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Induction of multiple plasmid recombination in *Saccharomyces cerevisiae* by psoralen reaction and double strand breaks

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ABSTRACT

DNA damage-induced multiple recombination was studied by cotransforming yeast cells with pairs of non-replicating plasmids carrying different genetic markers. Reaction of one of the plasmids with the interstrand crosslinking agent, psoralen, stimulated cellular transformation by the undamaged plasmid. The cotransformants carried copies of both plasmids cointegrated in tandem arrays at chromosomal sites homologous to either the damaged or the undamaged DNA. Plasmid linearization, by restriction endonuclease digestion, was also found to stimulate the cointegration of unmodified plasmids. Disruption of the *RAD1* gene reduced the psoralen damage-induced cotransformation of intact plasmid, but had no effect on the stimulation by double strand breaks. Placement of the double strand breaks within yeast genes produced cointegration only at sequences homologous to the damaged plasmids, while digestion within vector sequences produced integration at chromosomal sites homologous to either the damaged or the undamaged plasmid molecules. These observations suggest a model for multiple recombination events in which an initial exchange occurs between the damaged DNA and homologous sequences on an undamaged molecule. Linked sequences on the undamaged molecule up to 870 base pairs distant from the break site participate in subsequent exchanges with other intact DNA molecules. These events result in recombinants produced by reciprocal exchange between three or more DNA molecules.

INTRODUCTION

Damage affecting both strands of a DNA molecule, such as double strand breaks or interstrand crosslinks, results in the complete loss of genetic information from the duplex. The missing information can be restored, and the lesion can be repaired in an error-free manner, by recombinational repair involving the damaged molecule and undamaged homologous sequences. Psoralens are photoreactive molecules which form covalent monoadducts and interstrand crosslinks in the presence of near UV light (1). DNA interstrand crosslinkers have been found to be effective inducers of recombination in both prokaryotic (2,3) and eukaryotic (4–6) cells.

A notable feature of the plasmid-chromosome recombination induced by double strand breaks (7), or by psoralen photoreaction (8), is the prevalence of multiple plasmid integrations. In some recombinants, more than 20 copies of the plasmid are integrated into the homologous chromosomal locus in a tandem array. In a previous study, we had found that the occurrence of psoralen damage-induced multiple integration depended on the function of *RAD1* (8), a gene which is involved in both excision repair (9) and in some forms of recombination (10, 11). Most of the recombinants in a *rad1* strain were produced by single integration or by gene conversion.

In the present study we have investigated the interactions between DNA carrying double strand damage and partially homologous undamaged molecules by cotransformation experiments in yeast cells. Undamaged plasmids were introduced into cells along with plasmid molecules bearing either psoralen adducts or double strand breaks. Cotransformation by the undamaged plasmids is stimulated by damage to partially homologous DNA molecules. The cotransformants contain both the damaged and undamaged plasmids cointegrated in tandem arrays within chromosomal DNA. The frequency and patterns of cointegration are consistent with a model in which an initial recombination intermediate between homologous damaged and undamaged molecules proceeds to participate in further exchanges with intact DNA molecules.

MATERIALS AND METHODS

Reagents

[^{3}H]4′-aminomethyl-4,5′,8-trimethylpsoralen (AMT) was obtained from HRI, Inc. (Emeryville, CA), and cold AMT was from Calbiochem. Restriction endonucleases were obtained from New England Biolabs (Beverly, MA), Bethesda Research Laboratories (Gaithersburg, MD) or International Biotechnologies, Inc. (New Haven, CT). Bacteriophage lambda DNA was from New England BioLabs.

Strains and plasmids

Yeast strain W303 is *MAT α leu2–3,112 trp1–1 ade2–1 ura3–1 can1–100 his3–11,15* (12). H32 (*rad1::LEU2*) was

derived from W303 by gene disruption (13). Plasmids were maintained in *E. coli* strain DH5. Plasmid pUC18-*HIS3* contains the *HIS3* gene of *Saccharomyces cerevisiae* inserted into the *Bam*HI site of pUC18, and pUC18-*URA3* contains the *URA3* gene inserted into the *Hin*DIII site. Plasmids were prepared by alkaline lysis and purified on CsCl gradients (14).

Plasmid treatment

Plasmid DNA samples, suspended in TE buffer at a concentration of 100–200 μM in base pairs, were incubated in the dark with [^{3}H]AMT, at concentrations from 0 to 10 μM, for 30 min. The samples were irradiated for 10 min in a Rayonet photoreactor (Southern New England Ultraviolet Co., Branford, CT). Unreacted AMT was removed by phenol extraction, followed by ethanol precipitation. The plasmid DNA was resuspended in TE buffer at a concentration of 200–400 μM, and the number of psoralen adducts per plasmid molecule was calculated from the level of bound [^{3}H]AMT (8). Linearized plasmid DNA was produced by restriction endonuclease digestion, following the conditions specified by the supplier, and resuspended at a concentration of 200–400 μM.

Yeast transformation

Yeast spheroplasts were prepared and transformed according to Beggs (15), adding 0.5 to 1.0 μg of each plasmid DNA to the spheroplasted cells. Aliquots of the spheroplasts were plated, in sorbitol-containing top agar, onto both SD-his and SD-ura omission plates (16) and incubated at 30°C for 5 days. Colonies appearing on these plates were transferred to YPD plates, for growth under non-selective conditions, and grown for 1 day at 30°C. They were replica plated onto both SD-his and SD-ura plates to test for cotransformation by the unselected marker.

Analysis of transformants

Yeast genomic DNA was prepared from 10 ml YPD cultures according to Sherman et al. (16), digested with *Eco*RI and run on 0.8% agarose gels in TAE buffer. The gels were transferred to Gene Screen Plus nylon membranes (DuPont) by alkaline blotting (17). Probes were prepared by nick translation of the *Hin*dIII *URA3* gene fragment or the *Bam*HI *HIS3* gene fragment with biotin-dUTP, using a BRL nick-translation kit, and hybridized to the membranes according to the manufacturer's directions. The filters were visualized by the Blue Gene system with alkaline phosphatase-conjugated streptavidin.

RESULTS

Experimental design

The vectors used are integrating plasmids which lack yeast replication sequences and cannot replicate extrachromosomally in yeast cells (18). Cellular transformation by these plasmids occurs by integration into the chromosomes, or by gene conversion of chromosomal alleles. Transformation by integrating plasmids is thus a measure of plasmid-chromosome recombination.

Unmodified integrating plasmids transform yeast cells with poor efficiency, but the level of transformation may be stimulated by the introduction of double strand breaks or gaps (7), or by psoralen photoreaction (8). In these experiments we examined the ability of damaged plasmid molecules to stimulate transformation by undamaged plasmids. The plasmids used were pUC18-*HIS3* and pUC18-*URA3*, which consist of the *HIS3* and *URA3* genes, respectively, inserted into the polylinker sequence of the *E. coli* plasmid pUC18 (Figure 1). We paired damaged with undamaged plasmids in cotransfections, and measured the extent of transformation by each marker.

Psoralen photoreaction of plasmid DNA stimulates transformation by undamaged homologous plasmids

Yeast cells were cotransfected with a mixture of psoralen-reacted plasmid pUC18-*HIS3* and unreacted pUC18-*URA3*, and transformants were selected on either histidine or uracil omission media. The number of His$^+$ transformants increased with psoralen modification (Figure 2). Transformation by undamaged pUC18-*URA3* was also stimulated by the photoreaction of pUC18-*HIS3*. The extent of Ura$^+$ transformation was lower than that of His$^+$ transformation at all damage levels, and rose linearly with the dose of psoralen. A similar cotransformation of undamaged pUC18-*HIS3* with damaged pUC18-*URA3* was also observed (data not shown).

In a previous study (8) we observed that, although psoralen damage-induced transformation reached similar levels in *RAD*

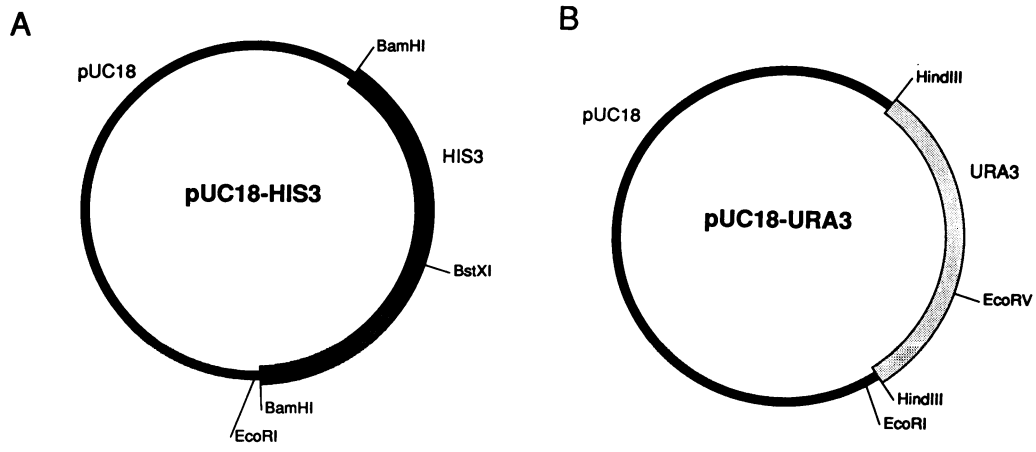

Figure 1. Restriction maps of the plasmids used in this work. Relevant restriction sites are shown. A. pUC18-*HIS3*; B. pUC18-*URA3*

and *rad1* strains, fewer than 10% of the *rad1* transformants, as compared with 80% of the *RAD* transformants, had multiple integrations. We therefore measured the cotransformation frequency in an isogenic *rad1* strain. In a cotransfection of psoralen-modified pUC18-*HIS3* DNA with unmodified pUC18-*URA3*, inactivation of *RAD1* decreased the level of Ura^+ transformation, relative to His^+ transformation, by about 4-fold (Figure 2).

Cotransformation depends upon the presence of homology between the damaged and undamaged transfecting molecules. When psoralen photoreacted lambda phage DNA, which shares no homology with the plasmids, was cotransfected with unmodified plasmid DNA no damage-dependent increase in the level of transformation was observed.

Individual transformants, initially selected on either histidine or uracil omission media, were next screened to measure the extent of cotransformation to His^+Ura^+. The results are presented in Figure 3. Most of the colonies initially selected on histidine omission plates were His^+Ura^- single transformants. The level of cotransformation to His^+Ura^+ rose with the extent of pUC18-*HIS3* modification, from 20% without psoralen addition up to 50% at three adducts per plasmid molecule. In contrast, of the colonies initially selected for transformation by the undamaged pUC18-*URA3* DNA, a high and nearly constant proportion of about 80% were His^+Ura^+ cotransformants. This suggests that, in most cases, transformation by the damaged plasmid is a requirement for cotransformation by the undamaged plasmid. These results indicate that His^+ transformation was directly induced by damage to pUC18-*HIS3*, but that transformation by pUC18-*URA3* was indirectly induced by the pUC18-*HIS3* photoreaction.

His^+Ura^+ co-transformants contain co-integrated pUC18-*HIS3* and pUC18-*URA3*

Histidine or uracil prototrophs may arise by several mechanisms: 1) reversion of the chromosomal mutant alleles to wild type, 2) gene conversion between plasmid and chromosome, or 3) plasmid integration. Plasmid integration into the homologous chromosomal sites produces changes in their restriction maps and is detectable by Southern blotting and hybridization. Genomic DNA was isolated from cotransformants, digested with *Eco*RI and probed on duplicate filters with *HIS3* and *URA3* fragments.

A representative hybridization is presented in Figure 4. In this experiment unmodified pUC18-*HIS3* was cotransfected with psoralen photoreacted pUC18-*URA3*. Hybridization to a *HIS3* probe is shown in Figure 4a and hybridization to *URA3* is shown in Figure 4b. Lanes 2, 3, 9, 11 and 12 have intact chromosomal *URA3* genes (compare to the parental strain in lane C of Fig. 4b), but disrupted *HIS3* genes (compare to lane C of Fig. 4a) and so contain both plasmids integrated into the chromosomal *HIS3* locus. Lanes 2, 3, 9 and 11 contain an additional 4.2 kb band hybridizing to *HIS3* and thus contain multiple integrated copes of pUC18-*HIS3*. In contrast, the other lanes have intact *HIS3* genes (Fig. 4a) but disrupted *URA3* genes (Fig. 4b), and so carry plasmids integrated at the chromosomal *URA3* locus. Lane 8 has a novel band, of 5.5 kb, which hybridizes to both *HIS3* and *URA3*; this was the only deviation from the basic integration pattern seen among the 59 psoralen-induced cotransformants analyzed.

Several phenotypically single transformants were also tested for plasmid integration. We analyzed twelve transformants, initially selected for the marker on the undamaged plasmid, and not expressing the marker on the damaged plasmid. Eight were His^+Ura^- and four were His^-Ura^+. Of these twelve, seven were found to have cointegration of both plasmids at the chromosomal locus homologous to the damaged plasmid. The loss of expression of one of the plasmid markers was probably the result of gene conversion of the plasmid allele by the mutant chromosomal allele during integration. Thus the cotransformation frequency, measured by phenotypic analysis, gives a minimum estimate of the frequency of cointegration.

Plasmid co-integration sites

The cointegrants were divided into two classes, according to whether the integrations were at the chromosomal locus

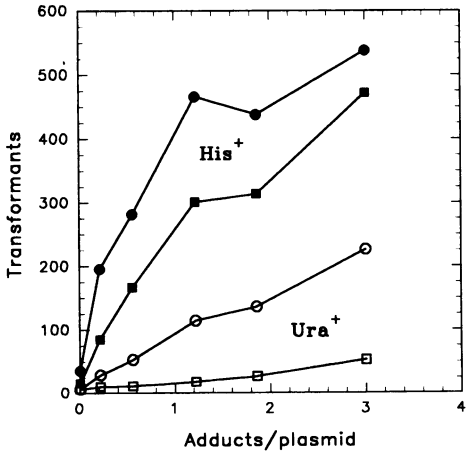

Figure 2. Cotransformation of psoralen-reacted pUC18-*HIS3* and undamaged pUC18-*URA3*. Yeast spheroplasts were transfected with 1 μg of each plasmid and aliquots were plated onto histidine and uracil omission media. For comparison of the two strains, the number of colonies transformed by the replicating plasmid YRp12 was determined in each experiment, and the ratio of YRp12 transformants was used to normalize the *rad1* values to the *RAD* transformants. circles, *RAD* cells; squares, *rad1* cells ●, ■ His^+ transformants; ○, □ Ura^+ transformants.

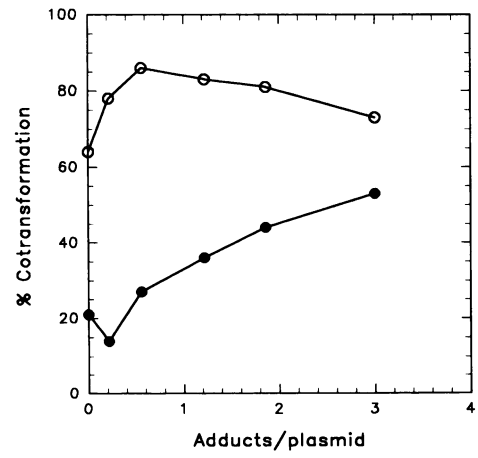

Figure 3. Proportion of His^+Ura^+ cotransformants. Repair proficient cells, transformed with psoralen reacted pUC18-*HIS3* plus undamaged pUC18-*URA3*, were initially selected on either histidine or uracil omission medium. They were tested for stable His^+Ura^+ cotransformation by growth on YPD plates, followed by replica plating onto both histidine and uracil omission plates. ●, initial selection as His^+ transformants; ○, initial selection as Ura^+ transformants.

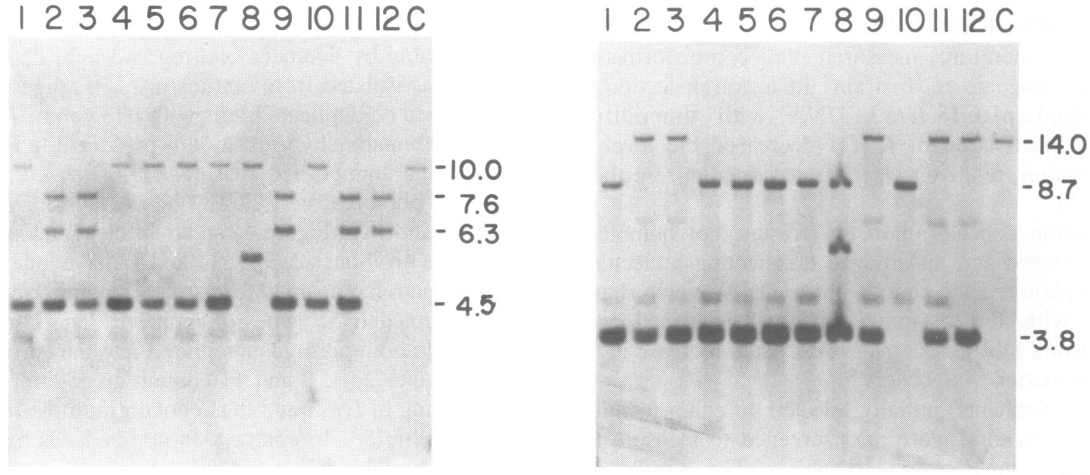

Figure 4. Cointegration sites of psoralen damaged pUC18-*URA3* and undamaged pUC18-*HIS3*. Psoralen reacted pUC18-*URA3* was cotransfected with undamaged pUC18-*HIS3* and His^+Ura^+ cotransformants were identified among His^+ transformants. Genomic DNA from cotransformants was digested with *Eco*RI and duplicate portions were run on agarose gels. Lanes 1 to 12 are cotransformants and lane C is the parental strain. A. Hybridization to *HIS3* probe. Plasmid integration disrupts the 10 kb chromosomal *HIS3* band, producing 2 new bands of 6.3 and 7.6 kb. Integration of two or more copies of pUC18-*HIS3* produces another band of 4.5 kb. B. Hybridization to *URA3* probe. The 14 kb chromosomal *URA3* band is disrupted by plasmid integration, producing an 8.7 kb band upon single integration and a 3.8 kb band upon multiple pUC18-*URA3* integrations. The faint bands seen on both filters are due to contamination of the probes by pUC18 sequences, which hybridize to both integrated plasmids.

homologous to the damaged (Class I) or the undamaged (Class II) plasmid. For example, the samples shown in Figure 4 derive from cotransfection of unmodified pUC18-*HIS3* and reacted pUC18-*URA3*. Plasmid cointegrations at the *URA3* locus are termed Class I events, while integrations at the *HIS3* locus, homologous to the undamaged plasmid molecules, are termed Class II events. In a cotransfection of damaged pUC18-*HIS3* with undamaged pUC18-*URA3*, the Class I integrations are at *HIS3* and the Class II integrations are at *URA3*. Most of the cotransformants were found to be Class I cointegrants at the locus homologous to the damaged plasmid (Table 1). Of the cotransformants in which pUC18-*HIS3* was damaged, 23 of 24 (96%) were Class I, and 1 of 24 (4%) was Class II. When pUC18-*URA3* was the modified plasmid 27 of 35 tested (77%) were Class I events and 8 (23%) were Class II.

Cotransformation of linearized and uncut plasmids

Double-strand breaks were also investigated for their ability to stimulate the cointegration of unmodified plasmid DNA. Double strand breaks were placed within the yeast sequences of the plasmids by digesting pUC18-*HIS3* with *Bst*XI or pUC18-*URA3* with *Eco*RV. Alternatively, cuts were made in the vector polylinker sequences by digesting either plasmid with *Eco*RI. Yeast cells were then cotransfected with pairs of linearized and circular plasmids, as with the psoralen damaged plasmids. The results are presented in Table 2.

Addition of linearized DNA stimulated cotransformation of the circular plasmids by from 10 to over 50-fold. Cleavage within vector sequences, in a region where the plasmids are homologous, induced cotransformation by circular DNA when pUC18-*HIS3* was the linearized plasmid and also, to a lesser extent, when pUC18-*URA3* was cut. Interestingly, cotransformation was also stimulated when the double-strand break was located within the yeast sequences of the plasmids, which are not homologous. Cleavage within the *HIS3* gene of pUC18-*HIS3* stimulated transformation by uncut pUC18-*URA3*, while cleavage within *URA3* induced transformation by circular pUC18-*HIS3*.

Table 1. Co-integration sites of unmodified and psoralen damaged plasmids

Plasmid treatment	Class I[a]	Class II[b]
Psoralen reacted pUC18-*HIS3* + unmodified pUC18-*URA3*	23 (96%)[c]	1 (4%)
Psoralen reacted pUC18-*URA3* + unmodified pUC18-*HIS3*	27 (77%)	8 (23%)

[a]Plasmid co-integration at the site homologous to the damaged plasmid.
[b]Plasmid co-integration at the site homologous to the undamaged plasmid.
[c]Number (percent) of co-transformants analyzed.

The cotransformation frequency to His^+Ura^+ was also measured. As with the psoralen-induced transformants, the frequency of His^+Ura^+ cotransformation was high, ranging from 78% to 97%, when the initial selection was for the gene present on the unmodified plasmid. This is consistent with a need for interaction with the linearized plasmid molecule in order for transformation by the circular plasmid to occur. The majority of transformants selected for the yeast gene present on the linearized plasmid were single transformants; from 19% to 35% of these colonies were His^+Ura^+. The dependence of double strand break-induced cotransformation on *RAD1* function was examined by comparing the wild type and *rad1* strains (Table 3). In contrast to psoralen damage-induced cotransformation, there was no significant decrease in the extent of cotransformation by circular plasmids in the *rad1* strain.

The chromosomal structures of His^+Ura^+ cotransformants were analyzed by Southern blotting and hybridization, as described above. All the samples were found to have copies of the two plasmids integrated into the same site, and some multiple integrations, of three or more plasmids, were observed, as for the psoralen damage-induced cointegrations of Figure 4 (data not shown).

The sites of cointegration depended on the placement of the double-strand break. Plasmid digestion within the yeast genes of either pUC18-*HIS3* or pUC18-*URA3* produced Class I

Table 2. Cotransformation of linearized and uncut plasmids

Plasmid treatment	His^+		Ura^+	
	No. of transformants	%His^+Ura^+	No. of transformants	%His^+Ura^+
None				
Uncut pUC18-*HIS3*	3		0	
Uncut pUC18-*URA3*	0		4	
Cut within yeast sequences				
Cut pUC18-*HIS3*	96		0	
Cut pUC18-*HIS3* + uncut pUC18-*URA3*	266	19	48	85
Cut pUC18-*URA3*	0		200	
Cut pUC18-*URA3* + uncut pUC18-*HIS3*	84	86	352	34
Cut within vector sequences				
Cut pUC18-*HIS3*	202		0	
Cut pUC18-*HIS3* + uncut pUC18-*URA3*	257	34	118	97
Cut pUC18-*URA3*	0		31	
Cut pUC18-*URA3* + uncut pUC18-*HIS3*	22	78	48	35

Yeast spheroplasts were transfected with 0.25 μg of each plasmid, and duplicate 20 μl aliquots were plated onto histidine and uracil omission media. The number of transformants is the average of two independent transfections. The cotransfections with plasmids cut within yeast and vector sequences were performed on different occasions, and the absolute numbers of transformants are not directly comparable. His^+ and Ura^+ transformants from each cotransfection were grown on YPD, then replica plated to both histidine and uracil omission media to determine the frequency of His^+Ura^+ cotransformation.

cointegrations, at the chromosomal locus homologous to the site of cleavage, in all of the cotransformants analyzed (Table 4). A different pattern was observed with plasmids linearized within vector polylinker sequences. The cotransfection of *Eco*RI-cut pUC18-*URA3* and uncut pUC18-*HIS3* produced roughly equal numbers of Class I cointegrations at the *URA3* locus (15/30) and Class II cointegrations at the *HIS3* locus (12/30). Nearly all the cotransformants produced by pairing *Eco*RI-digested pUC18-*HIS3* with circular pUC18-*URA3* were Class I integrations at *HIS3*. There were, in addition, a small number of co-transformants in which the two plasmids were integrated at unknown sites, as both the *HIS3* and *URA3* chromosomal copies were intact; these strains were not investigated further.

Table 3. Cotransformation of linearized and uncut plasmids. Comparison of *RAD* and *rad1* strains.

Selection	Uncut plasmid cotransformants / Linearized plasmid transformants	
	RAD	*rad1*
His^+	0.24 ± 0.08	0.26 ± 0.08
Ura^+	0.19 ± 0.08	0.18 ± 0.09

RAD and *rad1* yeast spheroplasts were cotransfected with mixtures of uncut and linearized plasmids, either *Bst*XI digested pUC18-*HIS3* + uncut pUC18-*URA3* or *Eco*RI digested pUC18-*URA3* + uncut pUC18-*HIS3*. Aliquots were plated onto histidine or uracil omission media, and the ratio of uncut plasmid transformants/linear plasmid transformants was calculated for His^+ and Ura^+ colonies. The results presented are the mean ± S.D. of 4 independent experiments.

DISCUSSION

Studies of damage-induced plasmid recombination in yeast showed that plasmids bearing double-strand breaks (7) or psoralen adducts (8) often produced multiple, tandem integrations into homologous chromosomal loci. We have investigated the mechanism of these multiple recombination events by performing cotransfection experiments with damaged and undamaged plasmid molecules. Damage to transfecting plasmid molecules was found to stimulate the chromosomal integration not only of the damaged DNA, but of partially homologous unmodified plasmids as well.

Indirect induction of recombination between undamaged DNA molecules has been previously reported in *E. coli* (19) and yeast (20). In mammalian cells, integration of extrachromosomal DNA is sometimes associated with a transient chromosomal destabilization, leading to further DNA rearrangement (21–23). We have found that, in yeast cells, damaged plasmid molecules stimulate multiple recombination events, involving three or more DNA molecules, at a high frequency; about one quarter of the transformants induced by a double strand break in plasmid DNA have cointegrated undamaged plasmid.

We propose a model involving multiple exchanges between the damaged DNA molecule and homologous sequences on undamaged molecules. Multiple integrations may take place by repeated plasmid insertions at a single locus, or may involve extrachromosomal interplasmid recombination, generating plasmid multimers which then integrate into a chromosome. These two pathways may be distinguished by the sites at which the cotransfected plasmids integrate.

If multiple recombinations occur by sequential integrations of plasmid molecules into a chromosome, all the cointegrations should be located at the site homologous to the damaged plasmid, which is the recombinogenic substrate and is presumed to initiate the recombination events. This mechanism would produce only Class I cointegrations. The second pathway, involving an extrachromosomal recombination between damaged and

Table 4. Co-integration sites of linearized and unmodified plasmids

Site of linearization	Class I	Class II	Other[a]
Within yeast sequences			
Cut pUC18-*HIS3* + uncut pUC18-*URA3*	32 (100%)[b]	0	0
Cut pUC18-*URA3* + uncut pUC18-*HIS3*	32 (100%)	0	0
Within vector sequences			
Cut pUC18-*HIS3* + uncut pUC18-*URA3*	29 (97%)	0	1
Cut pUC18-*URA3* + uncut pUC18-*HIS3*	15 (50%)	12 (40%)	3

[a]Integrations at sites other than *HIS3* or *URA3*
[b]Number (percent) of samples in each class

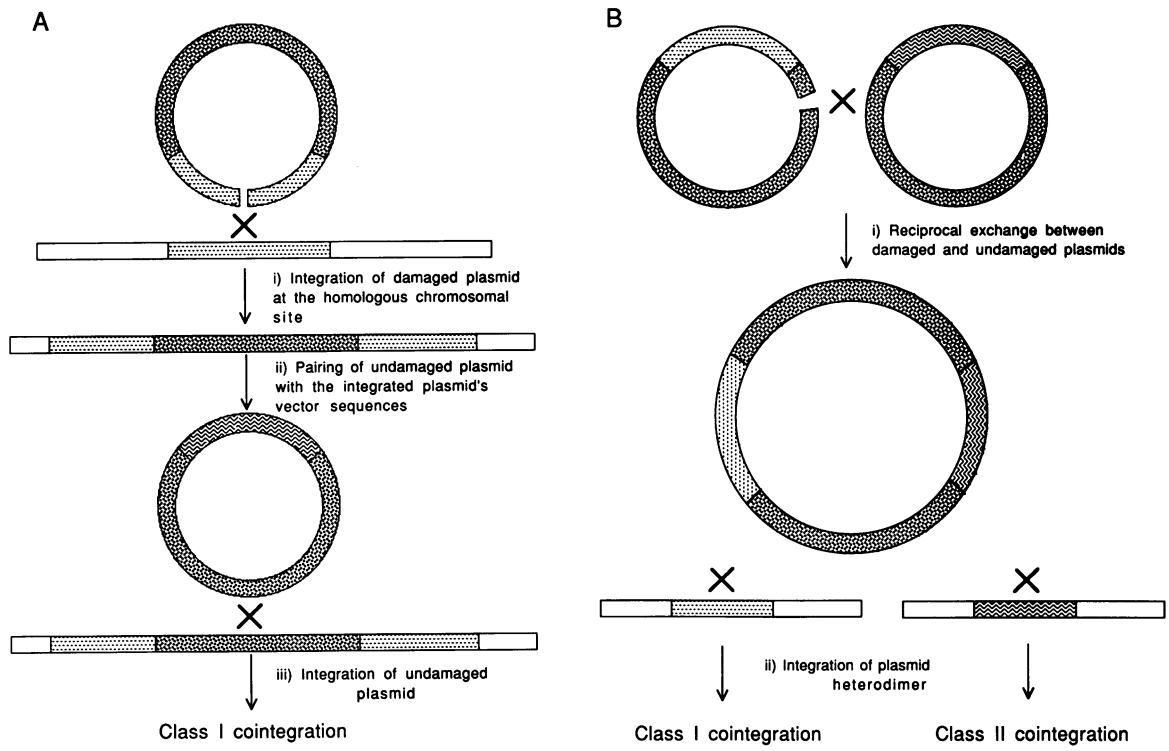

Figure 5. Model for cointegration of damaged and undamaged plasmids. A. Damage within yeast sequences. Reciprocal exchange between the damaged plasmid and homologous sequences on a chromosome results in integration of the plasmid. Integrated vector sequences form recombinogenic intermediates which pair with the vector sequences of undamaged plasmid. A second reciprocal exchange results in cointegration of the undamaged with the damaged plasmid. Since these events occur at the chromosomal locus homologous to the damaged plasmid, all cointegrations are Class I. B. Damage within vector sequences. Reciprocal exchange between the damaged plasmid and homologous vector sequences on an undamaged plasmid results in formation of a plasmid heterodimer. The yeast sequences on the dimeric plasmid molecule form recombinogenic intermediates which pair with homologous sequences on chromosomes. Reciprocal exchange produces integration of the dimeric plasmid. Since either yeast gene on the recombinant plasmid may pair with its chromosomal homolog, both Class I and Class II events are possible. dashed lines, yeast sequences on damaged plasmid; wavy lines, yeast sequences on undamaged plasmid; dots, vector sequences.

undamaged plasmid molecules prior to the integration step, may result in integration of the plasmid multimer at chromosomal sites homologous to either of the cotransfected plasmids, producing both Class I and Class II cointegrations. We observed both Class I and Class II cointegrants, a result indicating that extra-chromosomal reciprocal exchange between plasmids does occur. In the experiments with double strand breaks, placement of breaks within yeast sequences resulted in only Class I events, while breaks within vector sequences produced both Class I and Class II integrations.

The following model, diagrammed in Figure 5, is consistent with these results. When the DNA lesion is placed within yeast sequences (Figure 5a), recombination takes place between the damaged DNA and homologous undamaged sequences on the chromosome, leading to integration of the damaged plasmid into the homologous chromosomal locus. The plasmid vector sequences are now located on the chromosome. A second reciprocal exchange between the undamaged plasmid and the chromosomal vector sequences produces Class I cointegration of the two plasmids.

When the damage is located within vector sequences (Figure 5b), the initial exchange occurs between the damaged plasmid and homologous vector sequences on a cotransfected undamaged plasmid. This produces a plasmid heterodimer, carrying yeast genes of both the reacted and unreacted plasmids, which can undergo a subsequent reciprocal exchange with chromosomal DNA at either locus to produce both Class I and Class II cointegrants.

In both cases, the first exchange occurs between the damage site on the reacted plasmid and homologous sequences on an undamaged DNA molecule, producing a recombinant molecule. A later exchange can then take place between other markers on the recombinant and homologous sequences on a third, undamaged, DNA molecule. In order for this second exchange to take place linked sequences, at a distance from the initial lesion site, must themselves become recombinogenic. In the case of cotransfection by *Bst*XI-digested pUC18-*HIS3* and unmodified pUC18-*URA3*, the primary recombinogenic lesion is a double-strand break placed in the middle of the *HIS3* gene, at a distance of 875 base pairs from the upstream vector sequences and 912 base pairs from the downstream vector sequences. This indicates that regions on recombining DNA molecules at least 875 base pairs distant from the initiating lesion can form secondary recombinogenic intermediates. Nickoloff et al. (24) have observed stimulation of both intrachromosomal and plasmid-chromosome recombination of genes 2.1 kb from an *HO* nuclease recognition site, while Ray et al. (20) reported *HO*-induced interchromosomal recombination of genes at a distance of 8.6 kb from the cutting site.

There are several possible mechanisms for the stimulation of recombination at a distance from the primary lesion. Nuclease degradation may produce a double strand gap reaching to the

secondary recombination sites. However, cases in which the intervening sequences were retained in recombinants cannot be explained by this model. Recombination proteins may enter a broken DNA molecule at the break site, but act at a distance, as for the RecBCD system of *E. coli* (24,25). This model predicts that, in cotransfections with double strand breaks placed within yeast sequences, the initial exchange could involve vector sequences and generate Class II cointegrations, while we observed only Class I events. Alternatively, there may be extensive single strand degradation of the recombination intermediate (20).

There were differences between the linearized pUC18-*HIS3* and pUC18-*URA3* plasmids in the proportions of Class I and Class II cointegrants produced by double strand breaks within vector sequences. Integration at *HIS3* appears to be preferred, perhaps due to the longer region of homology to chromosomal sequences, 1.8 kb in pUC18-*HIS3*, but only 1.2 kb in pUC18-*URA3*. The rate of mitotic recombination has been found to be correlated with the extent of homology in intraplasmid (26), plasmid-chromosome (27,28), and interchromosomal reciprocal recombination, but not intrachromosomal recombination (29), although there appeared to be sequence-specific effects as well in these systems.

Although similar frequencies of transformation are induced by psoralen damage in *RAD* and *rad1* cells, few of the *rad1* transformants have multiple plasmid integrations (8). This indicates that there is an alternative, *RAD1*-independent pathway of recombination, producing fewer multiple crossovers. Here we have found that loss of *RAD1* function reduced the frequency of cotransformation stimulated by psoralen photoreaction, but not by double strand breaks. Psoralen crosslinks have been observed to produce double strand breaks in yeast DNA (30,31), and incision of psoralen damage depends on *RAD1* function (32). This suggests that double strand breaks in plasmid DNA produced by excision repair activity on crosslinks are responsible for most, but not all, of the cotransformation. The residual psoralen damage-induced cotransformation in *rad1* cells may involve recombination intermediates other than double strand breaks.

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