Effects of Post-treatment Incubation on Recombinogenesis in Incision-proficient and Incision-deficient Strains of *Saccharomyces cerevisiae*:

II. Recombinogenesis after the Photoaddition of Furocoumarins

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After the photoaddition of mono- and bifunctional furocoumarins to G1 phase cells, most gene conversion and crossing-over occurred without post-irradiation incubation of these cells in incision-proficient strains. In contrast, incision-deficient cells showed marked induction of both recombinational events only after treated cells had been incubated for several hours before selection. These results indicate that when furocoumarins are photoadded to G1 cells, initiation of recombinational events occurs during the same G1 phase in the incision-proficient cells; whereas, it occurs only after post-irradiation DNA replication in incision-deficient cells. The action of the *PSO2* gene product specific for the repair of DNA crosslinks in recombination induction is discussed and compared to the actions of the excision repair genes *RAD1* and *RAD2*.

INTRODUCTION

Bifunctional furocoumarins react photochemically with DNA to form monoadducts with pyrimidine bases and crosslinks between two pyrimidines on opposite strands; whereas, the monofunctional derivatives of psoralen form only monoadducts (for a review, see 1). These agents have mutagenic and recombinogenic activities in both prokaryotes and eukaryotes (for a review, see 2), the bifunctional derivatives in general being more effective than the monofunctional ones

1. In the yeast *Saccharomyces cerevisiae* all three repair pathways that are involved in the repair of UV- or *γ*-ray-induced lesions, or both (for review, see 3, 4), and that act on the mutagenic, or recombinogenic responses, or both (for review, see 5, 6), also have roles in the repair of lesions by furocoumarin-photoaddition

The existence of mutants specifically sensitive to crosslinking agents13-16 suggests that specific steps also are involved in the repair of DNA inter-strand crosslinks. For one such mutant

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psol-1, a loss of resistance of diploid relative to haploid and of S/G2 phase cells as compared to G1 cells was found following the photoaddition of psoralens13. This indicates that the recombinational function responsible for this resistance present in the wild-type is altered in psol-1. Indeed, mutants of the RAD52 repair pathway that are known to be defective in spontaneous and UV or X-ray-induced recombinations17,18 are defective both in ploidy effect and S/G2 resistance after a psoralen photoaddition8.

A difference in the timing of mutation, gene conversion and crossing-over has been reported between incision-proficient and -deficient strains following UV-irradiation19-25; therefore, it is important to examine the effects of post-irradiation incubation in excision-deficient rad strains treated with a furocoumarin photoaddition on the induction of recombination and to compare these effects with those for the psol-1 strain. The induction of mitotic gene conversion and crossing-over after photoaddition of mono- or bifunctional furocoumarins was compared in strains homozygous for the wild-type RAD+, for the excision-deficient mutations rad1-1, rad3-2 and rad14-2, and for psol-1 mutation26.

MATERIALS AND METHODS

Strains

The diploid strains, wild-type, rad1-1, rad3-2, rad14-2 and psol-1, are described elsewhere25.

Media

The complete liquid medium (2 × YEPD), minimal medium (MM), synthetic complete medium (SC) and selection media (SC-leucine, -lysine, -methionine, -adenine and SC + cycloheximide) are described elsewhere25.

Chemicals

Chromatographically purified 3-carbethoxypsoralen (3CPs) and psoralen were the gift of Dr. E. Moustacchi (Institut Curie, Paris). 8-methoxypsoralen (8MOP, Sigma, St. Lewis, USA) also was used. After treatment with 365 nm ultraviolet light (UVA), 8MOP and psoralen induce both monoadducts and crosslinks in DNA; whereas, 3CPs forms only monoadducts1,27. The former compounds are consequently bifunctional, the latter monofunctional.

Cell culture

Conditions for cell culture are described elsewhere25.

Treatment with furocoumarins and UVA

Stationary phase cells were washed three times with saline, after which the cell suspension was sonicated as described previously25. Cell density and the number of budding cells were determined in a counting chamber. A suspension of 2 × 10⁷ cells per ml then was incubated for 20 min at 4°C with 5 × 10⁻⁵ M furocoumarins, after which it was irradiated with 365 nm of
light (UVA) emitted from a Blak-Ray B-100 lamp (Ultra-Violet Products, Inc., San Gabriel, Calif., USA). The UVA dose rate was measured with a UVX radiometer (Ultra-Violet Products, Inc.). The procedure used was that described by Averbeck et al.2).

Immediate plating and post-irradiation incubation experiments and the test for the induction of mitotic recombinational events after treatment

The immediate plating conditions, post-irradiation incubation experiments and procedures to test the induction of mitotic recombinational events are described elsewhere25).

RESULTS

Induction of recombinational events by furocoumarin + UVA treatment in repair-deficient mutant strains: immediate plating experiments

Under the conditions in which cells were plated on SC-leucine or SC + cyclo-heximide immediately after UVA irradiation in the presence of the furocoumarin, 8MOP, 3CPs or psoralen, marked induction of Leu+ convertants and CYHf reciprocal recombinants took place in the RAD, pso2-1 and rad14-2 strains, but almost no induction occurred in the rad1-1 and rad3-2 strains (Fig. 1). The dose-response curves shown are regression lines obtained by the plotting the experimental data from several repeated experiments on one graph. To compare the induction of gene conversion (Leu+ ) and crossing-over (CYHf) among the five strains with their different sensitivities to the lethal effects of a furocoumarin photoaddition, we calculated the average values of both the convertants and reciprocal recombinants in 105 survivors at the 50% and 10% survival doses from the data obtained from several repeated experiments. At 50% survival after 8MOP + UVA, 1350, 60 and 590 convertants, and 450, 30 and 390 recombinants respectively were induced in the wild-type, pso2-1 and rad14-2 strains; whereas, in the rad1-1 and rad3-2 diploids respectively, only 3 and 20 convertants were obtained. Furthermore, these latter strains did not show any induction of crossing-over at the 50% or 10% survival doses. At the 50% survival dose after a 3CPs photoaddition, 1090, 450 and 400 convertants, and 380, 170 and 400 recombinants respectively were induced in the wild-type, pso2-1 and rad14-2 cells, but only 5 and 8 convertants, and 3 and 0 recombinants were induced in the diploids rad1-1 and rad3-2. After the psoralen photoaddition resulting in 50% survival, 1100, 120 and 500 convertants, and 300, 60 and 570 recombinants were induced in the wild-type, pso2-1 and rad14-2 strains, but in the rad1-1 diploid only 1 convertant and 1 recombinant were induced (not examined in rad3-2).

The pso2-1 was reduced during induction of both recombinational events after a photoaddition of bifunctional 8MOP; whereas, induction appeared almost normal after a photoaddition of the monofunctional agent 3CPs (Fig. 1)1). The mutant seems to induce both recombinational events effectively as in the wild-type (Fig. 1). The pso2-1 cells, however, are highly sensitive to the lethal effect of photoadditions of the bifunctional furocoumarins 8MOP and psoralen. Moreover, when frequencies are compared, those of the two induced recombinational events in this mutant are much more reduced than those of the wild-type for the same survival level as shown elsewhere (Fig. 1, reference 1). The effects of rad1-1 and rad3-2 mutations appear to be far more severe
Fig. 1. Induction of gene conversion at the *leu1* locus and crossing-over between the *cyh2* and centromere in RAD/RAD, rad1-1/rad1-1, rad3-2/rad3-2, pso2-1/pso2-1 and rad14-2/rad14-2 diploid strains by different furocoumarins at equimolar concentrations + UVA under immediate plating. A) convertants (CON); B) recombinants (REC). The ordinate label "CON, REC Induction/10^5 survivors (x10^2)" means that the ordinate numerals in A), 0 to 40 represent 0 to 4000 and those in B), 0 to 15, 0 to 1500.
Fig. 2. Effects of post-irradiation incubation in 2x YEPD on the induction of gene conversion and crossing-over following furcoumarin photoadditions in rad1-1/rad1-1 and rad3-2/rad3-2 diploid strains. a), b) Induction of convertants and reciprocal recombinants per 10^5 survivors as a function of the UVA dose in rad1-1/rad1-1; a) 8MOP, b) 3CPs. c), d) Induction of convertants and recombinants per 10^5 survivors as a function of the UVA dose in rad3-2/rad3-2; c) 8MOP, d) 3CPs. Squares, convertants (CON); triangles, recombinants (REC). Open symbols, no post-irradiation incubation; closed symbols, post-irradiation incubation for 2 h. Ordinate labelling is the same as in Fig. 1. (a) 0 to 10 = 0 to 100, b) 0 to 30 = 0 to 300, c) 0 to 10 = 0 to 100, d) 0 to 10 = 0 to 100
than the effect of *psp2-I* as almost no induction of recombinational events took place in either incision-deficient strain even after a photoaddition of the monofunctional agent 3CPs.

*Effects of post-irradiation incubation on the induction of recombinational events after a furocoumarin photoaddition*

*Incision-deficient strains:* After incubation in 2× YEPD post-furocoumarin + UVA treatment, the incision-deficient strains, *rad1-I* and *rad3-2*, showed marked induction of both gene conversion and crossing-over (Fig. 2). At 50% survival after 8MOP photoaddition, 80 and 120 convertants, and 20 and 55 recombinants were induced in the *rad1-I* and *rad3-2* strains. Incubation after a 3CPs photoaddition produced 100 and 95 convertants, and 258 and 35 recombinants at the same survival level. The magnitude of induction was, however, considerably smaller than that for the wild-type without post-irradiation incubation. These results indicate that the initiation of recombinational events in incision-deficient cells after a furocoumarin photoaddition requires post-irradiation incubation.

![Graph](https://example.com/graph.png)

**Fig. 3.** Effects of the post-irradiation incubation of *rad1-I/rad1-I* cells in 2× YEPD (closed symbols) and 0.9% saline (open symbols) after the photoaddition of 8MOP with a 1.5 KJ/m² UVA dose on the induction of gene conversion (squares) and crossing-over (triangles) as a function of the period of incubation. Labelling of the ordinate is the same as in Fig. 1. (0 to 1.5 = 0 to 150)
To clarify the effects of post-irradiation incubation on the induction of recombinational events in incision-deficient strains after a furocoumarin photoaddition, we examined the effects of post-irradiation incubation in saline. The UVA dose used was 1.5 KJ/m². Survival levels for the various incubation times did not change (data not shown). After incubation of the rad1-1 cells in saline, scarcely any induction of gene conversion and crossing-over was observed, in contrast to the results after incubation in 2×YEPD (Fig. 3). Induction of both recombinational events in the diploid rad3-2 following a 3CPs photoaddition was not affected by post-irradiation incubation in saline (Fig. 4). Although a slight difference was observed between immediate and delayed plating, in terms of the yield of gene conversion after 6 KJ/m² of UVA, it is negligible if one notes the scale of the vertical axis. These results demonstrate that post-irradiation incubation only is effective for the induction of recombinational events in incision-deficient cells after a furocoumarin photoaddition provided there is incubation in growth medium.

**Incision-proficient strains:** The induction of recombinational events after post-irradiation incubation of wild-type and pso2-1 cells treated with 8MOP + UVA is shown in Fig. 5. In contrast to the results for UV-induced recombinogenesis, post-irradiation incubation increased recombinant yields but decreased the induction of convertants in the wild-type strain treated with 8MOP + UVA. The increment or reduction obtained after post-irradiation incubation was, however, not as marked as for incision-deficient cells.

Another incision-proficient strain, pso2-1, which probably is defective in a specific step after incision near DNA crosslinks, showed that post-irradiation incubation increased the induction of both recombinational events after an 8MOP photoaddition. At 50% survival, the yields for gene conversion and crossing-over after post-irradiation incubation were both 2-fold the values

![Graph](https://example.com/graph.png)

**Fig. 4.** Effects of post-irradiation incubation of rad3-2/rad3-2 in 0.9% saline after 3CPs photoaddition on the induction of gene conversion (squares) and crossing-over (triangles) as a function of the UVA dose. Open symbols, no post-irradiation incubation; closed symbols, post-irradiation incubation for 2 h.
for immediate plating. Effects of post-irradiation incubation in saline on the induction of both recombinational events were examined in $RAD$ and $pso2-1$ cells. No remarkable difference was found for either event between yields obtained with and without post-irradiation incubation in saline (Fig. 6). Similar results were obtained for $pso2-1$ cells after an 8MOP photoaddition (data not shown).

**DISCUSSION**

In summary, in the incision-proficient strains $RAD$, $pso2-1$ and $rad14-2$ homozygous diploids, after a furocoumarin photoaddition most gene conversion and crossing-over occurred without post-irradiation incubation of the cells in growth medium. The frequencies were not markedly changed by selection after incubation. In contrast, the incision-deficient strains $rad1-1$ and $rad3-2$ showed almost no induction of either recombinational event after a furocoumarin photoaddition when cells were selected immediately after UVA irradiation. But, when furocoumarin + UVA-treated cells were incubated for several hours in liquid growth medium before selection, there
was marked induction of both recombinational events. In addition, post-irradiation incubation of the incision-deficient cells in saline yielded no increase in induction for either recombinational event. Because the stationary phase cells (G1) were treated with furocoumarin + UVA, these results demonstrate that initiation of both gene conversion and crossing-over occurred during the same G1 phase in the incision-proficient strains, but did not occur before post-irradiation replication in the incision-deficient strains. Similar results have been reported for the induction of mutation\textsuperscript{19-21}, gene conversion and crossing-over\textsuperscript{23-25} for incision-proficient and -deficient G1 cells exposed to UV.

The results we obtained for incision-deficient strains, as well as the results of UV
experiments\textsuperscript{25}, indicate that post-replication nicks or gaps produced on the initiation of both recombinational events after the replication of DNA containing furcocumarin photoproducts, which have not been incised during G1 phase, have the same function as the incision nicks or gaps in the incision-proficient strains.

In the \textit{psd2-1} mutant, both recombinational events occur without post-irradiation incubation, and the frequencies of these events are greater than those of the incision-deficient strains but less than those of the wild-type, especially after the photoaddition of bifunctional furcocumarins\textsuperscript{1}. In order to explain these differences in deficiency between the incision-deficient strains and \textit{psd2-1} in the induction of the two recombinational events after a furcocumarin photoaddition, it is necessary to discuss the nature of the \textit{psd2-1} mutant.

The \textit{psd2-1} mutant is highly sensitive to photoaddition of bifunctional 8MOP but only moderately sensitive to a photoaddition of monofunctional 3CPs as compared with the wild-type\textsuperscript{3}. In the \textit{psd2-1} diploid there was a reduction of both recombinational events after the photoaddition of a bifunctional furcocumarins; whereas, this mutant is almost normal for the induction of the two recombinational events after a 3CPs photoaddition (Fig. 1)\textsuperscript{5}. Following the photoaddition of a bifunctional furcocumarin, both monoadducts and crosslinks were induced in the chromosomal DNA, the proportion of monoadducts being much greater than that of crosslinks after exposure to biological doses of UVA\textsuperscript{28,29}. Some portion of the monoadducts are converted to crosslinks on secondary irradiation with UVA\textsuperscript{29}. Because the excision repair system of \textit{psd2-1} cells is not defective\textsuperscript{8}, most monoadducts formed after the photoaddition of mono- or bifunctional furcocumarins are considered to be repaired in a genetically silent fashion, as expected from the low sensitivity to 3CPs\textsuperscript{29}. If, however, two monoadducts are formed to complementary DNA strands that are sufficiently close together, a double-strand breaks may occur owing to the matching of the gaps on the opposite strands after the incision of the monoadducts. As stated elsewhere\textsuperscript{25}, double-strand breaks of DNA, considered the main substrate of recombination in yeast\textsuperscript{30,31}, are repaired by the action of the RAD52 repair pathway\textsuperscript{17,18}. The almost normal induction of the two recombinational events that took place after a 3CPs photoaddition and the reduced but marked induction after an 8MOP photoaddition in the \textit{psd2-1} diploid suggest that the double-strand breaks that occurred after the incision of the monoadducts are repaired by the recombinogenic repair system without involvement of the \textit{PSD2} gene product, unlike in the repair of the double-strand breaks that occurred during the processing of the crosslinks. The \textit{psd2-1} mutant shows double-strand breaks in its DNA after a very short period of incubation after an 8MOP photoaddition, but it does not show the recovery of high molecular weight DNA found for the wild-type, even after prolonged incubation. A similar feature is seen for the \textit{rad52} mutant that belongs to the recombinogenic repair pathway of double-strand breaks. In contrast, the incision-deficient \textit{rad3} mutant does not show such extensive double-strand breaks following incubation after an 8MOP photoaddition\textsuperscript{32}. These observations suggest that the double-strand breaks formed from DNA crosslinks by the action of incision repair genes require some processing by the action of \textit{PSD2} gene product before they can be repaired by the RAD52 repair pathway.

On the basis of these considerations, the induction of the two recombinational events must
depend both on excision and recombination repair pathways after the formation of DNA monoadducts; whereas, their induction depends on both repair pathways and the function of the PSO2 gene product after formation of DNA crosslinks. Recombination induction after DNA monoadduct formation is similar to that after pyrimidine dimer formation preceded by UV exposure. Under direct plating the effects of the deficiencies in incision-deficient mutants are very severe after photoadditions of both types of furocoumarins; but, these deficiencies can be circumvented by passage through replication of the damaged DNA which induces gaps in the DNA strand opposite to the DNA damage. In contrast, circumvention of PSO2-L block through delayed plating to repair DNA crosslinks appears to be unsuccessful, as shown by our results. The reduced induction of the two recombinational events following photoaddition of bifunctional furocoumarins to PSO2-L may be the results of this mutant’s lack of ability to repair DNA crosslinks, although it induces these recombinational events by DNA monoadducts.

REFERENCES


