M. Kupiec, 1992 Involvement of cDNA in homologous recombination between Ty elements in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1613–1620.

Modrich, P., and R. Lahue, 1996 Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. **65:** 101–133.

Moore, J. K., and J. E. Haber, 1996 Capture of retrotransposon DNA at the sites of chromosomal double-strand breaks. Nature **383:** 644–646.

Moreau, S., J. R. Ferguson and L. S. Symington, 1999 The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. Mol. Cell. Biol. **19:** 556–566.

Mortensen, U. H., C. Bendixen, I. Sunjevaric and R. Rothstein, 1996 DNA strand annealing is promoted by the yeast Rad52 protein. Proc. Natl. Acad. Sci. USA **93:** 10729–10734.

Nairz, K., and F. Klein, 1997 mre11S—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. **11:** 2272–2290.

Nevo-Caspi, Y., and M. Kupiec, 1994 Transcriptional induction of Ty recombination in yeast. Proc. Natl. Acad. Sci. USA **91:** 12711–12715.

Nevo-Caspi, Y., and M. Kupiec, 1996 Induction of Ty recombination in yeast by cDNA and transcription: role of the RAD1 and RAD52 genes. Genetics **144:** 947–955.

Nevo-Caspi, Y., and M. Kupiec, 1997 cDNA-mediated Ty recombination can take place in the absence of plus-strand cDNA synthesis, but not in the absence of the integrase protein. Curr. Genet. **32:** 32–40.

New, J. H., T. Sugiyama, E. Zaitseva and S. C. Kowalczykowski, 1998 Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature **391:** 407–410.

Nugent, C. I., G. Bosco, L. O. Ross, S. K. Evans, A. P. Salinger *et al.*, 1998 Teleomere maintenance is dependent on activities required for end repair of double-strand breaks. Curr. Biol. **8:** 657–660.

Ogawa, T., X. Yu, A. Shinohara and E. H. Egelman, 1993 Similarity of the yeast Rad51 filament to the bacterial RecA filament. Science **259:** 1896–1899.

Orphanides, G., T. Lagrange and D. Reinberg, 1996 The general transcription factors of RNA polymerase II. Genes Dev. **10:** 2657–2683.

Orr-Weaver, T. L., J. W. Szostak and R. J. Rothstein, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

Paques, F., and J. E. Haber, 1997 Two pathways for removal of nonhomologous DNA ends during double-strand break repair in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:** 6765–6771.

Paques, F., and J. E. Haber, 1999 Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404.

Paquin, C. E., and V. M. Williamson, 1984 Temperature effects of the rate of Ty transposition. Science **226:** 53–55.

Petes, T. D., R. E. Malone and L. S. Symington, 1991 *Recombination in Yeast. The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis and Energetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Petukhova, G., S. Stratton and P. Sung, 1998 Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature **393:** 91–94.

Prolla, T., 1998 DNA mismatch repair and cancer. Curr. Opin. Cell Biol. **10:** 311–316.

Rattray, A. J., and L. S. Symington, 1995 Multiple pathways for homologous recombination in *Saccharomyces cerevisiae*. Genetics **139:** 45–56.

Roeder, G. S., and G. R. Fink, 1980 DNA rearrangements associated with a transposable element in yeast. Cell **21:** 239–249.

Rolfe, M., 1985 UV-inducible transcripts in *Saccharomyces cerevisiae*. Curr. Genet. **9:** 533–538.

Rose, M. D., F. Winston and P. Heiter, 1990 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rothstein, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

Ruby, S. W., and J. W. Szostak, 1985 Specific *Saccharomyces cerevisiae* genes are expressed in response to DNA-damaging agents. Mol. Cell. Biol. **5:** 75–84.

Rudin, N., E. Sugarman and J. E. Haber, 1989 Genetic and physical analysis of double-strand break repair and recombination in *Saccharomyces cerevisiae*. Genetics **122:** 519–534.

Saeki, T., I. Machida and S. Nakai, 1980 Genetic control of diploid recovery after γ-irradiation in the yeast *Saccharomyces cerevisiae*. Mutat. Res. **73:** 251–265.

Sandmeyer, S. B., 1992 Yeast retrotransposons. Curr. Opin. Genet. Dev. **2:** 705–711.

Saparbaev, M., L. Prakash and S. Prakash, 1996 Requirement of mismatch repair genes *MSH2* and *MSH3* in the *RAD1-RAD10* pathway of mitotic recombination in *Saccharomyces cerevisiae*. Genetics **142:** 727–736.

Schiestl, R., and S. Prakash, 1988 *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. **8:** 3619–3626.

Schiestl, R. H., J. Zhu and T. D. Petes, 1994 Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 4493–4500.

Sharon, G., T. J. Burkett and D. J. Garfinkel, 1994 Efficient homologous recombination of Ty1 element cDNA when integration is blocked. Mol. Cell. Biol. **14:** 6540–6551.

Sharples, G. J., and D. R. F. Leach, 1995 Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. Mol. Microbiol. **17:** 1215–1220.

Shinohara, A., and T. Ogawa, 1992 Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature **391:** 404–407.

Shinohara, A., H. Ogawa and T. Ogawa, 1992 Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell **69:** 457–470.

Strathern, J., A. Klar, J. Hicks, J. Abraham, J. Ivy *et al.*, 1982 Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the *MAT* locus. Cell **31:** 183–192.

Sugawara, N., E. L. Ivanov, J. Fishman-Lobell, B. L. Ray, X. Wu *et al.*, 1995 DNA structure-dependent requirements for yeast RAD genes in gene conversion. Nature **373:** 84–86.

Sugawara, N., F. Paques, M. Colaiacovo and J. E. Haber, 1997 Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. Proc. Natl. Acad. Sci. USA **94:** 9214–9219.

Sugiyama, T., J. H. New and S. C. Kowalczykowski, 1998 DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. Proc. Natl. Acad. Sci. USA **95:** 6049–6054.

Sun, H., D. Treco, N. P. Schultes and J. W. Szostak, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

Sung, P., 1994 Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science **265:** 1241–1243.

Sung, P., 1997a Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. J. Biol. Chem. **272:** 28194–28197.

Sung, P., 1997b Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. Genes Dev. **11:** 1111–1121.

Sung, P., and D. L. Robberson, 1995 DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. Cell **82:** 453–461.

Sung, P., P. Reynolds, L. Prakash and S. Prakash, 1993 Purification and characterization of the *Saccharomyces cerevisiae* RAD1/RAD10 endonuclease. J. Biol. Chem. **268:** 26391–26399.

Svejstrup, J., Z. Wang, W. Feaver, X. Wu, D. Bushnell *et al.*, 1995 Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. Cell **80:** 21–28.

Svejstrup, J. Q., P. Vichi and J. M. Egly, 1996 The multiple roles of transcription/repair factor TFIIH. Trends Biochem. Sci. **21:** 346–350.

Temin, H. M., 1985 Reverse transcription in the eukaryotic genome: retroviruses, pararetroviruses, retrotransposons, and retrotranscripts. Mol. Biol. Evol. **2:** 455–468.

Teng, S. C., B. Kim and A. Gabriel, 1996 Retrotransposon reverse-transcriptase-mediated repair of chromosomal breaks. Nature **383:** 641–644.

Thomas, B. J., and R. Rothstein, 1989 The genetic control of direct-repeat recombination in Saccharomyces: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Genetics **123:** 725–738.

Tomkinson, A. E., A. J. Bardwell, L. Bardwell, N. J. Tappe and E. C. Friedberg, 1993 Yeast DNA repair and recombination proteins Rad1 and Rad10 constitute a single-stranded-DNA endonuclease. Nature **362:** 860–862.

Tsukamoto, Y., J. Kato and H. Ikeda, 1996 Effects of mutations of *RAD50*, *RAD51*, *RAD52*, and related genes on illegitimate recombination in *Saccharomyces cerevisiae*. Genetics **142:** 383–391.

Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa *et al.*, 1998 Complex formation and functional versatility of Mre11 of budding yeast in recombination. Cell **95:** 705–716.

Weinstock, K. G., M. F. Mastrangelo, T. J. Burkett, D. J. Garfinkel and J. N. Strathern, 1990 Multimeric arrays of the yeast retrotransposon Ty. Mol. Cell. Biol. **10:** 2882–2892.

Wilke, C. M., E. Maimer and J. Adams, 1992 The population biology and evolutionary significance of Ty elements in *Saccharomyces cerevisiae*. Genetica **86:** 155–173.

Winston, F., K. Durbin and G. Fink, 1984 The *SPT3* gene is required for normal transcription of Ty elements in *S. cerevisiae*. Cell **39:** 675–682.

Youngren, S. D., J. D. Boeke, N. J. Sanders and D. J. Garfinkel, 1988 Functional organization of the retrotransposon Ty from *Saccharomyces cerevisiae*: Ty protease is required for transposition. Mol. Cell. Biol. **8:** 1421–1431.

Communicating editor: L. S. Symington