Induction in *Saccharomyces cerevisiae* of Mitotic Recombination by Mono and Bifunctional Agents: Comparison of the *pso2-1* and *rad52* Repair Deficient Mutants to the Wild-Type

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Summary. The induction in *Saccharomyces cerevisiae* of mitotic gene conversion and crossing-over by photoaddition of mono and bifunctional psoralen derivatives as well as by mono (HN\(_1\)) and bifunctional (HN\(_2\)) nitrogen mustards or 254 nm ultraviolet (UV) radiation was compared in wild type and in a mutant, *pso2-1*, initially selected for sensitivity to DNA cross-linking agents. The induction of the same recombinational events by photoaddition of one mono and one bifunctional derivative of psoralen was also examined in the *rad52-1* diploid strain.

The non-reciprocal (gene conversion) and the reciprocal (crossing-over) events follow the same patterns for each agent tested regarding the relative sensitivities of the wild type and repair-deficient strains.

The *pso2-1* strain retains the same or even a greater ability to perform recombination than the wild type following exposure to monofunctional agents, as well as to HN\(_2\) and UV. In contrast, this mutant is not able to perform mitotic recombination as efficiently as the wild type specifically after photoaddition of bifunctional furocoumarins. Such a difference is not simply due to the relative toxicity of the inducing agents.

The *rad52-1* mutant is blocked in induced recombination by the photoaddition of both the mono and bifunctional furocoumarins tested and the same was already established for UV and \(\gamma\)-rays.

The *rad52-1* and *pso2-1* mutant strains have in common a high sensitivity in the G2 phase of the cell cycle and a loss of the ability to reconstitute high molecular weight DNA after the appearance of DNA double strand breaks related to the incision of inter-strand cross-links. This leads to a generalised block in recombination ability in *rad52* whereas in the *pso2-1* mutant the nature of the deficiency in recombination is much more specific.

Introduction

Bifunctional furocoumarins are known to react photochemically with DNA forming monoadducts with pyrimidine bases and cross-links between two pyrimidines on opposite strands whereas monofunctional derivatives of psoralen form only monoadducts (for review see Scott et al. 1976; Song and Tapley 1979; Parsons 1980). On the other hand bifunctional nitrogen mustards induce monoadducts as well as intra and inter-strand cross-links involving purines on DNA. Monofunctional nitrogen mustards produce only monoaalkylation on purines (for review see Fox and Scott 1980). All these agents demonstrate mutagenic and recombinogenic activities in both proaryocytes and eucaryotes (Scott et al. 1976; Cassuto et al. 1977; Bridges et al. 1979; Burger and Simons 1979; Averbeck and Moustacchi 1979, 1980; Grant et al. 1979; Ruhland and Brendel 1979; Schimmel et al. 1980; Venturini et al. 1980; Fox and Scott 1980; Abel and Schimmer 1981). Differences in genotoxicity according to functionality have been reported and in general bifunctional derivatives were found to be more effective than monofuncional ones.

In the yeast *Saccharomyces cerevisiae* it has been demonstrated that the three major repair pathways involved in repair of UV and/or X-ray-induced damage (for review Haynes and Kunz 1981) and known to interfere with the mutagenic and/or the recombinogenic response (for reviews Lemontt 1980; Lawrence 1981; Kunz and Haynes 1981), also play a role in the repair of lesions induced by furocoumarins photoaddition (Averbeck and Moustacchi 1975; Henriques and Moustacchi 1980a) or by alkylating nitrogen mustards (Brendel and Haynes 1973; Ruhland and Brendel 1979; Siede and Brendel 1982).

The existence of mutants essentially sensitive to cross-linking agents (Henriques and Moustacchi 1980b; Cassier and Moustacchi 1981; Ruhland et al. 1981; Siede and Brendel 1982) suggests that specific steps may be also involved in the repair of DNA inter-strand cross-links. For one such mutant, *pso2-1*, a loss of the resistance of diploids relative to haploids and of G2 phase cells compared to G1/S phase cells, was demonstrated following photoaddition of psoralens (Henriques and Moustacchi 1980b). This indicated that the recombinational function responsible for such a resistance in wild type may be altered in *pso2-1*. Indeed the *rad52* type mutants known to be defective in spontaneous and ultraviolet or X-ray-induced recombination (Resnick 1975; Prakash et al. 1980; Malone and Esposito 1980; Game et al. 1980; Saeki et al. 1980; Prakash and Taillon-Miller 1981) have lost the ploidy effect and G2 resistance following psoralens photoaddition (Henriques and Moustacchi 1980a).

In view of these observations, it became of interest to determine the role of the PS02 gene in induced recombination. The induction of gene conversion and mitotic crossing-
over by a variety of agents were compared in wild type and in homozygous ps02-1 diploids. Bisfunctional derivatives which induce both monoadducts and cross-links were compared to monofunctional compounds producing only monoadducts in order to determine the specific role of cross-links. For the same aim different bisfunctional furcoumarins known to produce different proportions of monoadducts over cross-links in vitro were used. Bisfunctional nitrogen mustards inducing cross-links between purines in DNA were compared to bisfunctional psoralens which produce cross-links between pyrimidines in order to assess the possible importance of the molecular nature of the cross-links.

The recombinogenic response of rad52 homozygous diploids to photoaddition of furcoumarins was also examined. This was justified by the common phenotypic features of rad52 and ps02-1 toward exposure to psoralens plus 365 nm radiation mentioned above.

Materials and Methods

Strains. The diploid wild-type strain XS2316 has been previously described (Machida and Nakai 1980) and its genetic constitution was as follows:

\[
\begin{align*}
+ leul-1 & \quad trp5-48 & + + & a \  his1-1 \\
ade6 & leul-12 & + cyh2 & met13 & lys5-1 & a \ his1-1
\end{align*}
\]

TSC1-1 diploid strain was constructed for the present study and its genetic constitution was the following:

\[
\begin{align*}
+ leul-1 & \quad trp5-48 & + + & pso2-1 & \ his1-1 & a \\
ade6 & leul-12 & + cyh2 & lys5-1 & pso2-1 & his1-1 & a
\end{align*}
\]

The homozygous rad52 strain XS1898 (Saeki et al. 1980) had the following genotype:

\[
\begin{align*}
+ leul-1 & \quad trp5-48 & + + & rad52-1 & a \\
ade6 & leul-12 & + cyh2 & met13 & lys5-1 & rad52-1 & a
\end{align*}
\]

Media. The complete liquid medium (YEP) contained 0.5% Yeast Extract Difco, Detroit, USA, 2% bactopeptone Difco and 2% glucose. The Minimal Medium (MM) contained 0.67% Yeast Nitrogen Base without amino acids Difco, 2% glucose and 2% bacto-agar Difco. The synthetic complete medium (SC) was MM supplemented with 2 mg adenine, 5 mg lysine, 1 mg histidine, 3 mg leucine, 2 mg methionine and 2 mg tryptophan, per 100 ml MM. The omission media (SC-leucine, -lysine, -adenine) were a series of media in which one of the amino acids or bases had been omitted from SC: e.g., SC minus leucine, etc. The cycloheximide medium (SC+CyH) was SC supplemented with 200 µg cycloheximide (Calbiochem, Los Angeles, USA) per 100 ml SC.

Chemicals. Furocoumarins: chromatographically purified 3-carbethoxypsoralen (3-CPs) and psoralen were kindly provided by Dr. E. Bisagni. Trioxalen 4-aminomethyl hydrochloride (AMT) (Calbiochem) and 8-methoxypsoralen (8-MOP) (Sigma, St. Louis, USA) were also used. After treatment with 365 nm radiation, 8-MOP, AMT and psoralen induced on DNA both monoadducts and cross-links (for review Parsons 1980) whereas 3-CPs forms only monoadducts (Magaña-Schwencke et al. 1980). The former compounds are consequently bifunctional whereas the later is monofunctional. Nitrogen mustards: bifunctional nitrogen mustard (HN2), 2-2'-dichloro-N-methyl diethylamine hydrochloride, and monofunctional nitrogen half mustard (HNI), 2-dimethylamino ethyl chloride, were purchased from Aldrich Europe.

Cell Culture. In each independent experiment, a loopful of slant culture was inoculated in YEP and incubated at 30°C with vigorous shaking for 3 days.

Treatment with Furocoumarins and UVA. Stationary phase cells were harvested, washed three times with saline (0.9% NaCl) and the cell suspension was sonicated for 15 s in an MSE 150 W ultrasonic disintegrator MK2 (needle probe 38121-108). The number of cells and proportion of budding cells was measured in a counting chamber. A cell suspension at 2 x 10^7 cells per ml was then incubated for 20 min at 4°C with 5 x 10^-7 M furocoumarins and irradiated with 365 nm light source (UVA) as described previously (Averbeck et al. 1978). Treatment with UV (254 nm) radiation was performed as previously described (Moustacchi 1969).

Treatment with Nitrogen Mustards. Stationary phase cells were harvested, washed three times in phosphate buffer (0.067 M, pH 7) and resuspended at 4 x 10^6 cells per ml. The treatment was performed as described by Ruhland and Brendel (1979). Cells were incubated for 2 h at 36°C with various concentrations of nitrogen mustards. The reaction was stopped by adding an equal volume of 4% thiosulphate.

Test of the Induction of Mitotic Recombinational Events Following Treatment. Strains heterozygous for cyh2 and heteroallelic for leul-1 were used to study the induction of mitotic recombinational events. The cells treated with various recombinogens were diluted in saline, plated on three kinds of medium, SC, SC-LEU and SC+CYH, and incubated for 7 days at 30° C. This was followed by the examination of cell killing on SC, intragenic recombination (gene conversion) on SC-LEU and intergenic recombination (crossing-over) on SC+CYH. It is noteworthy that the reversion frequency either from leul-1 or leul-12 was lower than the gene conversion frequency by at least one order of magnitude after a recombinogenic treatment. To measure the exact frequency of reciprocal crossing-over, it is necessary to eliminate the possibility that some cycloheximide-resistant colonies had resulted from reversion or gene conversion at the cyh2 locus, as well as monosomy on chromosome VII. For this purpose, the cycloheximide-resistant colonies were replica-plated on a series of omission media, SC-lys, SC-met and SC-ade, for screening the linked markers of cyh2.

For each strain and each agent used experiments were repeated at least three times. Plating were done in triplicate for each dose so that a minimum of 200 survivors and of 50 recombinants per point were scored.

Results

For all the treatments the homozygous diploid ps02-1 mutant strain was compared to the corresponding wild type. The recombinogenic response of the rad52-1 diploids to furcoumarins photoaddition in particular, was also studied for comparison.