The Genetic Control of Direct-Repeat Recombination in Saccharomyces: The Effect of *rad52* and *rad1* on Mitotic Recombination at *GAL10*, a Transcriptionally Regulated Gene

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ABSTRACT

We have previously shown direct-repeat recombination events leading to loss of a plasmid integrated at the *GAL10* locus in *Saccharomyces cerevisiae* are stimulated by transcription of the region. We have examined the role of two recombination- and repair-defective mutations, *rad1* and *rad52*, on direct repeat recombination in transcriptionally active and inactive sequences. We show that the *RAD52* gene is required for transcription-stimulated recombination events leading to loss of the integrated plasmid. Similarly, Gal$^+$ events between the duplicated repeats that retain the integrated plasmid DNA (Gal$^+$ Ura$^+$ replacement events) are reduced 20-fold in the *rad52* mutant in sequences that are constitutively expressed. In contrast, in sequences that are not expressed, the *rad52* mutation reduces plasmid loss events by only twofold and Gal$^+$ Ura$^+$ replacements by fourfold. We also observe an increase in disome-associated plasmid loss events in the *rad52* mutant, indicative of chromosome gain. This event is not affected by expression of the region. Plasmid loss events in *rad1* mutant strains are reduced only twofold in transcriptionally active sequences and are not affected in sequences that are repressed. However, the *rad1* and *rad52* double mutant shows a decrease in plasmid loss events greater than the sum of the decreases in the rates of this event displayed by either single mutant in both constitutive and repressed DNA, indicating a synergistic interaction between these two genes. The synergism is limited to recombination since the *rad1 rad52* double mutant is no more sensitive when compared with either single mutant in its ability to survive radiation damage. Finally, the recombination pathway that remains in the double mutant is positively affected by transcription of the region.

GENETIC recombination in most organisms occurs by multiple pathways. In prokaryotic systems such as *Escherichia coli* or the bacteriophage λ, the isolation and characterization of mutations defective in various steps of the recombination process has contributed greatly to the understanding of the mechanism of these events *in vivo* (reviewed in SMITH 1987). In the yeast *Saccharomyces cerevisiae*, mutants defective in meiotic or mitotic recombination or both have been isolated by their sensitivity to various types of radiation or by direct recombinational screens (GAME and MORTIMER 1974; HAYNES and KUNZ 1981; KUNZ and HAYNES 1981; ESPOSITO *et al.* 1984; HOLLINGSWORTH and BYERS 1989). Meiotic recombination mutants have also been recovered in screens for sporulation-defective cells (ESPOSITO and KLAPHOLZ 1981) or in screens for meiotic lethals (ENGEBRECHT and ROEDER 1989). However, although some of these mutations have been studied quite extensively in specific assay systems, there has been little progress in ordering these genes into genetic pathways (GAME and MORTIMER 1974; HAYNES and KUNZ 1981; KUNZ and HAYNES 1981; MALONE 1983).

The *RAD52* gene was originally identified by a mutation that confers sensitivity to x-rays (RESNICK 1969). Based on physical analysis of irradiated cells, it was concluded that the *RAD52* gene product is necessary for the repair of double-strand breaks in the DNA (HO 1975; RESNICK and MARTIN 1976). In addition, the *RAD52* gene product is required to repair the double-strand lesion induced in HO-catalyzed mating-type interconversion (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981). Detailed analyses of specific mitotic recombination events have shown differing effects of the *rad52* mutation in different assay systems. In one case, direct-repeat reciprocal exchange events leading to loss of a plasmid integrated at the *HIS4* locus were found to be only slightly affected by a mutation in *rad52* (JACKSON and FINK 1981). However, events described as gene conversions were dramatically reduced (JACKSON and FINK 1981). Recombination events leading to loss of a *SUP4-o* allele located within a cluster of the repeated element, delta, on chromosome *X* were reduced 100-

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fold by a mutation in the *RAD52* gene (ROTHSTEIN, HELMS and ROSENBERG 1987). Similarly, recombination between small inverted repeats displays a 100-fold decrease in the *rad52* mutant (WILLIS and KLEIN 1987). Recombination occurring by sister chromatid exchange is dependent on *RAD52* (FASULLO and DAVIS 1987). Integration of a gapped plasmid into the genome is blocked in the *rad52* mutant (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). Finally, direct repeat recombination events initiated by an HO-induced double-strand-break also require a functional *RAD52* gene (RUDIN and HABER 1988; NICKOLOFF *et al.* 1989). In general, the *rad52* mutation results in a striking reduction in what have classically been termed gene conversion events. In contrast, reciprocal exchange events are reduced by varying amounts in *rad52* cells. It is clear that the *RAD52* gene plays a major role in recombination in mitotic cells; however, since recombination is not completely eliminated in *rad52* mutant cells, it suggests that another, *RAD52*-independent recombination pathway must also be operating (HABER and HEARN 1985; HOEKSTRA, NAUGHTON and MALONE 1986).

The *RAD1* gene is a component of the excision-repair pathway for UV damage in yeast (REYNOLDS and FRIEDBERG 1981; WILCOX and PRAKASH 1981). Recently, studies of mitotic recombination events stimulated by the PolI rDNA promoter, *HOT1* (KEIL and ROEDER 1984; ROEDER, KEIL and VOELKEL-MEIMAN 1986), show that a mutation in *RAD1* reduces *HOT1*-stimulated recombination events (R. KEIL, personal communication). Similarly, the *RAD1* gene has been shown to be required for recombination between direct-repeats, as well as for homologous integration of linear plasmids (SCHIESTL and PRAKASH 1988). Based on analysis of recombination events between heteroallelic duplications, it was suggested that *RAD1* may be involved in the processing of recombination intermediates containing short stretches of heteroduplex DNA (KLEIN 1988). In addition, studies of *RAD52*-independent recombination demonstrate that exposure of *rad52* cells to UV-irradiation results in a wild-type spectrum of recombination events (E. SUGARMAN and J. HABER, personal communication). These experiments suggest a role for excision-repair gene products in genetic recombination in yeast.

We examined the effect of the *rad1* and *rad52* mutations in transcription-stimulated and transcription-independent recombination in the hope of elucidating the genetic pathways of these recombination events. The results of these experiments show that the transcription-stimulated recombination we observe in *gal80* constitutive cells is specifically eliminated by a mutation in the *RAD52* gene. Interestingly, Gal^+ recombination events that retain the integrated plasmid DNA (Gal^+ Ura^+ replacement events; see below) are recovered at nearly wild-type levels in transcriptionally inactive *rad52* cells, although this class of recombinant was previously shown to be extremely sensitive to a mutation in *rad52* (JACKSON and FINK 1981). On the other hand, the rate of this event is reduced 20-fold in transcriptionally active cells in the *rad52* mutant, although transcription does not affect the rate of this event in *RAD* wild-type strains. In contrast to other studies (R. KEIL, personal communication), a mutation in *RAD1* results in only a twofold reduction in plasmid loss events in expressed sequences, and shows no effect on plasmid loss events in repressed DNA. The *rad1 rad52* double mutant displays a dramatic decrease in the rate of plasmid loss events. These experiments show that a wild-type copy of *RAD52* can compensate for a defect in *rad1*, but a functional *RAD1* gene only partially rescues the *rad52* mutant phenotype, which suggests that *RAD1* and *RAD52* are components of two overlapping pathways for mitotic recombination in yeast. Furthermore, based on the different sensitivities of these events to a mutation in *rad52* in transcribed versus repressed DNA, we propose that transcription-stimulated recombination probably proceeds via a different pathway from recombination in transcriptionally silent sequences. Finally, since the *rad1 rad52* double mutant does not exhibit a concomitant synergistic increase in sensitivity to damage by radiation when compared with either single mutant, the recombination function of these gene products is independent of their role in survival of radiation damage.

MATERIALS AND METHODS

Strains: The strains used in these experiments are described in Table 1. A *TRP1* disruption of *rad52* (SCHILD *et al.* 1983a, b) was introduced into the genome by transforming linearized DNA (ROTHSTEIN 1983) into yeast made competent by the LiCl method (ITO *et al.* 1983). The presence of the disruption was confirmed by analysis of the genomic DNA of transformants by blots (SOUTHERN 1975), genetic linkage analysis to known mutations, and sensitivity of the mutant strain to ionizing radiation (50 krad, in a cesium source emitting 7.8 krad/hr). The *rad1* disruption was previously constructed in this laboratory in an isogenic genetic background (RONNE and ROTHSTEIN 1988), and is marked with the *LEU2* selectable marker. Segregants containing either a single *rad* mutation or both were made by crossing the disrupted strains to previously constructed isogenic strains containing null mutations in the *GAL* regulatory genes *gal4* or *gal80* and the plasmid pWJ227 (Figure 1) integrated at the *GAL10* locus (THOMAS and ROTHSTEIN 1989).

Plasmid integration: The plasmid pWJ227 was described previously (THOMAS and ROTHSTEIN 1989; Figure 1). The plasmid was targeted to integrate at the *GAL10* locus by linearizing plasmid DNA at the unique *Hpa*I site within the *GAL10* fragment on the plasmid (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981), and transforming yeast cells made competent by the LiCl method (ITO *et al.* 1983).

Fluctuation analysis of recombination events: Fluctuation analyses were performed following the methods of

TABLE 1

Yeast strains

Strain	Genotype[a]
W586-6C	*MAT***a** *gal80::LEU2 gal10::pWJ227-URA3*
W586-8D	*MAT***a** *gal80::LEU2 gal10::pWJ227-URA3*
W586-9A	*MATα gal80::LEU2 gal10::pWJ227-URA3*
W586-6D	*MATα gal4::LEU2 gal10::pWJ227-URA3*
W586-8A	*MATα gal4::LEU2 gal10::pWJ227-URA3*
W586-9D	*MAT***a** *gal4::LEU2 gal10::pWJ227-URA3*
W625-17B	*MAT***a** *gal4::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W625-20D	*MAT***a** *gal4::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W628-3A	*MAT***a** *gal80::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W628-5A	*MAT***a** *gal80::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W628-13B	*MATα gal80::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W672-4B	*MAT***a** *gal4::LEU2 rad1::LEU2 gal10::pWJ227-URA3*
W672-14D	*MATα gal4::LEU2 rad1::LEU2 gal10::pWJ227-URA3*
W673-2B	*MAT***a** *gal80::LEU2 rad1::LEU2 gal10::pWJ227-URA3*
W673-3C	*MAT***a** *gal80::LEU2 rad1::LEU2 gal10::pWJ227-URA3*
W673-7C	*MATα gal80::LEU2 rad1::LEU2 gal10::pWJ227-URA3*
W674-6A	*MATα gal80::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W674-11C	*MATα gal80::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W674-12B	*MATα gal80::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W675-7D	*MATα gal4::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W675-8B	*MATα gal4::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W675-11C	*MATα gal4::LEU2 rad1::LEU2 rad52::TRP1 gal10::pWJ227-URA3*
W569-1B	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 can1-100 SUP4-o::HIS3 trp5-2 trp1-1 lys2-1 gal10::LEU2*
W569-7D	*MATα leu2-3,112 his3-11,15 ura3-1 ade2-1 can1-100 SUP4-o::HIS3 trp5-2 gal10::LEU2*
W569-2A	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 can1-100 SUP4-o::HIS3 trp1-1 gal10::LEU2*
W569-5A	*MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 can1-100 SUP4-o::HIS3 trp1-1 gal10::LEU2*

[a] All strains except the W569 segregants are isogenic to W303 as described in MATERIALS AND METHODS, and contain the following markers: *leu2-3,112 his3-11,15 trp1-1 can1-100 ade2-1 ura3-1*. Disruption alleles are followed by "::" and the gene contained in the disruption. The *gal10* allele is disrupted with the plasmid pWJ227 containing the *URA3* selectable marker, as shown in Figure 1.

LURIA and DELBRUCK (1943) and the data was analyzed by the method of the median of LEA and COULSON (1949) as described in THOMAS and ROTHSTEIN (1989). Briefly, cells were plated to single colonies on nonselective medium containing a noninducing, nonrepressing carbon source to allow full expression of the *gal80* constitutive allele, which displays some glucose repression (TORCHIA *et al.* 1984). Seven or nine independent colonies were used for each experiment, and experiments were repeated at least three times. Typically, YPS medium was used (1% yeast extract, 2% bactopeptone, 2% sucrose), although the results were identical if the carbon source was raffinose (2%) or glycerol-lactate (3% each; data not shown). A plug containing an entire colony was picked, and cells were resuspended in water, sonicated briefly, and appropriate dilutions were plated to the following media: Com, synthetic complete medium, to determine viable colony-forming units (cfu) per ml; 5-FOA, synthetic complete medium containing 750 μg/ml 5-fluoro-orotic acid (5-FOA) and 50 μg/ml uracil, to select for Ura$^-$ cells; Gal Com, synthetic complete media containing 2% galactose as a carbon source, with 0.25% Tween-80 and 7.2 μg/ml ergosterol to permit membrane synthesis under anaerobic conditions (MORPURGO *et al.* 1964). Com and 5-FOA plates were incubated at 30°C for three days. Gal Com plates were incubated at 30° for 5–7 days under anaerobic conditions (BBL GasPak Anaerobic System) to reduce nonspecific background growth (DOUGLAS and HAWTHORNE 1966).

Mating rescue of *GAL10* recombinants that arise in a *gal4* mutant strain: Cells containing a mutation in *gal4* that become Gal$^+$ at the *GAL10* locus cannot be selected directly on galactose medium because the strains are constitutively repressed for expression of the *GAL* genes. *GAL10* recombinants can be rescued by mating with a *GAL4 gal10* tester strain. Since mating rescue does not occur at 100% efficiency, it is necessary to determine the proportion of mated cells in each experiment. The proportion of mated cells can be determined using a color assay system as described previously (THOMAS and ROTHSTEIN 1989). This proportion is used to determine the frequency of Gal$^+$ recombinants in a *gal4* population. Cells were mated in suspension by mixing equal numbers of each parent in 5 ml of YPD pH 4.5 (YPD is 1% yeast extract, 2% bactopeptone, and 2% dextrose), to a total cell concentration of 6×10^6 cells/ml. After 90 min of gentle shaking (150 rpm in a rotary shaker) at 30°, cells were pelleted gently to force cells of opposite mating type into contact with each other. Cells were gently resuspended, and were placed at 30° to shake for another 60 min (250–300 rpm). Alternatively, cells were mated on nitrocellulose filters on a YPD plate (DUTCHER and HARTWELL 1982). Equal numbers of cells were mixed to a final concentration of about 4×10^7 cells in 1 ml of sterile H_2O. Cells were forced through a sterile filter unit (Gelman Sciences, Inc.) onto a 13-mm nitrocellulose disc. Discs were removed onto a YPD plate and incubated at 30° for 2 hr. After mating,

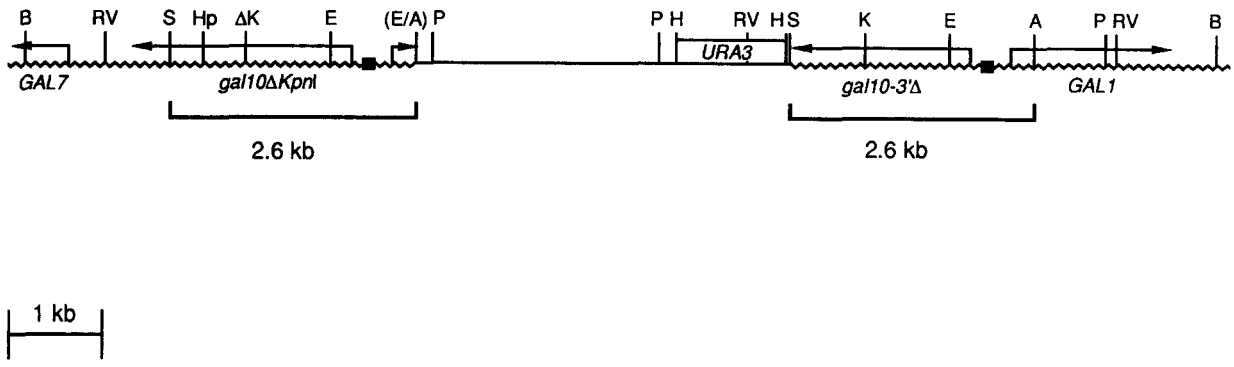

FIGURE 1.—Structure of the *GAL* locus on chromosome *II* bearing the integrated plasmid pWJ227. The 2.6 kb of duplicated *GAL10* sequence are indicated below the figure. The homology extends from an *Ava*I site in *GAL1*, which was destroyed at the plasmid junction in the left repeat, to the *Sal*I site in *GAL10*. The left repeat is a full-length copy of *GAL10* with a 4-bp deletion of a *Kpn*I restriction site, and the right repeat is a 3'-truncated copy of *GAL10*. The wavy line represents yeast chromosomal sequences, the straight line represents plasmid DNA. The *URA3* gene is depicted by an open box, and the direction of transcription is shown by an arrow below the gene. The small filled box is the *GAL1-10* UAS (upstream activating sequence). The arrows indicate the presence of functional *GAL* promoters, and the arrowhead is in the direction of transcription. S, *Sal*I, K, *Kpn*I, E, *Eco*RI, A, *Ava*I; P, *Pvu*II; RV, *Eco*RV; H, *Hin*dIII; B, *Bgl*II; Hp, *Hpa*I; (E/A), a fusion between *Eco*RI and *Ava*I that does not restore either site; ΔK, the *Kpn*I deletion mutation.

the discs were washed in 1 ml H_2O to remove cells. In both procedures, appropriate dilutions were made before and after mating to determine the proportion of mated cells and to determine the number of cell divisions occurring in the rescued population. The remaining mated cells were pelleted and resuspended in H_2O to plate on galactose medium. For the suspension mating procedure, the mating efficiency was generally on the order of 1–3% diploids formed, while for the filter procedure, this value was normally 3–10%. To avoid expansion of the diploid population during incubation on YPD, assays were included in the analysis only if growth of the rescued haploid strain was limited to ≤1 doubling. Since the rate of recovery of the Gal^+ Ura^- class of recombinant is the same after selection on galactose and 5-FOA (Table 2), we are confident that the mating rescue procedure accurately reflects the Gal^+ proportion of the recombinant population.

Genomic blot analysis of recombinants: The genomic blot procedure (SOUTHERN 1975) was described previously (THOMAS and ROTHSTEIN 1989).

Yeast colony hybridization: Yeast colony hybridization (HICKS, HINNEN and FINK 1979) was performed as modified by ROTHSTEIN (1985). Briefly, cells were patched onto a nitrocellulose filter placed on a YPD plate and incubated at 30° overnight. Colonies were lysed by removing the filter and placing it on a piece of Whatman 3MM paper in a Petri dish soaked in 3 ml of a solution of 1 M sorbitol, 20 mM EDTA, 5 μg/ml zymolyase-20T at 37° overnight. The plate was sealed with plastic to prevent the filter from drying out. The following day, the filters were processed as for bacterial colony hybridization by placing them for 5 min onto Whatman 3MM paper soaked in the following solutions: (1) 0.5 M NaOH, 1.5 M NaCl; (2) 1.5 M Tris-Cl (pH 7.6), 1.5 M NaCl; (3) same as (2). The filters were then air-dried for several hours, baked and hybridized as usual.

Genetic analysis of disomes: Putative gene convertants were distinguished from disomes by analyzing genetic linkage between the Gal^+ and Ura^+ phenotypes. Gal^+ Ura^+ recombinants recovered in a *gal80* mutant background were crossed to a *gal*10::*LEU2 lys2-1 ura3-1* strain of the appropriate mating type. The Gal^+ and Ura^+ phenotypes segregate as unlinked genes if the markers are contained on two disomic chromosomes. Additionally, the recombinant strains contain the wild-type *LYS2* allele on chromosome *II*. If the chromosome becomes disomic, an extra *LYS2* gene segregates in the cross and $4^+:0^-$ and $3^+:1^-$ tetrads for lysine prototrophy result in addition to the expected $2^+:2^-$ pattern. Because *GAL10*$^+$ recombinants that occur in a *gal4* mutant background are rescued by mating, these diploids can be sporulated directly and analyzed. Lysine segregation could not be analyzed in all of these recombinants due to the genotype of the rescuing strain; however Gal-Ura linkage was determined.

Heteroallelism analysis: The 5-FOA^R mutants arising in the *rad1 rad52 gal80* strain were examined for their *ura3* allele by heteroallelism analysis to determine whether or not they arose by an ectopic gene conversion event with the chromosomal *ura3-1* allele. Cells were patched to a YPD master plate, and mated to a strain containing the markers *ura3-1* and *trp5-2*. The mated cells were plated either directly to sporulation medium to induce meiotic levels of recombination, or first to medium lacking tryptophan to select for diploids, and then to sporulation medium. There was no qualitative difference in the result using either procedure. Control *ura3-1/ura3-1* diploids produced no papillae under this regimen. Of 25 of the 5-FOA^R cells tested, 23 gave rise to Ura^+ papillae when mated to a strain that was *ura3-1*. The frequency of Ura^+ papillae varied considerably from diploid to diploid, probably reflecting the distance between the heteroalleles. Of the two remaining Ura^- derivatives examined, one displayed complementation for growth on medium lacking uracil when mated to a *ura3-1* strain, and likely represents a mutation in another gene in the uracil biosynthetic pathway, such as *ura5* (BOEKE, LACROUTE and FINK 1984). The second derivative did not produce Ura^+ papillae in this assay.

Analysis of radiation sensitivity in the *RAD* mutants: Cells were grown to mid-log phase in YPD liquid medium, were sonicated to break up any clumps of cells, and appropriate dilutions were plated to YPD plates. The plates were exposed to the radiation source for the indicated lengths of times, and then were incubated at 30° until colonies were visible. The gamma source was a cesium source emitting 7.8 krad/hr. Viable cfu/ml were plotted as a function of time.

Statistical analysis of rates: Significance was determined by chi-square analysis and by the Fisher exact probability test (SIEGEL 1956).

TABLE 2

Effect of mutations in *rad1* and *rad52* on direct repeat recombination in transcriptionally active and inactive cells

Genotype[a]		Selected on 5-FOA		Selected on galactose			
		Rate of Ura$^-$	Rate of Ura$^-$ Gal$^+$	Rate of Gal$^+$	Rate of Gal$^+$ Ura$^-$	Rate of Gal$^+$ Ura$^+$	Relative rate of Ura$^-$[b]
wt	*gal4*	2.0×10^{-5}	0.7×10^{-5}	1.3×10^{-5}	0.9×10^{-5}	4.3×10^{-6}	1
	gal80	29.3×10^{-5}	12.5×10^{-5}	14.2×10^{-5}	12.5×10^{-5}	3.5×10^{-6}	15
rad1	*gal4*	1.7×10^{-5}	0.4×10^{-5}	1.5×10^{-5}	1.2×10^{-5}	4.0×10^{-6}	1
	gal80	13.4×10^{-5}	6.1×10^{-5}	8.6×10^{-5}	8.0×10^{-5}	5.9×10^{-6}	7
rad52	*gal4*	1.1×10^{-5}	0.5×10^{-5}	0.5×10^{-5}	0.3×10^{-5}	1.8×10^{-6}	0.5
	gal80	1.8×10^{-5}	0.7×10^{-5}	1.0×10^{-5}	0.9×10^{-5}	0.8×10^{-6}	1
rad1 rad52	*gal4*	2.0×10^{-7}	ND[c]	ND[c]			0.01
	gal80	8.0×10^{-7}	5.0×10^{-7}	6.0×10^{-7}	6.0×10^{-7}	6.0×10^{-7}	0.04

Experiments were done by fluctuation analysis as described in MATERIALS AND METHODS. The "Rate of Ura$^-$" is the total rate of 5-FOA^R cells in the population; these cells were screened by replica-plating for their galactose phenotype, and the rate of Ura$^-$ Gal$^+$ was determined independently. Similarly, the "Rate of Gal$^+$" is the total rate of galactose-positive recombinants. These cells were screened for their uracil phenotype to determine the rates of Gal$^+$ Ura$^-$ and Gal$^+$ Ura$^+$.

[a] The full genotypes of the strains used in these experiments is in Table 1.

[b] Relative to the rate in *RAD1 RAD52 gal4* cells.

[c] Not determined.

EXPERIMENTAL RESULTS

An assay system for direct repeat recombination at *GAL10*: We used a system described previously (THOMAS and ROTHSTEIN 1989), in which a plasmid containing the first three-fourths of the *GAL10* gene and the *GAL1-10* divergent promoter is targeted to integrate at the chromosomal *GAL10* locus (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; Figure 1). This creates a 2.6-kb duplication of *GAL10* coding sequences flanking the integrated plasmid and the *URA3* selectable marker. Neither repeat contains a functional *GAL10* gene; the repeat on the right is deleted for the 3′-portion of the gene, and the repeat on the left contains an *in vitro* constructed frameshift mutation that results in a translation termination codon. Expression of the repeats is regulated in isogenic strains by null mutations in either the positive activator, *GAL4* (JOHNSTON and HOPPER 1982), or the repressor, *GAL80* (TORCHIA *et al.* 1984). A *gal4* mutant cannot express the *GAL* structural genes; a *gal80* mutant constitutively expresses these genes to high levels (reviewed in OSHIMA 1982). Isogenic strains either wild type or containing disruption mutations in *RAD1* (YANG and FRIEDBERG 1984; RONNE and ROTHSTEIN 1988) and *RAD52* (SCHILD *et al.* 1983a, b) were used in all experiments, as described in MATERIALS AND METHODS. The relevant genotypes of the strains used in this study are listed in Table 1.

Because the integrated plasmid results in a cell that is *gal10* and *URA3*, recombination events between the duplicated *GAL10* repeats can be detected in two ways. By selecting for loss of the *URA3* marker with the drug 5-FOA (BOEKE, LACROUTE and FINK 1984), events are obtained that have deleted the integrated plasmid sequences, regenerating a single full-length copy of the *GAL10* gene (Figure 2). Ura$^-$ cells will be either Gal$^+$ or Gal$^-$, depending on whether or not the *Kpn*I-deletion mutation has been left in the chromosome. Alternatively, events that restore *GAL10* gene function can be selected. Since the *GAL* genes are constitutively expressed in a *gal80* mutant, Gal$^+$ events can be selected directly. However, we cannot directly select for *GAL10* recombinants in the *gal4* mutant background, since *gal4* cells are unable to grow on galactose-containing media. *GAL10* recombinants in the *gal4* mutant background can be rescued by mating, as described in MATERIALS AND METHODS. Gal$^+$ recombinants that occur by loss of the integrated plasmid will result in a cell that is Gal$^+$ and Ura$^-$. Therefore, the Gal$^+$ Ura$^-$ class of recombinant can be independently recovered under both selective conditions, ensuring that the selections used are not biasing the recombination events that we detect.

Three types of Gal$^+$ Ura$^+$ events are obtained (Figure 2). One class is disomic for chromosome *II*, where one chromosome has lost the plasmid sequences leaving a wild-type copy of the *GAL10* gene, and the other chromosome contains the original plasmid pWJ227 integrated at *gal10*, which contains *URA3*. A second class is a novel structure containing a triplication of *GAL10* sequences, with two copies of the integrated plasmid and the *URA3* gene on a single chromosome. A third class results from replacement of the *Kpn*I-deletion mutation with wild-type information from the truncated repeat without loss of the integrated plasmid. This results in a single-copy insertion of the plasmid containing a functional *GAL10* gene and the *URA3* marker.

This third class of Gal$^+$ Ura$^+$ recombinants can occur by a variety of mechanisms (see Figure 3 of

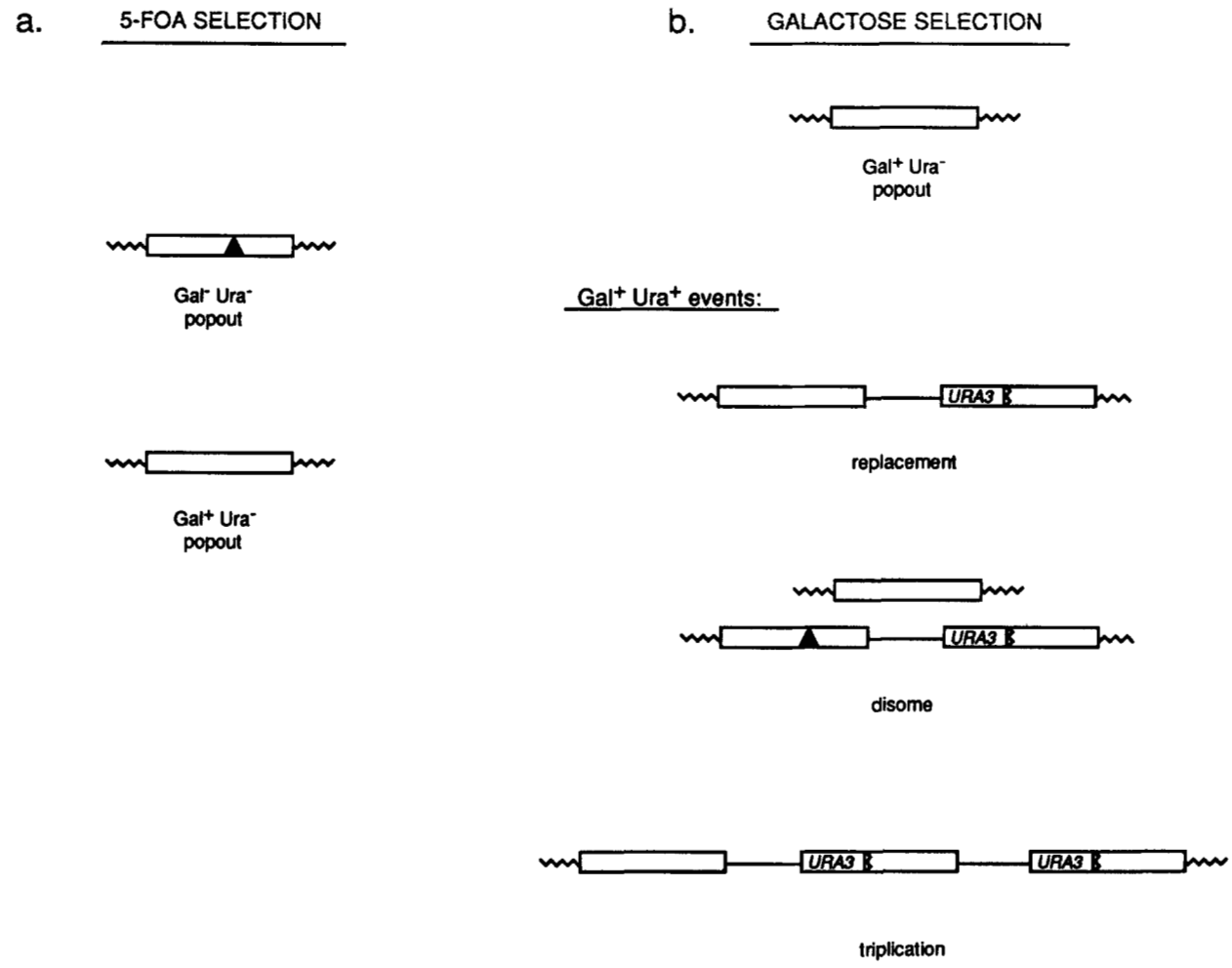

FIGURE 2.—Types of recombinants obtained under galactose and 5-FOA-selection. **a,** Selection on 5-FOA results in loss of one copy of the duplicated region, the plasmid sequences and the *URA3* selectable marker. The *GAL10* gene left in the chromosome is either Gal$^+$ or Gal$^-$, depending on whether or not the *Kpn*I frameshift mutation has been left in the chromosome. **b,** Galactose selection can also result in loss of the plasmid, producing Gal$^+$ Ura$^-$ cell. In addition, three types of Gal$^+$ Ura$^+$ events are recovered. Gal$^+$ Ura$^+$ replacements contain a single copy of chromosome *II*, where the *Kpn*I frameshift mutation in the full-length repeat has been replaced with wild-type information from the truncated repeat. Gal$^+$ Ura$^+$ disomes contain one copy of chromosome *II* with the original plasmid pWJ227, which is Gal$^-$ and Ura$^+$, and a second copy of chromosome *II* which has undergone a plasmid loss event to become Gal$^+$. The final Gal$^+$ Ura$^+$ event observed is a triplication of *GAL10* sequences. The full-length repeat on the left is Gal$^+$.

THOMAS and ROTHSTEIN 1989). For example, a gene conversion of the *Kpn*I-deletion mutation with wild-type information contained in the 3′-truncated repeat in G_1 or G_2 can result in a Gal$^+$ Ura$^+$ chromosome. Alternatively, a double crossover event flanking the frameshift mutation can restore a functional *GAL10* gene while maintaining the integrated plasmid. Finally, a Gal$^+$ Ura$^+$ chromosome can occur by mismatch repair of heteroduplex DNA covering the mutant site and subsequent segregation of daughter strands after DNA replication. Historically, this type of event has been referred to as gene conversion (JACKSON and FINK 1981). The term gene conversion was originally defined as the nonreciprocal transfer of genetic information, based on the recovery of non-Mendelian segregation patterns during meiotic recombination in fungi. Therefore, in order to truly designate an event as a gene conversion, all the products of the recombination event must be recovered. Since the reciprocal products of these mitotic direct repeat recombination events are generally not recoverable under the selective conditions used, this type of Gal$^+$ Ura$^+$ recombinant cannot be rigorously classified as a gene conversion. In addition, gene conversion may also be responsible for popout events (ROTHSTEIN, HELMS and ROSENBERG 1987; SCHIESTL, IGARASHI and HASTINGS 1988). Thus this term is ambiguous since it too narrowly defines the mechanism. Therefore, we will refer to these Gal$^+$ events that retain a single copy of the plasmid DNA as Gal$^+$ Ura$^+$ replacements, which is a mechanistically neutral description of these events.

Transcription-stimulated recombination events specifically require the *RAD52* gene product: We examined recombination in *gal4* and *gal80* cells in the presence of the *rad1* and *rad52* disruption mutations. The results are shown in Table 2. The rate of plasmid

loss as measured by 5-FOA-resistance in *RAD1 RAD52* wild-type cells that do not express the *GAL* genes is 2×10^{-5}. Cells that actively transcribe the *GAL* region display a 15-fold increase in recombination events leading to loss of the plasmid (Table 2; THOMAS and ROTHSTEIN 1989). We examined the effect of a disruption allele of the *RAD1* gene on direct repeat recombination at *GAL10* (Table 2). In *rad1* mutant cells that constitutively express the *GAL* region, a reproducible twofold reduction in the rate of plasmid loss is observed when compared to cells that are *RAD1* ($P < 0.0001$; Table 2). No effect of the *rad1* mutation is observed in *gal4* cells. Thus, it appears that recombination events in the *rad1* mutant can occur relatively efficiently.

In contrast, the *rad52* disruption mutation completely abolishes the transcription-dependent stimulation in recombination seen in *gal80* constitutive cells, decreasing the rate of Ura$^-$ recombinants to 1.8×10^{-5} (Table 2). Transcription-independent recombination in the *gal4* mutant is reduced only about twofold in the *rad52* strain ($P = 0.0007$). Because the stimulation in recombination at *GAL10* is dependent on expression of the *GAL10* region (THOMAS and ROTHSTEIN 1989), it was possible that the effect of the *rad52* mutation was to reduce or eliminate expression of the *GAL10* repeats. We examined steady-state transcripts of the *GAL10* construct in the *rad52* mutant strains, and find no effect of the *rad52* mutation on expression of the *GAL10* region (data not shown). These results suggest transcriptional activation of the *GAL10* construct results in a recombination intermediate that requires processing by the *RAD52* gene product.

Mutations in *rad1* and *rad52* display synergism in their effects on direct repeat recombination: We examined recombination in a *rad1 rad52* strain and found that the double mutant has a dramatic effect on recombination events leading to 5-FOA-resistance. Of the 5-FOA^R events recovered in the *rad1 rad52* strains, 98% are mutations of the *URA3* selectable marker carried on the integrated plasmid. Southern analysis (SOUTHERN 1975) revealed that these Gal$^-$ Ura$^-$ cells still maintain the plasmid insertion at the *GAL10* locus. We examined all the 5-FOA^R events in both *gal4* and *gal80* cells by colony hybridization (HICKS, HINNEN and FINK 1979) with nick-translated pUC18 sequences to determine the proportion of cells arising by recombination (pUC18$^-$) *vs.* mutation (pUC18$^+$). After adjusting for mutations to *ura3* we find that the *rad1 rad52* double mutant reduces direct repeat recombination relative to wild type by approximately 100-fold in *gal4* cells, to 2×10^{-7}, and by 400-fold in *gal80* cells, to 8×10^{-7}. The resulting fourfold greater rate of recombination in the transcriptionally active *rad1 rad52* mutant implies that the residual recombination left in the double mutant is affected positively by transcription.

TABLE 3

Fraction of 5-FOA^R events that are Gal$^+$ in *rad1* and *rad52* strains

Genotype	*gal4*	*gal80*
Expected[a]	0.33	0.33
wt	0.42	0.41
rad1	0.24	0.46
rad52	0.45	0.41

Fractions were determined by dividing the rate of Gal$^+$ from the 5-FOA^R population by the total rate of 5-FOA^R.

[a] Expected values were determined by assuming that the probability of a crossover in a region is linearly related to the physical size of that region.

The *rad52* and *rad1* mutations do not change the distribution of crossovers in the duplication: We determined the position of the exchange event that leads to plasmid loss by examining the Ura$^-$ population of recombinants for their Gal phenotype. In models for loss of the plasmid by a reciprocal exchange, a crossover to the left of the *Kpn*I-deletion mutation will produce a Ura$^-$ cell that is Gal$^+$; a crossover to the right will result in a Gal$^-$, 5-FOA^R cell. A change in the distribution of crossover events through the *GAL10* repeat might therefore reflect a different pattern of recombination initiation or resolution in the *rad* mutants.

In wild-type cells, the distribution of crossovers is approximately related to the physical size of the regions undergoing the recombination event; about 40% of the crossovers occur in the 0.9 kb to the left of the *Kpn*I-deletion mutation to give a Gal$^+$ Ura$^-$ recombinant, and about 60% are in the 1.7 kb to the right, producing a Gal$^-$ Ura$^-$ cell (Table 3). The distribution of crossover events in wild-type cells is not affected by expression of the region. Ura$^-$ recombinants in the *rad52* mutant display a pattern of crossover events identical to wild-type cells, and similarly, the distribution of the exchanges is not affected by expression of the repeats. This pattern is also observed in the *gal80 rad1* mutant. A slight decrease in the recovery of Gal$^+$ Ura$^-$ derivatives is observed in the repressed *gal4 rad1* strain, however, this difference is not significantly different from wild-type ($P = 0.08$).

Analysis of the Gal$^+$ Ura$^+$ events in wild-type and *rad* mutant strains: Previous analyses of mitotic recombination in yeast suggested that the *rad52* mutation has a severe effect on replacement events, but has a smaller, more variable effect on events resolving reciprocally (JACKSON and FINK 1981; HABER and HEARN 1985; HOEKSTRA, NAUGHTON and MALONE 1986; ROTHSTEIN, HELMS and ROSENBERG 1987; RUDIN and HABER 1988; SCHIESTL and PRAKASH 1988; KLEIN 1988). We examined Gal$^+$ Ura$^+$ events in wild-

type *rad1* and *rad52* cells by screening the Gal$^+$ population of cells for their Ura phenotype. These Gal$^+$ Ura$^+$ recombinants were screened by genomic blot analysis to detect the three classes of events described below.

The first class of Gal$^+$ Ura$^+$ events we will consider are those that replace the *Kpn*I frameshift mutation with wild-type information. In Rad$^+$ cells, the majority of Gal$^+$ Ura$^+$ events observed are replacements. The total rate of Gal$^+$ Ura$^+$ replacements is unaffected by transcription, and is about 4×10^{-6} in both *gal4* and *gal80* cells (Table 4). The *rad1* mutation has no effect on the rate of Gal$^+$ Ura$^+$ replacements. In the *rad52* mutant, we observe a difference in the recovery of Gal$^+$ Ura$^+$ replacements depending on the transcriptional state of the region. In transcriptionally active cells, Gal$^+$ Ura$^+$ replacements are reduced 20-fold relative to wild type. However, in transcriptionally inactive cells we observe only a fourfold reduction in the rate of Gal$^+$ Ura$^+$ replacement when compared with wild type.

Disomes associated with a Gal$^+$ popout were also observed (Table 4). Representative disomic recombinants were also analyzed genetically, showing that the Gal$^+$ and Ura$^+$ phenotypes were not linked in these strains (see MATERIALS AND METHODS for a description of the crosses). The rate of this event in cells wild type for the *RAD* mutations is about 0.1×10^{-6}. The *rad52* strains show a rate of disomy associated with plasmid loss of 0.6×10^{-6}, sixfold elevated over the level observed in wild-type cells. The rate of this event is not dependent on the transcriptional state of the *GAL* locus. In *rad1* cells, disomes are recovered at wild-type levels in the transcriptionally inactive *gal4* strain. However, in the transcriptionally active *rad1 gal80* cells, a tenfold increase relative to wild type in the rate of disomes associated with plasmid loss is observed (to 1.0×10^{-6}). This increase in the rate of disomes may be associated with a decrease in the rate of a novel class of recombinant in the *gal80 rad1* strain (triplications, see below).

The last class of Gal$^+$ Ura$^+$ recombinants observed consists of a novel event, a triplication of *GAL10* sequences (Table 4). A total of six triplication events were observed in the transcriptionally repressed *gal4* mutant strain: five in the *rad1* mutant, and one in *rad52*. In addition, one event containing four copies of the *gal10* repeat was also recovered in a *gal4 rad1* strain (Figure 3). This event was not observed in cells wild type for the *RAD* genes, nor in any other *gal80* constitutive strain examined.

Gal$^+$ Ura$^+$ events are recovered in the transcriptionally active *rad1 rad52* double mutant: No Gal$^+$ events were recovered in the *rad1 rad52 gal4* strain due to the reduced recombination rates in these cells and the low efficiency with which Gal$^+$ recombinants are recovered by mating rescue (see MATERIALS AND METHODS; *gal4* strains that are *GAL10*$^+$ are phenotypically Gal$^-$). However, we examined Gal$^+$ Ura$^+$ cells arising in a *rad1 rad52 gal80* strain to see whether the *rad* double mutant also displays a synergistic reduction in the recovery of these events. In one experiment in the *rad1 rad52 gal80* strain, nine Gal$^+$ Ura$^+$ colonies representing a minimum of six independent events were examined molecularly and genetically for evidence of recombination leading to conversion of the *Kpn*I deletion mutation (Table 5). Analysis of total genomic DNA revealed that three of the colonies (recombinants 2-1, 2-4 and 4-1 in Table 5) contain restriction fragments consistent with disomy for chromosome *II*, where one chromosome contains a single copy of the *GAL10* gene and the other contains the original integrated plasmid. The remaining six colonies (1-1, 2-2, 2-3, 3-1, 3-2, and 3-3) appear to have a single chromosome containing the plasmid insertion, which is indicative of a replacement event. However, when these cells were analyzed for the presence of the *Kpn*I restriction site, only one of the six (2-3) has the *Kpn*I restriction site restored, suggesting that the remaining five are not true replacements (data not shown).

Genetic analysis was also performed on each of the nine recombinants. One of the putative disomes (2-1) displays no linkage between the Gal$^+$ and Ura$^+$ phenotypes, as anticipated if the markers were segregating independently on disomic chromosomes. In addition, this recombinant contains an extra copy of the *LYS2* gene, which is located on chromosome *II* distal to the *GAL* locus, indicating a duplication of this marker. A second putative disomic event (2-4) lost the *URA3* containing chromosome before the genetic analysis, and was not analyzed further. The final disomic event (4-1) displays an unusual segregation pattern; although this strain appears disomic when analyzed by Southern blots, genetic analysis reveals only one *LYS2* wild-type chromosome segregating in the cross. In addition, this strain displays linkage between

TABLE 4

Distribution of Gal$^+$ Ura$^+$ events between replacements, disomes and triplications in the *rad* mutants

Mutant[a]		Rate of replacement	Rate of disomy	Rate of triplication
wt	*gal4*	4.3×10^{-6}	$<0.2 \times 10^{-6}$	$<0.2 \times 10^{-6}$
	gal80	3.4×10^{-6}	0.1×10^{-6}	$<0.1 \times 10^{-6}$
rad1	*gal4*	3.4×10^{-6}	$<0.2 \times 10^{-6}$	0.6×10^{-6}
	gal80	4.9×10^{-6}	1.0×10^{-6}	$<0.2 \times 10^{-6}$
rad52	*gal4*	1.0×10^{-6}	0.6×10^{-6}	0.2×10^{-6}
	gal80	0.2×10^{-6}	0.6×10^{-6}	$<0.6 \times 10^{-7}$

The Gal$^+$ Ura$^+$ events were screened by Southern analysis of genomic DNA (SOUTHERN 1975) to detect the different classes of events.

[a] The full genotypes of the strains are given in Table 1.

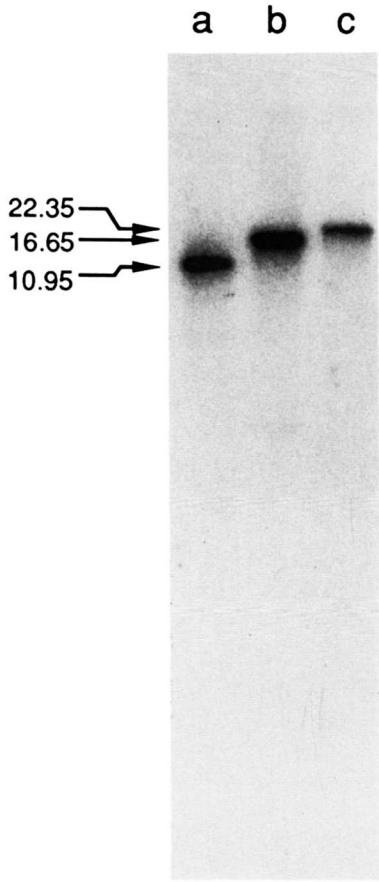

FIGURE 3.—Genomic blot analysis of Gal$^+$ Ura$^+$ recombinants containing multiple copies of the *gal10* repeat. Genomic DNA was isolated as described (THOMAS and ROTHSTEIN 1989) and was digested with *Bgl*II, which cleaves outside of the integrated construct (see Figure 1). **a,** Contains two copies of the *gal10* repeat; **b,** contains three copies; **c,** contains four copies of the *gal10* repeat. The size of each fragment is indicated next to the bands in kilobases.

the Gal$^+$ and Ura$^+$ phenotypes. No simple model for this recombinant can be proposed.

The six single copy integrants display 2:2 segregation for lysine prototrophy, demonstrating that they contain a single copy of chromosome *II*. In addition, the Gal$^+$ and Ura$^+$ phenotypes segregate as linked genes. However, we noticed that after crossing four of these strains (3-1, 3-2, 3-3 and 4-1), the Ura$^+$ segregants are either weakly Gal$^+$, or only papillate to Gal$^+$ after several days incubation on galactose medium. Because these events did not restore the *Kpn*I deletion mutation to wild type, it is likely that these cells represent mutation events that partially restore *GAL10* function. Therefore, taking into account these mutation events, we estimate the rate of replacement to Gal$^+$ Ura$^+$ to be about 6×10^{-8} in the transcriptionally active *rad1 rad52* double mutant.

The *ura3* mutants arising in the *rad1 rad52* double mutant do not occur by ectopic gene conversion: Ectopic gene conversion occurs between homologous sequences located at nonhomologous positions in the genome (MIKUS and PETES 1982; JINKS-ROBERTSON and PETES 1986; LICHTEN, BORTS and HABER 1987). It is possible that the *ura3* mutations recovered in the *rad1 rad52* double mutant arose by an ectopic gene conversion between the endogenous *ura3-1* allele on chromosome *V* and the *URA3* selectable marker integrated at *GAL10*. Heteroallelism tests were performed on 25 of the Ura$^-$ mutants arising in the *rad1 rad52* double mutant as described in MATERIALS AND METHODS. We found no evidence for ectopic gene conversion in these experiments.

The *rad1 rad52* double mutant shows no synergism in radiation sensitivity: To determine whether the *rad1 rad52* double mutant displays a synergistic effect in response to radiation damage in addition to its dramatic effect on recombination, we examined the ability of the double mutant to survive either gamma-irradiation or UV treatment as compared to either single mutant and the wild-type strain. The results are shown in Figure 4. The double mutant is no more sensitive to either gamma or UV irradiation than either single mutant. We conclude that the synergism displayed by the double mutant is limited to its effect on genetic recombination.

DISCUSSION

We have previously shown that direct-repeat recombination leading to loss of a plasmid integrated at *GAL10* is stimulated 15-fold when the region is expressed (THOMAS and ROTHSTEIN 1989). We show here that the transcription-stimulated recombination that we observe is dependent on a functional *RAD52* gene. A mutation in *RAD1* alone has only a small effect on the rate of plasmid loss events in constitutively transcribed sequences, and shows no effect on recombination in repressed sequences. However, we observe synergism between the *rad1* and *rad52* mutations on recombination events in both constitutive and repressed sequences in this system in that the rate of plasmid loss when both mutations are present is much lower than the sum of the rates of either single mutation.

Replacements: We were surprised to see very little effect of the *rad52* mutation on Gal$^+$ Ura$^+$ replacement events in transcriptionally inactive cells. Previous studies of intrachromosomal direct repeat recombination have shown this event to be extremely sensitive to *rad52* (JACKSON and FINK 1981; ROTHSTEIN, HELMS and ROSENBERG 1987; SCHIESTL and PRAKASH 1988; KLEIN 1988). In contrast, the rate of Gal$^+$ Ura$^+$ replacements in the *gal80* constitutive strain was reduced 20-fold relative to wild type. This is consistent with the previous studies cited above, which have reported up to a 100-fold reduction in replacement events in the *rad52* mutant. Therefore, although the absolute rate of Gal$^+$ Ura$^+$ replacement is the same in *gal4* and *gal80 RAD52* wild-type cells

TABLE 5

Analysis of the Gal$^+$ Ura$^+$ events recovered in the *rad1 rad52 gal80* strain

Plate#-recombinant[a]	Chromosome structure[b]	*KpnI*[c]	*LYS2* segregation[d]	Gal-Ura segregation	Conclusion
1-1	Integrant	−	2:2	Linked	Reversion of frameshift mutation at *GAL10*
2-1	Integrant + wild type	Disome	Multiple copies	Unlinked	Disome
2-2	Integrant	−	2:2	Linked	Reversion of frameshift mutation at *GAL10*
2-3	Integrant	+	2:2	Linked	Replacement
2-4	Integrant + wild type	Disome	2:2	All segregants Ura$^-$	Putative disome; lost the Ura$^+$ chromosome before analysis
3-1	Integrant	−	2:2	Linked; Gal +/− or pap	Reversion of frameshift mutation at *GAL10*
3-2	Integrant	−	2:2	Linked; Gal +/− or pap	Reversion of frameshift mutation at *GAL10*
3-3	Integrant	ND	2:2	Linked; Gal +/−	Reversion of frameshift mutation at *GAL10*
4-1	Integrant + wild type	Disome	2:2	Linked; Gal +/− or pap	Multiple events?

Cells were screened by analysis of genomic DNA by the method of SOUTHERN (1975) as described (THOMAS and ROTHSTEIN 1989). Cleavage with *Pvu*II reveals an extra disomic copy of chromosome *II*, while cleavage with *Kpn*I detects the presence of a restored *Kpn*I site in the full-length repeat. Chromosome number was also determined genetically by crossing the cells with a *gal10 ura3 lys2* strain as described in MATERIALS AND METHODS and analyzing segregants from this cross.

[a] Refers to the origin of each recombinant. Recombinants from the same plate may be clonally related.

[b] "Integrant" is a single copy of the integrated plasmid at *GAL10*; "integrant + wild type" is one copy of the integrated plasmid and one copy of the wild-type parental *GAL10* gene.

[c] A "−" indicates no cutting of the full-length repeat by *Kpn*I; a "+" indicates that the full-length repeat is sensitive to *Kpn*I digestion; "disome" refers to the presence of fragments indicative to chromosome *II* disomy; ND is not done.

[d] Cells containing one copy of chromosome *II* will display 2:2 segregation for lysine prototrophy. Cells disomic for chromosome *II* will contain two copies of *LYS2*, and will display 4:0, 3:1 and 2:2 tetrads for lysine prototrophy.

(3–4 × 10^{-6}, Table 4), this result implies that the pathways for this event are different depending on whether the DNA is expressed. We speculate that the variations in recombination frequencies observed between different loci in the *rad52* mutant may reflect the level of transcription of the regions being measured.

Disomes: A second class of Gal$^+$ Ura$^+$ recombinants recovered in these experiments consists of a disome associated with a plasmid loss (Figure 2 and Table 4). One mechanism to generate a disome is by chromosome nondisjunction. The rate of chromosome nondisjunction as measured by chromosome loss in yeast is about 10^{-5} (HARTWELL and SMITH 1985). In *RAD* wild-type cells the rate of plasmid loss is 2 × 10^{-5} in *gal4* cells and 3 × 10^{-4} in *gal80* cells. Therefore, if plasmid loss and chromosome nondisjunction were two independent events, we would expect the rate of Gal$^+$ Ura$^+$ disomy to be the product of the rates of these two events (10^{-11} in the *gal4* mutant and 2 × 10^{-10} in *gal80*). However, in these experiments disomy occurs at a rate of about 0.05–0.1 × 10^{-6} in constitutive and repressed *RAD* wild-type cells and the *gal4 rad1* mutant (Table 4, and B. J. T. and R. R., unpublished observations), and is sixfold higher in the *rad52* mutant in both transcriptional states. The transcriptionally active *gal80 rad1* strain shows a tenfold elevation in the rate of disome recombinants (1.0 × 10^{-6}, Table 4). Therefore, it is likely that the recombination event leading to loss of the plasmid is linked mechanistically to the chromosome nondisjunction in these cells. One prediction of this model is that at least a fraction of the plasmid loss events we observe occur in G_2.

In addition to defects in recombination and repair, the *rad52* mutation has also been associated with an increase in chromosome loss (MORTIMER, CONTOPOULOU and SCHILD 1981; HABER and HEARN 1985). HABER and HEARN (1985) proposed that *RAD52*-independent recombination proceeds via events initiated by single-strand nicks. They suggested that chromosome loss was mediated by the inability of the *RAD52*-independent recombination pathway to resolve a half-chiasma structure generated by single-stranded invasion, resulting in a chromosome containing unrepaired breaks. In their model, the broken chromosome is lethal and is therefore not recovered. This contrasts with our observation that the disomic events represent a chromosome gain. It is possible that heteroallelic recombination in diploids proceeds by a different mechanism in *rad52* cells from the haploid direct repeat recombination events presented here.

Triplications: A third, novel class of Gal$^+$ Ura$^+$ recombinants was also observed at a low frequency in transcriptionally inactive *rad1* and *rad52* strains. This

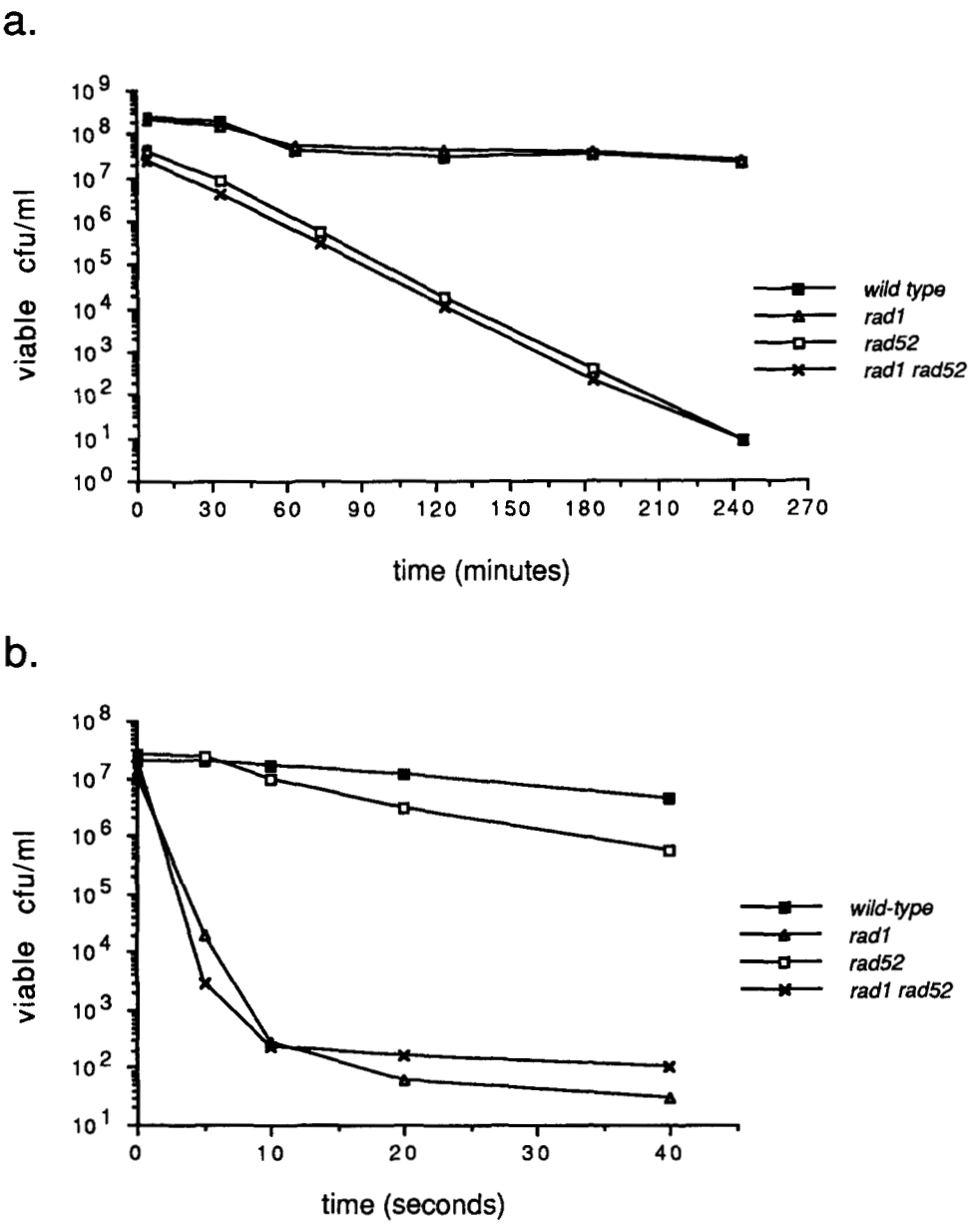

FIGURE 4.—Comparison of the sensitivities to gamma- and UV-irradiation of the rad mutant strains with an isogenic wild-type strain. **a,** Viable colony forming units were determined by plating cells and exposing the plates for varying times to a cesium source emitting 7.8 krad/hr of ionizing radiation. **b,** Viable colony forming units were determined by plating cells and exposing the plates for varying times to UV-irradiation.

event consists of a triplication of *GAL10* sequences. Triplications are normally produced during direct repeat recombination by an unequal sister chromatid exchange (Figure 5). However, using this construct, a simple crossover between unequally aligned sister chromatids is not sufficient to generate a functional full-length *GAL10* gene in the leftmost repeat (Figure 5). One possibility is that two, possibly concerted, events are required to generate the triplication class. However, as argued for the disome recombinants above, the rate of this event in the *gal4 rad1* strain is too high to be the product of two independent events.

The majority of the triplications observed occur in the transcriptionally inactive *gal4 rad1* strains. In the *gal80 rad1* mutant, triplications are not observed; however, these strains exhibit a tenfold elevated rate of popout-associated disomes (to 1.0×10^{-6}, Table 4). It appears that, in the transcriptionally inactive *rad1*

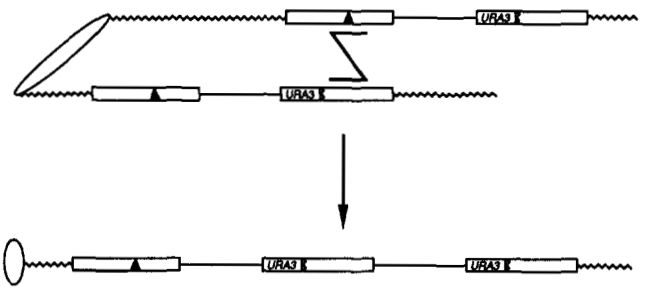

FIGURE 5.—Illustration of a sister chromatid exchange event in the *gal10* duplication. Unequal pairing occurs after DNA replication between the truncated repeat on one chromatid and the full-length repeat containing the Δ*Kpn*I mutation from the other chromatid. A recombination event, indicated by the heavy zig-zag line, can occur between these repeats. A recombination event that resolves by a crossover will produce the triplicated structure, shown below. However, the final structure will always contain the frameshift mutation in the full-length repeat.

strains, Gal$^+$ triplications occur at the expense of the disome recombinants. One explanation for this observation is that the two events possess a common intermediate and that transcription drives the resolution of this intermediate towards the popout disome recombinant. A second model proposes that amplification of the region through multiple rounds of DNA replication causes the triplicated structures (reviewed in STARK and WAHL 1984). A replicative model would explain the recovery of the recombinant containing four copies of the *gal10* repeat.

The role of *rad1* and *rad52* on direct repeat recombination in transcriptionally active and inactive DNA: In contrast to the *rad52* mutation, the effects of *rad1* alone on recombination at *GAL10* are subtle. In transcriptionally active cells in the *rad1* mutant, we see a reproducible twofold decrease in the rate of plasmid loss when compared with *RAD1*. The distribution of the crossover events through the *GAL10* gene is unaffected by a mutation in *rad1*, as is the rate of Gal$^+$ Ura$^+$ replacement. In transcriptionally inactive cells, the rate of plasmid loss is not affected by a *rad1* mutation. Similarly, the rate of Gal$^+$ Ura$^+$ replacement is no different from wild type in *gal4 rad1* strains. These results suggest that, in the absence of *rad1* function, recombination events proceed efficiently by a recombination pathway that probably involves *RAD52*.

The results of these experiments are similar to other reports examining the role of excision-repair gene products in genetic recombination in yeast (SCHIESTL and PRAKASH 1988; KLEIN 1988). In the experiments reported by SCHIESTL and PRAKASH (1988), duplications containing a small region of homology, *ca.* 400 bp, showed a four- to sevenfold reduction in the *rad1* mutant; however, a much larger duplication containing *his4* heteroalleles showed either no effect or slightly higher recombination frequencies when compared to wild type. Similarly, KLEIN (1988) saw little effect of a *rad1* point mutation on popout frequencies between duplications of *leu2* and *his3* containing heteroalleles in the same linear arrangement as the *his4* repeats examined by SCHIESTL and PRAKASH (1988).

The *RAD1* gene has been shown to be involved in the excision repair of UV-induced damage (REYNOLDS and FRIEDBERG 1981; WILCOX and PRAKASH 1981). Based on physical analysis of irradiated DNA, *RAD1* has been grouped with several genes thought to be required for the incision step in the repair process. Recent work by SCHIESTL and PRAKASH (1988) has shown that *RAD1* is required for integration of a linear plasmid into the chromosome, suggesting that *RAD1* acts at a step after the initiating lesion is formed. Furthermore, they show that the hyperrecombination phenotype of *cdc9*, which contains a temperature-sensitive mutation in DNA ligase, is dependent on *RAD1*. It is possible that single-stranded nicks left in the DNA after replication in the *cdc9* mutant are recombinogenic. This is consistent with the results presented here that show that only the transcription-stimulated recombination events are lost in the *rad52* mutant, which we propose initiate by a double-strand lesion.

One possible model for the role of *RAD1* in genetic recombination can be drawn by analogy with excision repair in *E. coli*. In this process, known as "short-patch" repair, one to several nucleotides surrounding the damage are removed and replaced by a polymerase activity using the intact strand as a template (for review, see MODRICH 1987). The single-strand gap in the DNA formed after the removal of the lesion may be a target for a single-strand invasion, such as proposed in the Aviemore model of recombination (MESELSON and RADDING 1975). In yeast, *RAD1* might be involved in the formation or processing of such an intermediate, for example, by facilitating the strand invasion process. A similar model for the role of *RAD1* in genetic recombination has been proposed by SCHIESTL and PRAKASH (1988).

The *RAD52* gene is thought to be involved in the repair of double-strand breaks, probably by a recombinational-repair mechanism (HO 1975; RESNICK 1976; RESNICK and MARTIN 1976). We have proposed a model whereby the transcription-stimulated recombination events in *gal80* constitutive cells are initiated by double-strand breaks within the duplicated *gal10* repeats or the integrated plasmid sequences (THOMAS and ROTHSTEIN 1989). These breaks could be induced by a specific recombination enzyme or alternatively by a component of the transcription machinery, such as a polymerase-associated type II topoisomerase. Since double-strand breaks in the *rad52* mutant are lethal (RESNICK and MARTIN 1976; MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981), such transcription-stimulated, double-strand break-induced events would be lost in the constitutive *gal80 rad52* mutant. Transcriptional activation of a region has been shown to be important for accessibility of DNA repair enzymes in mammalian cells (BOHR *et al.* 1985; MADHANI, BOHR and HANAWALT 1986; MELLON *et al.* 1986). Transcribed DNA shows an increased rate of removal of pyrimidine dimers compared to nontranscribed DNA or bulk chromatin. In addition, MELLON, SPIVAK and HANAWALT (1987) have shown that UV-induced dimers are preferentially removed from the transcribed strand in hamster cells. These results support the notion that transcription renders the DNA more accessible to recombination and repair functions in the cell.

The *rad1 rad52* double mutant displays a much lower recombination rate in all the assays examined than that expected if the two genes acted in a common

pathway. This implies that *RAD1* and *RAD52* act in two overlapping pathways for recombination in yeast. Interestingly, this synergistic interaction of the two mutations does not extend to survival after exposure to radiation; the double mutant was no more sensitive to either gamma- or UV-irradiation than either single mutant (Figure 4). In fact, *RAD1* and *RAD52* were originally placed into separate groups on the basis of their Rad phenotypes. This result suggests that not all the lesions induced by exposure to radiation are processed by a recombination-repair type pathway. We are currently testing the hypothesis that the residual recombination seen in the *rad1 rad52* double mutant is mediated by yet another *RAD* epistasis group, such as the *RAD18* group. The genes in this group define an "error-prone" pathway for response to DNA damage [reviewed by KUNZ and HAYNES (1981) and HAYNES and KUNZ (1981)]. Finally, it is interesting to note that the residual recombination in the *rad1 rad52* double mutant is still affected positively by transcription.

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