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Single strand and double strand DNA damage-induced reciprocal recombination in yeast. Dependence on nucleotide excision repair and *RAD1* recombination

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ABSTRACT

Single strand and double strand DNA damage-induced recombination were compared in the yeast *Saccharomyces cerevisiae*. The non-replicating plasmid pUC18-*HIS3* was damaged *in vitro* and introduced into yeast cells; plasmid–chromosome recombinants were selected as stable His$^+$ transformants. Single strand damage was produced by UV irradiation at 254 nm or by psoralen photoreaction at 390 nm. Double strand damage was produced by psoralen photoreaction at 350 nm or by restriction endonuclease digestion. Recombinants were classified as resulting from gene conversion without crossing over, single plasmid integration, or multiple plasmid integration. Single and double strand DNA damage produced different patterns of recombination. In repair proficient cells double strand damage induced primarily multiple plasmid integrations, while single strand damage induced higher proportions of gene conversions and single integrations. Reciprocal recombination depended on the *RAD1* gene, which is involved in both excision repair and recombination; plasmid integration induced by all forms of damage was decreased in a *rad1* disruption strain. Mutation of the *RAD3* excision repair gene decreased plasmid integration induced by far UV irradiation and psoralen crosslinks, but not by double strand breaks, which are not substrates of nucleotide excision repair. Double strand break-induced plasmid integration was also decreased by disruption of *RAD10*, which forms a complex with *RAD1*; disruption of *RAD4* had no effect. Thus, while nucleotide excision repair genes are involved in the processing of damaged DNA to generate recombination intermediates, *RAD1* and *RAD10* are additionally involved in reciprocal exchange.

INTRODUCTION

Double strand DNA damage poses a particular challenge to cellular repair systems. The primary pathway for removal of DNA damage is excision repair (1–5), in which the affected portion is removed, leaving a single strand gap opposite the unmodified complementary strand. Repair synthesis can use the undamaged strand as a template to fill in the gap; ligation then completes the process. Damage affecting both strands, such as double strand breaks or interstrand crosslinks, leaves no intact template and must be repaired by other mechanisms.

One such pathway is recombinational repair, in which genetic information may be transferred from an undamaged homologous DNA molecule to the damaged molecule. Single strand or double strand breaks are postulated to initiate pairing of homologous DNA sequences, strand invasion of the paired duplex and formation of one or two Holliday junctions (6–8). Genetic information may be exchanged either by DNA synthesis, using the paired molecule as a template, or by branch migration of the Holliday junctions. The Holliday junctions may be resolved to yield either non-crossover products or reciprocal exchange between the two DNA molecules.

Although recombination is induced by both single and double strand damage, double strand damage is particularly effective. In the yeast *Saccharomyces cerevisiae*, psoralen interstrand crosslinks were found to be more recombinogenic than a similar number of psoralen monoadducts (9, 10). In this study we compare recombination induced by single strand or double strand damage in yeast, using 254 nm UV radiation and psoralen monoadducts as examples of single strand damage, and double strand breaks and psoralen crosslinks as examples of double strand damage. We find that single and double strand damage induce qualitatively different patterns of recombination; double strand DNA damage induces a higher proportion of recombination events with reciprocal exchange. We have also examined the role of the nucleotide excision repair pathway in damage-induced recombination. For both single strand and double strand lesions, reciprocal exchange depends on the nucleotide excision repair genes *RAD1* and *RAD3*. *RAD1* and *RAD10* have roles in some forms of recombination, in addition to their functions in excision repair (11–14). We have distinguished between these two functions by examining recombination induced by double strand breaks, which are not substrates for nucleotide

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excision repair. Mutations in *RAD3* and *RAD4* have no effect while mutations in *RAD1* and *RAD10* decrease reciprocal exchange.

MATERIALS AND METHODS

Yeast strains and plasmids

The DNA repair proficient strain W303 is *MATα leu2-3,112 trp1-1 his3-11,15 ade2-1 ura3-1 can1-100* (15). H32, WS41, WS101 and WS5 were derived from W303 by disruption of the *RAD1*, *RAD4*, *RAD10* and *RAD52* genes, respectively. WS1-4C is *MATa rad3-2 trp1-1 ade2-1 ura3-1 his3-11,15* (16). Plasmid pUC18-*HIS3* consists of *HIS3* inserted into the *Bam*HI site of pUC18; it does not replicate in yeast cells. Plasmid YRpHUT carries *HIS3*, *URA3* and *TRP1-ARS1* cloned into pBR322 (16). Plasmid DNA was prepared form *E.coli* DH5α cells by alkaline lysis and purified by CsCl gradients (17).

Plasmid DNA reactions

Plasmid DNA was photoreacted at 350 nm with [^{3}H]4'aminomethyl-4,5',8-trimethylpsoralen (AMT) as previously described, to produce a mixture of crosslinks and monoadducts (16). For the production of psoralen monoadducts by photoreaction at 390 nm, 60 μg/ml pUC18-*HIS3* DNA and 5 μM [^{3}H]AMT (specific activity 300 mCi/mmol) in 10 mM Tris−HCl, 1 mM EDTA, pH 8.0 (TE buffer), in a total volume of 1.0 ml, were incubated in the dark for 30 minutes. They were irradiated with monochromatic 390 light using a 350 W Xenon lamp and a Bausch and Lomb Model 33-86-08 0.25 m monochromator. Aliquots of 150 μl were removed at various times during the irradiation.

Noncovalently bound [^{3}H] AMT was removed from the samples by ethanol precipitation. The samples were made 0.3 M in sodium acetate and precipitated with 2 volumes of ethanol. The DNA pellet was resuspended in 150 μl 0.3 M sodium acetate, and re-precipitated with ethanol before a final resuspension in 75 μl TE buffer. The number of bound adducts was measured by scintillation counting of aliquots of the resuspended DNA, and the number of adducts per plasmid molecule was calculated from the DNA concentration. Re-irradiation of monoadducted DNA was carried out in a Rayonet photochemical reactor at 350 nm for 15 min.

For far UV irradiation, plasmid DNA was suspended in TE buffer at a concentration of 100 μg/ml. Droplets of 200 μl were placed on a plastic Petri dish and irradiated with a Model UVGL-25 lamp (UVP, San Gabriel, CA), emitting 254 nm light. Aliquots of 50 μl were removed at intervals. The light intensity was measured with a UVP Blak-Ray UV meter, model J-225. The number of pyrimidine dimers per plasmid was calculated from the UV dose, using a conversion factor of 0.007 dimers per kb per Jm^{-2} (18) and a length of 4.5 kb for pUC-*HIS3*; we calculate that 30 dimers per plasmid molecule were produced per kJm^{-2} under the conditions used.

Double strand breaks were produced by digestion with *Bst*XI, which cuts the plasmid once at position 437 of the *HIS3* gene.

Yeast transformation

Yeast culture media were prepared according to Sherman *et al.* (19). Yeast spheroplasts were transfected by the method of Beggs (20) with 1 μg plasmid DNA; no carrier DNA was used. The spheroplasts were suspended in 0.5 ml SOS medium and aliquots were plated onto SD-his media. The plates were incubated at 30°C for 4 days, then scored for transformation. Since the transformation efficiency of yeast spheroplasts shows variability between strains, and between spheroplast preparation for each strain, we included positive controls, consisting of the replicating plasmid YRpHUT. The transformation efficiency of the undamaged YRpHUT was used to normalize the numbers of damage-induced pUC-*HIS3* transformants.

Analysis of transformants

Yeast genomic DNA was prepared from His$^+$ transformants according to Sherman *et al.* (19), digested with EcoRI and resolved on 0.8% agarose gels run in TAE (40 mM Tris−acetate, 1 mM EDTA, pH 8.0) buffer, DNA was transferred to nylon membranes and hybridized to biotin-labeled pUC-*HIS3* probes as previously described (21).

RESULTS

Single strand DNA damage induces plasmid−chromosome recombination

DNA damage-induced recombination was measured by the transformation efficiency of damaged plasmid DNA. Plasmid pUC18-*HIS3* was modified *in vitro* by treatments that produced either single or double strand damage. The damaged plasmid DNA was then transfected into *his3* yeast cells and transformants were selected in histidine omission medium. Since pUC-*HIS3* is a non-replicating plasmid, stable His$^+$ transformants must arise by plasmid−chromosome recombination, resulting in a chromosomally located *HIS3* gene. This can occur either by plasmid integration into the chromosome, or by gene conversion of the chromosomal *his3* allele, without reciprocal exchange.

Previous studies have established that double strand damage, in the form of double strand breaks (22) or psoralen interstrand crosslinks (23) stimulate plasmid−chromosome recombination. We examined the ability of single strand damage to induce recombination. Psoralen photoreaction at wavelengths above 380 nm produces primarily 4',5' and 3,4 monoadducts; further irradiation at shorter wavelengths can convert 4',5' monoadducts

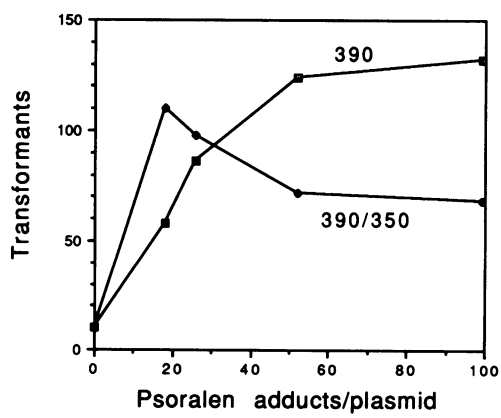


Figure 1. Transformation of *RAD* yeast cells by psoralen photoreacted pUC18-*HIS3*. Plasmid DNA was photoreacted with AMT at 390 nm (open symbols) to form monoadducts, and unbound psoralen was removed. A portion of the reacted plasmid was re-irradiated at 350 nm to convert monoadducts to crosslinks: 390/350 (closed symbols). 0.5 μg plasmid DNA was transfected into yeast cells and His$^+$ colonies were scored.

to interstrand crosslinks (24–26). Yeast transformation by psoralen monoadducted plasmid is shown in Fig. 1. Psoralen monoadducts stimulate transformation by pUC-*HIS3*, producing increasing numbers of transformants up to a damage level of about 50 adducts per plasmid. In comparison, only 1–2 crosslinked psoralen adducts were required to induce maximal transformation (23). When the monoadducted samples were re-irradiated at 350 nm to convert a portion of the monoadducts to crosslinks, the transformation frequency increased at the lowest adduct levels. However, at higher levels of damage the transformation efficiency decreases. This suggests that at low doses crosslinks are more recombinogenic than monoadducts, while at high doses the crosslinked plasmid cannot be efficiently repaired, and is lost from the cell without production of His$^+$ recombinants.

We also examined the ability of far UV irradiation to stimulate recombination (Fig. 2). Pyrimidine dimer-induced transformation of *RAD* cells increased linearly with UV dosage in the range examined. No induced transformation was seen in a strain deficient in the *RAD52* gene, which is required for most forms of mitotic recombination and for repair of double strand breaks (27–29).

In yeast cells nucleotide excision repair of DNA lesions requires the activity of the *RAD3* family of genes (30, 31). We examined induced recombination in yeast strains deficient in *RAD1* and *RAD3*, both members of this group. Mutations in these genes

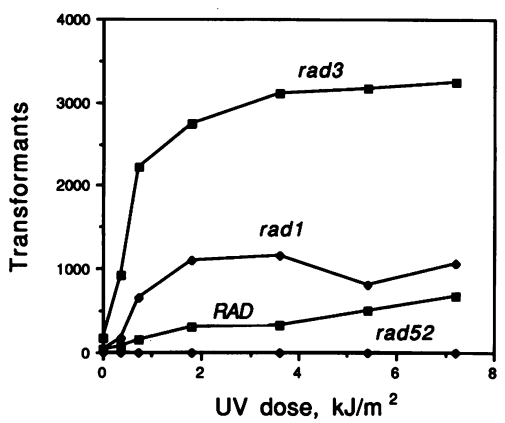
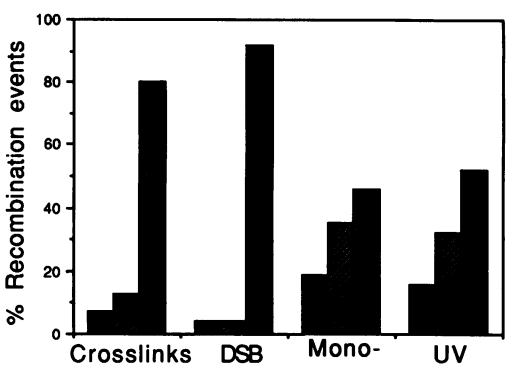
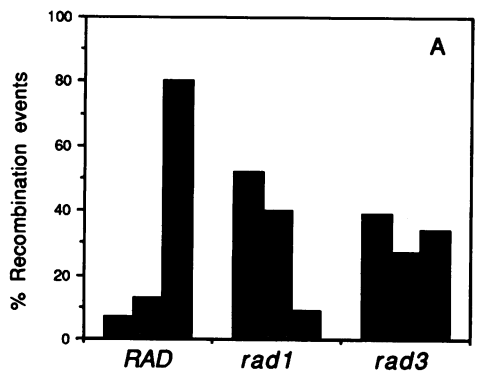


Figure 2. Transformation of repair proficient and repair deficient yeast cells by UV-irradiated plasmid. Plasmid pUC18-*HIS3* was irradiated at 254 nm, and 1.2 μg plasmid DNA was transfected into *RAD* (open squares), *rad1* (closed diamonds), *rad3* (closed squares) or *rad52* (open diamonds) yeast cells. His$^+$ transformants were scored. The transformation efficiency of the replicating plasmid YRpHUT in each transformation experiment was used to normalize the transformation efficiency of the repair-deficient strains to that of the *RAD* strain.

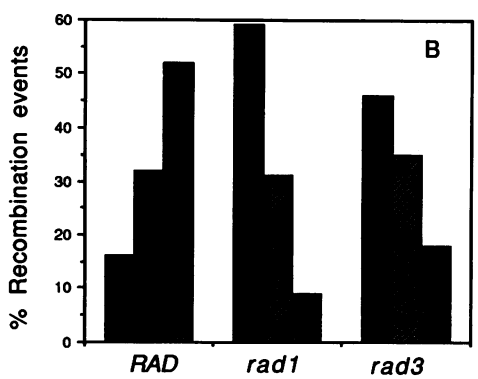


Conversions	5 (7%)	2 (4%)	7 (19%)	23 (16%)
Single integrations	9 (13%)	2 (4%)	13 (35%)	45 (32%)
Multiple integrations	55 (80%)	44 (92%)	17 (46%)	74 (52%)

Figure 3. Classes of recombination induced by double strand and single strand DNA damage in *RAD* cells. His$^+$ transformants induced by psoralen crosslinks, double strand breaks (DSB), psoralen monoadducts or 254 nm UV irradiation were examined by Southern analysis of EcoRI digested yeast genomic DNA. They were classified as gene conversions without plasmid integration (black bars), single plasmid integration (striped bars) or multiple plasmid integration (stippled bars). Recombination events induced by all doses of DNA damage were pooled. The number of samples in each class, and percent of total samples analyzed, are shown below the figure.



Conversions	5 (7%)	42 (52%)	30 (39%)
Single integrations	9 (13%)	32 (40%)	21 (27%)
Multiple integrations	55 (80%)	7 (9%)	26 (34%)



	23 (16%)	38 (59%)	38 (46%)
	45 (32%)	20 (31%)	29 (35%)
	74 (52%)	6 (9%)	15 (18%)

Figure 4. Recombination events induced by bulky DNA lesions in repair-proficient and excision repair-deficient strains. His$^+$ transformants in *RAD*, *rad1*, and *rad3* yeast cells were classified as gene conversion (black bars), single plasmid integration (striped bars) or multiple plasmid integration (stippled bars). Transformants from all damage doses were pooled. The number and percent of total samples are shown below the figures. **A.** Psoralen photoreaction at 350 nm. **B.** UV irradiation.

increased the level of UV-induced transformation. In the absence of excision repair, pyrimidine dimers persist in these strains and appear to be channeled into recombinational repair. In the *rad1* and *rad3* strains a plateau is reached at about 2 kJm^{-2}; this UV dose corresponds to about 60 dimers per plasmid molecule.

Double strand damage induces more reciprocal exchange than single strand damage

Transformation to His^+ may occur by reciprocal exchange, resulting in one or more integrated plasmids, or by gene conversion without crossing over. We analyzed chromosomal DNA from His^+ transformants for plasmid integration. Southern analysis of EcoRI digested DNA, hybridized to a pUC-*HIS3* probe, was used to classify transformants as gene conversion without exchange (no plasmid integrated), single plasmid integration, or multiple plasmid integrations (2 or more plasmids integrated) (21, 23).

There is a marked contrast between the types of recombination induced by single strand and double strand DNA damage (Fig. 3). The majority of the His^+ transformants produced in response to double strand breaks or psoralen interstrand crosslinks carried multiple copies of integrated plasmids. The single strand DNA damage produced by psoralen monoadducts or UV radiation generated fewer multiple integrations and more gene conversion or single integrations; 16–19% were gene conversion and 32–35% were single integrations, in comparison to levels of 4–13% for these classes in the double strand damaged samples.

Multiple plasmid integrations, rather than single integrations or gene conversions, were the most frequent class of recombinants induced by all forms of damage. The transfections were carried out with relatively large amounts of plasmid and no nonhomologous carrier DNA, allowing competent cells to take up more than one plasmid molecule. Damaged plasmids can therefore undergo successive integrations into the homologous chromosomal site (32). We have also observed (21) that an initial plasmid recombination stimulates subsequent reciprocal exchanges, involving undamaged as well as damaged homologous molecules. These events, which are dependent on *RAD1* function, lead to multiple plasmid integrations.

Reciprocal exchange induced by bulky DNA lesions depends on excision repair

Excision repair activity produces single strand breaks in yeast DNA after treatment with UV irradiation or psoralen monoadducts (33); double strand breaks are formed after psoralen crosslinking (34–36). These DNA breaks may be the initiators of recombination; we therefore analyzed damage-induced recombination in repair-deficient yeast strains. In a previous study we found that psoralen photoreaction at 350 nm, producing a mixture of single strand monoadducts and interstrand crosslinks, induced primarily gene conversions and single integrations, rather than multiple integrations, in a *rad1* strain (23). The *RAD1* gene is required for some forms of recombination, as well as for excision repair (11–13, 37); the *RAD3* gene has no comparable role in recombination. We therefore analyzed psoralen crosslink-induced recombination in a *rad3* strain in order to assess the contribution of excision repair alone. We found that in *rad3* cells the majority of transformants were the products of gene conversions or single integration, although the proportion of multiple integrations was higher than in the *rad1* strain (Fig. 4A).

UV light-induced recombination showed a similar pattern (Fig. 4B). The proportion of multiple integrations was lower in the excision repair-deficient strains, decreasing from 52% in *RAD* to 9% and 18% in *rad1* and *rad3* cells, respectively. Meanwhile, the proportion of gene conversion increased, from 16% in *RAD* cells to 59% in *rad1* and 46% in *rad3*. Thus, for DNA lesions which are subject to excision repair, reciprocal exchange induced by single strand or double strand damage depends upon excision repair activity.

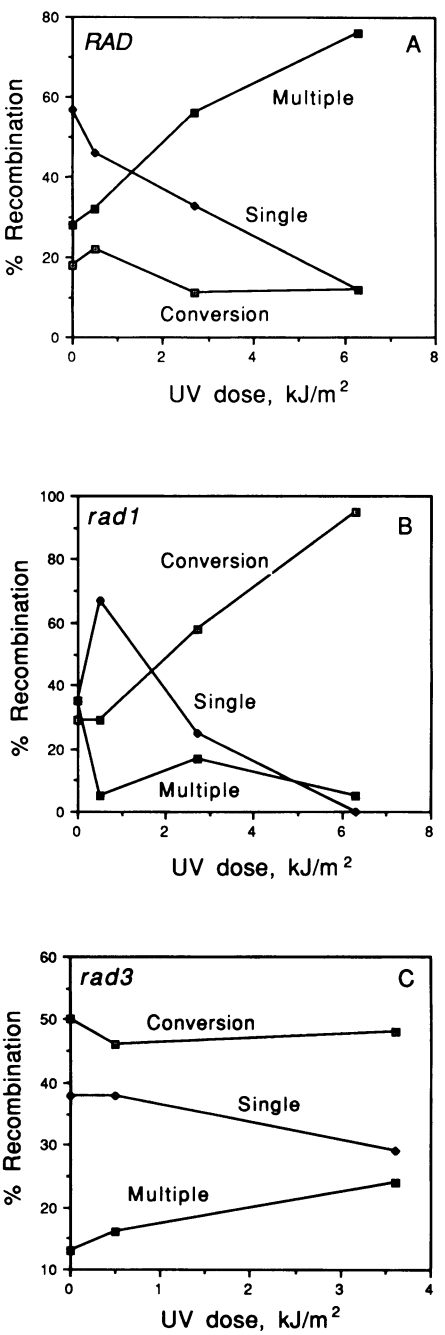


Figure 5. Dependence of recombination class on UV dose. Transformants were classified as gene conversion (open squares), single integration (closed diamonds) or multiple integration (closed squares). **A.** *RAD* **B.** *rad1* **C.** *rad3* yeast cells.

Although there was no stimulation of the plasmid transformation efficiency by DNA damage in *rad52* cells, there was a low, constant, frequency of transformation by pUC-*HIS3*. We analyzed 41 *rad52* colonies transformed by UV-damaged plasmid (data not shown). Of these, 25 had no integrated plasmid, and just 2 had a single integration at the chromosomal *his3* locus. The remaining 14 had plasmid integrated at sites other than the *his3* locus. Such nonhomologous recombination was only observed in the *rad52* strain.

Examining the classes of recombination induced by different doses of UV light, we found varying patterns as the DNA damage level increased (Fig. 5). In the repair proficient strain, the proportion of multiple integrations grew, at the expense of gene conversions and single integrations, as the UV dose increased (Fig. 5A). At the greatest dose, the pattern is similar to that of double strand damage induction. At these levels of damage, about 200 pyrimidine dimers per plasmid molecule, many of the plasmids are likely to carry dimers close to each other on opposite strands and have, in effect, double strand damage. The *rad1* cells, in contrast, have more gene conversions, and fewer integrations, with increasing doses of damage (Fig. 5B). The *rad3* strains showed no variation across the range of doses examined (Fig. 5C).

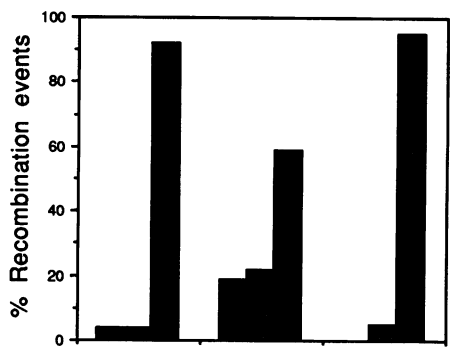


Figure 6. Recombination events induced by double strand breaks in *RAD*, *rad1* and *rad3* strains. pUC18-*HIS3* was linearized by BstXI digestion. His$^+$ transformants of were classified as gene conversion (black bars), single integration (striped bars) or multiple integration (stippled bars).

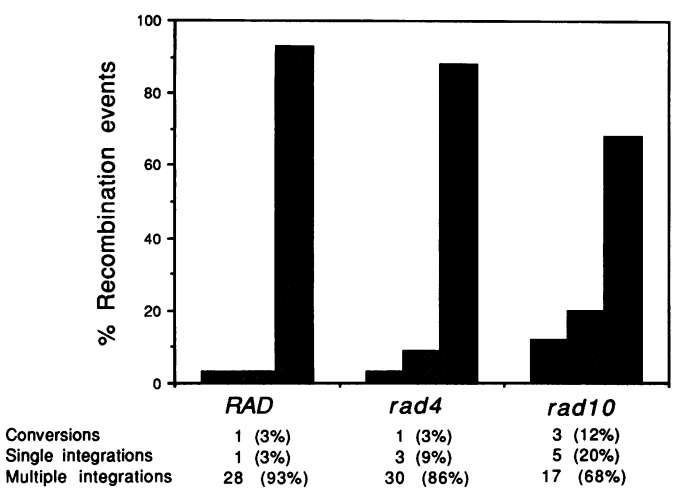


Figure 7. Recombination events induced by double strand breaks in *RAD*, *rad4* and *rad10* strains. pUC18-*HIS3* was linearized by BstXI digestion. His$^+$ transformants were classified as gene conversion (black bars), single integration (striped bars) or multiple integration (stippled bars).

Reciprocal exchange induced by double strand breaks depends on *RAD1* and *RAD10*, but not *RAD3* and *RAD4*

While bulky DNA lesions such as pyrimidine dimers or psoralen adducts are processed by the nucleotide excision repair pathway to generate recombinogenic DNA strand breaks, the double strand breaks produced by restriction endonucleases require no additional processing. Mutation in excision repair genes would therefore not be expected to affect double strand break induced recombination. However, the *RAD1* gene has been found to have an additional role in mitotic recombination.

Mutation in the *RAD3* gene had no effect on the type of recombination induced (Fig. 6); as in *RAD* cells, most of the transformants had multiple plasmid integrations. The *rad1* strain, however, showed a significantly larger proportion of gene conversions and single integrations. Mutations in *RAD10* affect recombination similarly to *RAD1* (14). In addition, the *RAD1* and *RAD10* gene products form a complex with single strand endonuclease activity (38–40). We therefore examined double strand break induced recombination in a *RAD10* deletion strain; gene conversions and single integrations were increased relative to the *RAD* strain (Fig. 7). Deletion of *RAD4*, a nucleotide excision repair gene which has not been implicated in recombination (14), did not affect the pattern of recombination. These results confirm that *RAD1* and *RAD10* play a role in recombination beyond the generation of DNA strand breaks.

DISCUSSION

In this study we have compared the forms of recombination induced by single strand and double strand DNA damage. We find that double strand damage results in a higher proportion of reciprocal exchange than single strand damage. The pattern of recombination products further depends upon nucleotide excision repair activity; mutations in *RAD1* or *RAD3* decrease damage-induced reciprocal exchange. Nucleotide excision repair in yeast acts on single strand lesions to produce single strand breaks, and on psoralen crosslinks to produce double strand breaks (33–36). Models of recombination initiated by both single strand and double strand breaks have been proposed. In the Meselson–Radding model and its variants (6,41) a single strand break or gap initiates pairing with an unbroken homologous duplex, followed by strand exchange and formation of a Holliday junction between the paired DNA molecules. In double strand break repair models (7, 8), the two 3′ ends flanking a double strand break or gap initiate pairing with a homologous DNA molecule, generating two Holliday junctions in the recombination intermediate.

In both types of model the Holliday junction or junctions are resolved by endonuclease cleavage to generate, depending on the orientation of cleavage, either crossover or noncrossover

products. There is no cleavage bias inherent in the models, predicting that the fraction of reciprocal exchange will be 50%. Alternative mechanisms of resolution, such as topoisomerase activity, or DNA replication through the junction, would result in noncrossover products. However, resolution by these mechanisms predicts that information can only be transferred from the intact to the damaged DNA molecule. In our system damaged plasmid is the donor of genetic information in the noncrossover gene conversions.

The observed fractions of reciprocal exchange vary with the system studied. Yeast transformation with replicating plasmids containing double strand breaks results in equal numbers of integrated and extrachromosomal plasmids (42), and HO endonuclease-induced double strand breaks between inverted repeats induce about 50% crossing over (43). However, in other systems the proportion of reciprocal exchange deviates from 50%. HO cutting in the *MAT* locus results exclusively in gene conversion without reciprocal exchange (44). Intrachromosomal recombination between direct repeats occurs primarily by gene conversion or by deletion of the intervening sequences, rather than by crossing over (45–47). Only 23% of DNA damage-induced sister chromatid recombination results in reciprocal exchange (48). Thus, resolution of recombination intermediates is often biased.

The contrasting patterns of recombination induced by single and double strand damage suggest that there are two different pathways for recombinational repair of DNA damage, one leading preferentially to non-crossover products and one leading to reciprocal exchange. The nature of the DNA repair intermediate formed from a lesion may affect entry into these pathways. The action of nucleotide excision repair on lesions in yeast DNA yields different products for single strand and double strand damage. Pyrimidine dimers and psoralen monoadducts generate single strand breaks, while psoralen interstrand crosslinks are processed to form double strand breaks. It is possible that single strand breaks are channeled into non-crossover, and double strand breaks into crossover, recombination.

The proportion of reciprocal exchange depends on the nucleotide excision repair gene activity. Loss of either *RAD1* or *RAD3* function decreases the fraction of crossover recombination after chemical or radiation damage. However, while *rad1* and *rad10* mutations affect the pattern of double strand break-induced recombination, *rad3* and *rad4* mutations do not.

The dependence of the observed pattern of recombination on *RAD1* and *RAD3* function is consistent with a role for excision repair-induced breaks in determining the course of recombination. The model predicts that mutants in the *RAD3* family genes, which do not process bulky lesions to strand breaks, will have lower frequencies of reciprocal exchange. However, directly formed double strand breaks such as the restriction endonuclease cuts in this study, which do not require this processing, should induce similar proportions of reciprocal exchange in both excision repair-proficient and -deficient cells. This prediction was followed in a *rad3* and *rad4* strains, but not in *rad1* and *rad10* cells, suggesting that *RAD1* and *RAD10* have functions in addition to their role in excision repair in this system.

RAD1 gene function has been previously found to affect reciprocal recombination. Crossing over in UV-induced sister chromatid recombination is decreased from 23% to 1% by a *rad1* mutation (48). In studies where both *RAD1* and *RAD3* function were examined, their properties differed. In both spontaneous recombination of an inverted repeat (13), and psoralen damage-induced recombination of a replicating plasmid (16) *rad1* strains had a lower percentage of crossing over than *RAD* or *rad3* strains. The *RAD1* gene product Rad1p, in a complex with Rad10p, has a single strand DNA endonuclease activity (40) which may function in the generation of reciprocal exchange products.

In conclusion, these experiments suggest that there are two pathways of DNA damage-induced recombination. The first, which produces reciprocal exchange, is initiated by double strand DNA damage, and requires *RAD1* function. The second, which generates non-crossover recombination, is initiated by single strand damage, and is *RAD1*-independent. Both pathways depend on *RAD52* function.

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