8-Methoxypsoralen photoinduced plasmid–chromosome recombination in *Saccharomyces cerevisiae* using a centromeric vector

Lisiane B. Meira*, Joao Antonio Pêgas Henriques 1 and Nieve Magaña-Schwencke

Institut Curie, Section Biologie, URA 1292 CNRS, 26 rue d’Ulm, F-75231 Paris Cedex 05, France and 1Laboratório de Radiobiologia Molecular, Centro de Biotecnologia, UFRGS, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil

Received November 21, 1994; Accepted March 10, 1995

ABSTRACT

The characterization of a new system to study the induction of plasmid–chromosome recombination is described. Single-stranded and double-stranded centromeric vectors bearing 8-methoxypsoralen photoinduced lesions were used to transform a wild-type yeast strain bearing the *leu2-3,112* marker. Using the SSCP methodology and DNA sequencing, it was demonstrated that repair of the lesions in plasmid DNA was mainly due to conversion of the chromosomal allele to the plasmid DNA.

INTRODUCTION

Despite being widely used as photo-therapeutic agents, psoralens are also DNA damaging agents, and can initiate a variety of molecular changes including base substitutions, insertions or deletions of nucleotides as well as recombination (1,2). Psoralens are photo-reactive molecules that intercalate into DNA and, in the presence of UVA, may form covalent monoadducts and interstrand crosslinks (3,4). The bifunctional furocoumarin 8-methoxypsoralen (8-MOP) is capable of forming both mono- and bivalent DNA-adducts. Monoadducts are thought to be mainly repaired by an excision repair pathway (5,6) while repair of crosslinks involves recombinational and/or translesional DNA synthesis in addition to excision repair. Psoralen photo-induced crosslinks, and to a lesser extent monoadducts, have been found to induce recombination in both prokaryotic (7,8) and eukaryotic cells (9–11). Plasmid–chromosome recombination is induced by psoralen DNA adducts in the yeast *Saccharomyces cerevisiae* (1,12). Saffran and co-workers (12) have used double-stranded (ds) non-replicating plasmids bearing psoralen adducts to demonstrate that cotransformation of undamaged and damaged plasmids resulted in integration in one or multiple copies of both plasmids at chromosomal sites homologous either to the damaged or the undamaged plasmid. Moreover, they found that the occurrence of psoralen damage-induced multiple integration depended on the function of the *RAD1* gene (2).

DNA in single-stranded (ss) form is known to participate in several metabolic processes in the cell. For example, it is assumed that ss regions are formed during DNA recombination and gene conversion as an essential step during strand exchange. Single-stranded vectors have been used to study the mechanisms of replication, repair and mutagenesis (13–16). After entering a cell, a ssDNA molecule must be converted into the duplex form, in order to be maintained in the cell. Cell free extracts of *Xenopus* eggs can convert M13 ssDNA into duplex molecules (17) and, in monkey COS7 cells, it was demonstrated that ss vectors replicate to form duplex molecules after transfection (13).

We have used ss and ds centromeric vectors to study the induction of plasmid–chromosome recombination by 8-MOP photoinduced lesions in *S.cerevisiae*. Two yeast genes, *URA3* and *LEU2*, were present in the vector used, allowing us to transform yeast cells, to select *Ura*<sup>+</sup> transformants and, among the *Ura*<sup>+</sup>, look for *Leu*<sup>−</sup> auxotrophs. The *Leu*<sup>−</sup> phenotype can result from mutagenic repair of the psoralen photoinduced lesion or by transfer of the *leu2* mutant marker from the chromosome to the plasmid by recombination. We have found that the majority of mutations recovered after psoralen treatment was due to plasmid–chromosome gene conversion events.

MATERIAL AND METHODS

Reagents

8-Methoxypsoralen (8-MOP, molecular weight = 216) was obtained from Sigma Chemical Company (St Louis, USA). *Taq* DNA polymerase and the dsDNA cycle sequencing kit were purchased from Gibco-BRL, Life Technologies, Inc. Oligonucleotides were synthesized using an Applied Biosystems DNA synthesizer or purchased from GeneSet, Paris, France (see Table 1). Restrictions endonucleases were purchased from Boehringer Mannheim, New England Biolabs and Gibco-BRL.

Phages, plasmids and strains

The helper phage R408 was purchased from Stratagene (La Jolla, CA). Plasmid pLU (15), was a kind gift from Dr Zgaga (University of Zagreb), psLU is the ss form of PLU (Fig. 1).

* To whom correspondence should be addressed at present address: University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9072, USA
plasmid minipreps were prepared by the boiling method (22). The obtained plasmid DNAs were treated with RNase I and extracted twice with phenol and another two times with phenol–chloroform prior to precipitation with 2 M NH₄Ac and 2 vol of cold ethanol. Untreated DNA and restriction digests were analyzed on agarose gels to verify plasmid size and restriction sites.

**PCR amplification**

DNA samples were generated for SSCP analysis using PCR (5 min at 94°C, once, followed by 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, for 30 cycles; then 3 min at 72°C and 10 min at 45°C) with different sets of primers. Primers LeuG and LeuH were used to PCR amplify the region covering the site of the leu2-112 marker. Primers LeuJ and LeuK were used to PCR amplify the region of the gene covering the site of the leu2-3 marker. The reaction mixture was as follows: 3–7 ng of plasmid DNA, 100 mM concentration of each deoxynucleoside triphosphate, 1 µM concentration of each primer, 15 mM Tris (pH 8.3), 60 mM KCl, 2.75 mM MgCl₂, 0.25 U Taq DNA polymerase and 0.1 µl [α³²P]dCTP (3000 Ci/µl) in a volume of 10 µl.

When analyzing total DNA preps from yeast transformants, a fragment of ~1 kb of the LEU2 gene was first amplified, containing almost the entire LEU2 open reading frame, using primers LeuY and LeuH (see Table 3) and without radioactivity incorporation. From this PCR reaction, 1 µl of a 10-fold dilution was used as a template for amplifying fragments of both the plasmid and genomic copies of the gene, using the desired set of primers, both with and without ³²P incorporation. When radioactive PCR was not performed, the ~200 bp fragments resulting from PCR using primers LeuG and H, or LeuJ and K were precipitated using 0.3 M sodium acetate and 2 vol of isopropanol. After 10 min at room temperature, tubes were centrifuged at 12 000 r.p.m. for 30 min. After washing with 70% ethanol, pellets were briefly vacuum dried and resuspended in 1/10 of the original reaction volume of MilliQ water. One µl of this DNA was used for end-labelling reactions.

**SSCP analysis**

PCR products were diluted 50-fold in 0.1% SDS, 10 mM EDTA, then 1:1 in 95% formamide, 20 mM EDTA, 0.05% bromophenol blue (BBP) and 0.05% xylene cyanol (XC). Products were heated at 95°C for 5 min, cooled on ice for 15 min then loaded onto 5% non-denaturing polyacrylamide gels containing 1 x TBE and 10% glycerol. The gels were run in a Gibco-BRL apparatus with fan, at 40 W. After electrophoresis, the gel was transferred to Whatman 3MM paper and dried. Autoradiography with Hyperfilm B-max (Amersham, UK) at −70°C with intensifying screen overnight was usually sufficient to detect bands on film. SSCP analysis was also performed by end-labeling the G–H or J–K fragments using T4 polynucleotide kinase (Gibco-BRL), following the manufacturer’s conditions. The labeled fragments, in a volume of 5 µl, were mixed with 20 µl of a solution containing 95% formamide, 20 mM EDTA, 0.05% BBP and 0.05% XC. After a denaturation step at 95°C for 5 min, the samples were rapidly frozen in liquid nitrogen and loaded into a 5% non-denaturing polyacrylamide gel, as described above.

**DNA sequencing**

DNA sequencing was performed using a dsDNA cycle sequencing kit (Gibco-BRL), following conditions specified by the supplier.

---

**Figure 1.** Plasmid pLU used in this study. pLU is the ss form of pLU.

Plasmids were maintained in *E.coli* strain DH5α. Yeast strain FF1852 is MATa leu2-3,112 trp1-289 ura3-52 ade5 can1 and was kindly provided by Dr Francis Fabre (Institut Curie, Orsay).

**Plasmid preparation**

Double-stranded plasmids were prepared using the QIAGEN plasmid purification kit (Gibco-BRL). Single-stranded DNA was isolated after infection of pLU plasmid-bearing bacteria with helper phage as described previously (18).

**Plasmid treatment**

Plasmid DNA, at a concentration of 10 ng/µl in TE for both ss and ds forms, was incubated with 8-MOP at 50 mM for 20 min in the dark at 4°C and irradiated with various doses, up to 5 kJ, of 365 nm UV radiation using a 2.5 kW Xenon lamp in a Schöeffel housing with a Kratos 252 high intensity grating monochromator (Kratos Analytical Instruments, Ramsay, NJ). The ssDNA samples were kept at 2°C during irradiation for better binding of 8-MOP to ssDNA (19).

**Yeast transformation and Leu⁺ mutants selection**

Yeast spheroplasts were prepared and transformed according to Zgaga (15), using 80 ng of plasmid. After transformation, the suspension was carefully mixed with melted SC-ura selection medium containing Sorbitol and agar, and poured into empty Petri dishes. After solidification, the plates were incubated at 30°C for 5 days. Isolated colonies of Ura⁺ transformants were carefully toothpicked in order to avoid cross-contamination; master plates were made and Ura⁺Leu⁻ transformants were selected by replica-plating in SC-leu medium. Thus, each Ura⁺Leu⁺ transformant carries an independent mutation.

**Analysis of the Leu⁺ mutants**

Yeast plasmid minipreps from the Ura⁺Leu⁺ transformants were performed according to Sherman *et al.* (20). DNA samples were used to transform DH5α super competent cells (21) and bacterial
We have sometimes performed the sequencing reaction using only the G-termination mix, to analyze different mutants for the presence or absence of the +G frameshift in ‘G-lanes’ (see Fig. 7).

RESULTS

Experimental strategy

We devised a system to monitor plasmid–chromosome recombination induction by psoralen phototreatment in ss and ds vectors (see Fig. 2). In this system, a haploid wild-type yeast strain is transformed with ss or ds plasmid DNA bearing 8-MOP photoinduced lesions. After entering a cell, a ssDNA molecule has to replicate in order to produce a duplex; in the presence of an 8-MOP photoadduct, this replication would stop at the point of the lesion, leaving a ss gap opposite to the damaged site. An intrastrand crosslink in a ss molecule (if allowed by the plasmid secondary structure) would cause the molecule to be topologically constrained, inhibiting replication and reducing viability. Only mutations resulting from repair of monoadduct lesions would then be detected. Thus, we believe that our system reflects the study of ss gap repair when using ssDNA, as opposed to crosslink repair when using dsDNA.

Taking advantage of the identification of both mutations present in the leu2-3,112 mutant strain (leu2-3, Edelman and Culbertson, unpublished results; leu2-112, this work), we devised a system, based on SSCP analysis, to determine which proportion of leu2 mutants recovered after transformation were due to recombinational repair using the chromosomal information as a template (Fig. 2).

Identification of the leu2-112 mutation

Both leu2-3 and leu2-112 markers were isolated after treatment with ICR-170, a frameshift inducing agent (23,24). The leu2-3 mutation was identified as a G insertion in a glycine codon at position +249 (Edelman and Culbertson, unpublished observation). We have designed several primers (Table 1), covering the most conserved regions of the LEU2 gene. The sequencing of the region covering a conformation change identified by SSCP and present in several leu2-3,112 mutant strains allowed us to identify the leu2-112 mutation as being a G insertion, also in a glycine codon, at position +792.

Table 1. Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeuY</td>
<td>5’ GTGCCTGACGCATATACT 3’</td>
<td>-256</td>
</tr>
<tr>
<td>LeuA</td>
<td>5’ GATCGTCGTTTTGCCAGGTG 3’</td>
<td>18</td>
</tr>
<tr>
<td>LeuP</td>
<td>5’ CACCTGCGCAAGACGATC 3’</td>
<td>37</td>
</tr>
<tr>
<td>LeuJ</td>
<td>5’ GGTCGTCGTTCTGCTAC 3’</td>
<td>145</td>
</tr>
<tr>
<td>LeuC</td>
<td>5’ GGTCGTCGCTAAAATGGG 3’</td>
<td>231</td>
</tr>
<tr>
<td>LeuM</td>
<td>5’ ATACATCAACAGATGGTGT 3’</td>
<td>251</td>
</tr>
<tr>
<td>LeuK</td>
<td>5’ TACATGTCCTTAAGTGTCG 3’</td>
<td>328</td>
</tr>
<tr>
<td>LeuE</td>
<td>5’ AAGACGATGTTGATGTGT 3’</td>
<td>444</td>
</tr>
<tr>
<td>LeuD</td>
<td>5’ ACCCATCACCACGTCTTC 3’</td>
<td>464</td>
</tr>
<tr>
<td>LeuG</td>
<td>5’ CTGCCGCCATGCTACGT 3’</td>
<td>675</td>
</tr>
<tr>
<td>LeuF</td>
<td>5’ AACTAGGATCATGGCCCG 3’</td>
<td>695</td>
</tr>
<tr>
<td>LeuH</td>
<td>5’ TGCAGCAGACAGAATGTGG 3’</td>
<td>926</td>
</tr>
</tbody>
</table>

Effects of psoralen treatment in transformation of yeast cells using ss or ds vectors

Wild-type yeast cells were transformed with ss or ds vectors (Fig. 1) bearing psoralen adducts. Transformants were selected in uracil omission medium. As shown in Figure 3, transformation using ssDNA was less effective than transformation with the plasmid in the ds form. Psoralen treatment reduces the transformation...
efficiency of psLU to a greater extent than the equivalent ds plasmid.

Among Ura\(^+\) transformants, Leu\(^-\) mutants were selected and the number of Ura\(^+\)Leu\(^-\) transformants increased with UVA dose, showing that the treatment was inducing the appearance of mutations in the LEU2 gene present in both ss and dsDNA. Figure 4 shows the mutation induction for pLU and psLU. As expected, the lesions induced by 8-MOP photoaddition were more mutagenic in the ssDNA than in dsDNA. Lower survival and higher mutagenesis after treatment of ssDNA had been already observed by Madzak and Sarasin (16) when analyzing the mutation spectrum in shuttle vectors treated with UV irradiation and transfected into monkey cells.

Molecular analysis of the leu2 mutants

In order to establish the role of recombination in the repair of plasmid DNA containing monoadducts or monoadducts and crosslinks, we have analyzed the plasmid DNA extracted from Ura\(^+\)Leu\(^-\) transformants selected after transformation with 8-MOP-treated ssDNA (82 transformants from four experiments) or 8-MOP-treated dsDNA (53 transformants from two experiments). Spontaneous mutants were also examined (five from ssDNA transformants and one from dsDNA transformants).

We first examined the vector size and the number and position of restriction sites in the recovered plasmids to determine whether there were any gross changes to the plasmid structure (data not shown). No changes were observed in plasmid DNA recovered from ssDNA transformants. For dsDNA transformants, two mutants show important deletions (4%).

Subsequently, we PCR amplified different fragments of the leu2 gene from each mutated plasmid using various pairs of primers (Table 1), and analyzed the so-obtained fragments by SSCP (Table 2).

In the experiments using ssDNA, we amplified the plasmid-borne LEU2 gene of the 82 induced mutants with primers LeuG and LeuH. The region amplified with these primers contains the site of the leu2-112 marker. Most of the mutants (55) presented the same conformational change on SSCP gels for this region (Fig. 5), indicating that the same mutation was present in all those mutants. Sequencing of this region revealed that this mutation was the leu2-112 frameshift originally present in the genomic DNA. Clearly, recombination had been induced after treatment and the information present in the chromosomal DNA was used as the template to correct the lesion. Two among the five spontaneous mutants analyzed also presented the same frameshift (Table 2), suggesting that plasmid–chromosome recombination can occur spontaneously during transformation with ssDNA but is greatly enhanced in the presence of lesions.

<table>
<thead>
<tr>
<th>Type of event originating the Leu(^-) mutant phenotype</th>
<th>Total</th>
<th>Recombination events</th>
<th>Other events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous mutants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssDNA</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>dsDNA</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Induced mutants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssDNA</td>
<td>82</td>
<td>57 (69.5%)</td>
<td>25 (30.5%)</td>
</tr>
<tr>
<td>dsDNA</td>
<td>53</td>
<td>46 (87%)</td>
<td>7 (13%)</td>
</tr>
</tbody>
</table>

The LEU2 gene of the plasmids from the remaining transformants was analyzed by SSCP after PCR amplification with the remaining set of primers. Only two mutants were found to possess the leu2-3 mutation alone. Thus, 57 out of 82 (69.5%) leu2 mutants arise from recombination events (Table 2). The remaining mutants...
carry different mutations consisting of transversions, transitions, small deletions and other frameshifts (data not shown). In one of the plasmids no mutation could be identified.

The same analysis was performed with Leu" mutants from cells transformed with dsDNA bearing 8-MOP photoinduced lesions. SSCP analysis revealed that 39 out of 53 Leu" mutants carried a mutation in the region defined by primers LeuG and LeuH. Sequencing of the mutated region revealed that all of them carried the leu2-112 frameshift insertion. The spontaneous mutant obtained for dsDNA transformed cells also presented the same frameshift.

The remaining mutants were analyzed further with different primers to determine the nature of the mutation present in the plasmid DNA. Seven of the mutants were found to carry the leu2-3 mutation alone. Thus, 46 out of 53 (87%) leu2 mutants are due to recombinational events (Table 2). In addition to the major deletions in the LEU2 coding region (4% of the mutants), the five remaining mutants were found to carry a different frameshift. No other types of mutations were found.

The recombination event involving the leu2-112 marker is mainly non-reciprocal

We performed SSCP analysis in some transformants whose plasmid DNA was already proven to bear the leu2-112 frameshift, using PCR amplified DNA from yeast DNA preps. Total DNA from 38 Leu" mutants (from both ss and ds) was used to amplify both chromosomal and plasmid copies of the LEU2 gene. This analysis revealed that four out of 38 samples presented two bands corresponding to the wild-type and mutant conformation (Fig. 6), suggesting that the transfer of information occurred in a reciprocal way. The majority (34 out of 38) presented only the mutant conformation band, showing that both the plasmid and the chromosome bear the leu2-112 frameshift, characterizing a gene conversion event.

In order to confirm that the two bands in the samples suggesting a reciprocal recombination actually corresponded to wild-type and mutant sequence we isolated the bands from the gel and sequenced them. The sequencing proved that no frameshift was present in the DNA coming from the wild type conformation band while the expected mutation was observed after sequencing of the mutant conformation band, confirming that a mixture of frameshift bearing molecules and wild-type molecules was present in the total DNA preps. As plasmid DNA from these mutants had already been sequenced and they all had the leu2-112 frameshift, it is clear that the information in the chromosome was corrected in a reciprocal event between plasmid and chromosome.

Analysis of the recombinational tract: the leu2-3 marker is often involved in the event

We attempted to determine how often the recombination tract would cover both the leu2-3 and the leu2-112 markers, by using two additional primers, LeuJ and LeuK (see Table 1), to amplify the region covering the leu2-3 frameshift. As both leu2-3 and leu2-112 frameshifts are caused by the insertion of a G, we devised...
a way of distinguishing yeast transformants containing only mutant, only wild-type or both sequences using G-lane sequencing of PCR products from total yeast DNA preps. The results are shown in Figure 7, where we can readily distinguish lanes 4, 6 and 7 as containing only the wild-type information (control DNA), lanes 1, 2 and 5 as transformants carrying only mutant information and lane 3 as a transformant bearing a mixture of wild-type and mutant information. When a mixture was detected, sequencing of the region present in the plasmid DNA was performed in order to determine whether plasmid or genomic DNA contained the mutant information. We analyzed 61 leu2 mutants by SSCP or by G-lane sequencing, most of them previously determined to be leu2-112 mutants, using total DNA and plasmid DNA, to determine the extension of the recombination tract in this interval and also, in the case of the reciprocal recombinants previously found, if gene conversion was associated with the reciprocal event.

For mutants originating from transformation using ssDNA, 15 DNA samples were analyzed for the presence of the leu2-3 marker. Of these, two were found to be the only mutants known to contain the leu2-3 frameshift alone on the plasmid and 13 were previously known to bear the leu2-112 mutation in the plasmid. As shown in Table 3, of the latter 13, five of the mutants were due to conversion of both markers, seven were due to conversion of the leu2-112 marker alone and one of the mutants, named E840, is a case of reciprocal recombination (in the leu2-112 marker) not associated with gene conversion. The percentages given correspond to the actual number of transformants analyzed for each category (Table 3).

For the mutants arising from transformation with dsDNA, all the 46 mutants arising from a recombination event were analyzed. Twenty-nine of them were due to conversion of both leu2-3 and leu2-112, seven to conversion of the leu2-112 marker alone, seven to conversion of the leu2-3 marker alone and three were due to reciprocal recombination in leu2-112 associated with gene conversion in leu2-3 (see Table 3).

**DISCUSSION**

We have shown that the induction of plasmid-chromosome recombination by psoralen photodaddition to replicating plasmid vectors in both ss and ds forms is the major pathway for correcting psoralen induced lesions in vector DNA. Studies of damage induced plasmid recombination in yeast have shown that non-replicating plasmids bearing double-strand breaks (25) or psoralen adducts (1,12) often produce multiple plasmid integration into homologous chromosomal loci. Orr-Weaver and Szostak (26) also used autonomously replicating ARS plasmids to show that double-strand breaks stimulated gene conversion which may or may not be associated with crossing-over. Using non-replicating plasmids bearing single-strand and double-strand DNA damage, Safran and co-workers were able to demonstrate that both ss and ds lesions can induce reciprocal recombination in yeast (27). We studied psoralen induced plasmid-chromosome recombination using centromeric plasmids which are unable to integrate in the chromosome. Thus, in our system, only gene conversions or double crossovers are allowed and we determined to what extent psoralen photoreaction would induce recombination in this system.

Double-stranded vectors bearing psoralen photoadducts were better repaired than ss vectors (Fig. 3). One could expect that lesions present in a ss vector would lead to less efficient transformation of damaged ssDNA when compared to damaged dsDNA since after entering a cell a ssDNA molecule has to replicate to produce fully duplex molecules which will then be perpetuated in the cell progeny. It has previously been shown that ssDNA exhibits a lower survival than dsDNA after UV irradiation (16). The higher mutagenesis observed after treatment of ssDNA could be attributed to the lack of excision repair on ssDNA, which could also account for the lower survival of the ss vector.

Zgaga (15) has used the same vector in a ss form to study the transforming efficiency of different yeast strains with UV-irradiated plasmid. He has found that the rev3, rad18 and rad52 mutations did not influence the efficiency of transformation with irradiated ssDNA. However, in the double mutant rev3 rad52 this efficiency was decreased. Of the Ura+ transformants in the wild-type strain, 5% were Leu-. The induction of Leu- mutants was dose-dependent and decreased in the rev3 mutant, suggesting that both mutagenic and recombinational repair processes operate on UV-damaged plasmid DNA on yeast (15). The possible pathways leading to the generation of Ura-Leu- transformants would be mostly restricted to mutagenic repair or conversion of chromosomal mutations in the leu2 gene to the homologous sequence in the plasmid. It has previously been shown that gene conversion of chromosomal information to extrachromosomal plasmids occurs at high frequency (26). In our studies gene conversion was responsible for 69.5% (ss) and 87% (ds) of the Ura-Leu- variants induced, therefore, this process was the most

<table>
<thead>
<tr>
<th>Type of event</th>
<th>ds DNA</th>
<th>ss DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>leu2-3 leu2-112</td>
<td>29/46 (63%)</td>
<td>5/13 (38%)</td>
</tr>
<tr>
<td>leu2-3 leu2-112</td>
<td>7/46 (15%)</td>
<td>7/13 (54%)</td>
</tr>
<tr>
<td>leu2-3 leu2-112</td>
<td>3/46 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>leu2-3 leu2-112</td>
<td>0</td>
<td>1/13 (8%)</td>
</tr>
<tr>
<td>leu2-3 leu2-112</td>
<td>7/46 (15%)</td>
<td>2/57 (3.5%)</td>
</tr>
</tbody>
</table>

**Table 3. Classification of the recombination events**
frequent event involved in the repair of 8-MOP-damaged ss or ds non-integrating plasmids. Moreover, the conversion tract can be long enough to cover both the leu2-3 and the leu2-112 markers, which are ~500 bp from one another. The frequency of those co-convertants in dsDNA transformed Leu2- mutant cells is higher (63% of the recombination events) than single leu2-3 and leu2-112 convertants (30%, see Table 3). On the contrary, among the ssDNA-induced mutants, only 37% are co-convertants while 56% are single convertants.

One interesting finding of our study is that, while some reciprocal recombination was observed at the leu2-112 marker (correction of the chromosomal information), no leu2 mutant carrying restored information at the leu2-3 site in the chromosome were recovered. However, as shown in Table 3, in the yeast transformed with ds plasmid single conversions at the leu2-3 site or leu2-112 site occurred at the same frequency (15%), suggesting that both sites are equally likely to be involved in the recombinational event. To determine whether this is related to the position of the lesions in the gene, an analysis of the spectrum of psoralen induced lesions in the plasmid copy of the LEU2 gene would be required.

Other repair processes could act on damaged plasmids by, for example, creating deletions or other gross changes. Psoralens have been found to induce deletions in the lacI gene in E.coli repair proficient and UvrB- strains (1,28). It was suggested that 8-MOP photoinduced deletions could result from the stabilization of misaligned intermediates by crosslinks. In our case, only two major deletions were found after transformation with ds vector, suggesting that this process is not frequent. It is likely that incisions at separate 8-MOP adducts, followed by rejoining, can account for the generation of the deletions observed. In addition, we cannot exclude the possibility that the repair of the psoralen adducts in ss or dsDNA is also performed in an error-prone manner. Indeed, we have found a few leu2 mutants that suggest that gap filling by mutagenic bypass of the lesion can occur. However, these mutants were infrequent, showing that, in our system, the main repair pathway was recombinational. Another gap repair pathway for ssDNA is translesion synthesis in which DNA polymerase switch from the damaged template, carries out translesion DNA synthesis by copying the undamaged strand in the sister duplex and then switches back to the damaged template after clearing the lesion, in this case copying the chromosomal information to convert the plasmid to a leu2-112 mutant. However, the reciprocal recombinants found suggest that recombinational interactions occurred at a high frequency, being mainly resolved as gene conversion events.

Our results show that recombinational repair of psoralen photoinduced lesions can be initiated either by a ss gap or by the processing of a ds crosslink. Moreover, we show that centromeric vectors, both ss and ds, can be useful in the study of recombinational repair in yeast, provided that regions of homology are available for the recombinational interaction.

ACKNOWLEDGEMENTS

We thank Dr Zoran Zgaga for the gift of pLU plasmid, Dr Francis Fabre for donating the FF1852 strain and Dr Dietrich Averbeck for many useful discussions. The help of Axel Nothurft in performing some experiments and discussing results is gratefully acknowledged. This work was supported by the Ministère de la Recherche et de la Technologie (MRT91/C/0540). LB.M thanks the Brazilian Council for Scientific Development (CNPq) for doctoral fellowship support.

REFERENCES