

THE EFFECTS OF THREE *PSO* GENES ON INDUCED  
MUTAGENESIS : A NOVEL CLASS OF  
MUTATIONALLY DEFECTIVE YEAST

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ABSTRACT

Reverse and forward mutation, induced by photoaddition of 8-methoxy-psoralen (8-MOP) and 3-carbethoxy-psoralen (3-CPs) or ultraviolet light (UV), are reduced in three *pso* mutants of *Saccharomyces cerevisiae*. The *pso1-1* strain exhibits a lower frequency of spontaneous reversion (anti-mutator) and is almost entirely unaffected by the three agents in both the haploid and diploid states. The *pso2-1* strain demonstrates very reduced frequencies of 8-MOP and 3-CPs plus 365 nm radiation-induced mutations in haploid and diploid cells. UV-induced mutations are slightly reduced, whereas survival is almost normal. The *pso3-1* strain is mutable by 8-MOP and 3-CPs photoaddition only in the low-dose range. After UV treatment, survival of *pso3-1* is nearly normal, whereas the frequencies of induced mutants are diminished as compared to the normal *PSO*<sup>+</sup>. An analogue of adenine, 6-N-hydroxyaminopurine, is capable of inducing reversions in wild type, as well as in *pso* and *rad6-1* mutant strains, indicating that this drug may act as a direct mutagen in yeast. The comparison of photoaddition of the bifunctional agent (8-MOP) to that of the monofunctional one (3-CPs) confirms that cross-links, as well as monoadditions, are mutagenic in *S. cerevisiae*. Repair, of the recombinational type, taking place in diploid cells or in haploid cells in G2 phase leads to higher survival, but appears to be error-free.

MUTATIONS induced by radiations or chemicals are thought to arise as a result of genetically controlled enzymatic repair processes utilizing DNA lesions as substrates. Indeed, when compared to repair-proficient strains, a number of repair-deficient mutants demonstrate a modified mutagenic response to various genotoxic agents. We examine here whether a novel class of *Saccharomyces cerevisiae* mutants selected for their sensitivity to the lethal effect of psoralens photoaddition (HENRIQUES and MOUSTACCHI 1980b) is affected in their mutagenic response.

Different repair pathways could lead either to an accurate (error-free) or to an inaccurate (error-prone) restoration of genetic information. The excision-resynthesis pathway for ultraviolet radiation (254 nm) or specific chemically induced DNA lesions is thought to be essentially error-free. Indeed, in prokaryotes, as well as in eucaryotes, mutants with a biochemical defect in excision repair of UV-induced pyrimidine dimers, for instance, exhibit enhanced fre-

quencies of UV-induced revertants compared with wild type at equal UV doses. This is the case in *uvr* mutants of *Escherichia coli* (HILL 1965; WITKIN 1966; BRIDGES and MUNSON 1968), in human fibroblasts derived from xeroderma pigmentosum patients (MAHER and McCORMICK 1976) and in the excision-defective mutants belonging to the epistasis group defined by the 10 independent loci of the *rad3* type in *S. cerevisiae* (MOUSTACCHI 1969; NAKAI and YAMAGUCHI 1969; RESNICK 1969; AVERBECK *et al.* 1970; ZAKHAROV, KOZINA and FEDOROVA 1970). In general, mutations in the excision-repair pathway (for review see MOUSTACCHI, CHANET and HEUDE 1977; HAYNES *et al.* 1978) lead to UV but not X-ray sensitivity to cell killing.

The identification of mutant genes conferring both increased induced lethality and defective induced mutability, compared with wild type, led to the concept of error-prone repair (WITKIN 1966). In *E. coli*, the *recA* and *lex* mutants behave in this manner. In the eucaryotic microorganism *S. cerevisiae*, mutagenesis induced by radiation or chemicals may be blocked by a number of mutations in different loci, namely, *rev1*, *rev2*, *rev3*, *rad6*, *rad9*, *rad18* and *rad8* (*rad6* type) (LEMONTT 1971, 1972; LAWRENCE and CHRISTENSEN 1976, 1978a,b, 1979). Such a block in induced mutagenesis is accompanied by an increased cellular sensitivity to UV, X rays (COX and GAME 1974) and a number of chemicals (PRAKASH 1974, 1976). In other words, such mutants share some common features with *recA* and *lex* mutants of *E. coli* (WITKIN 1969a; MOUNT, LOW and EDMISTON 1972). However, unlike *lexA*, which is dominant to the wild-type allele (MOUNT, LOW and EDMISTON 1972), the *rad* and *rev* genes of *Saccharomyces* are recessive, suggesting the loss of enzymatic steps required in the mutagenic mechanism. Moreover, mitotic recombination appears to be normal in the *rad6* mutants, whereas it is altered in the *rec* mutants of *E. coli* (WITKIN 1969b). It should be noticed that, although in *E. coli* the molecular nature of the error-prone repair pathway, including its inducible component, has been partially characterized (for review, see WITKIN 1967), very little is known about the nature of this type of repair in yeast.

The photoaddition (365 nm radiation) of psoralen derivatives induces both monoadditions and cross-links between DNA strands (for review, see SONG and TAPPLEY 1979). According to the molecular model proposed by COLE (1976), in *E. coli* both excision resynthesis and recombinational (*recA*-dependent) repair processes are required for the repair of cross-links. In yeast, we have previously shown that the three major genetically identified repair pathways are involved in the repair of such lesions (AVERBECK and MOUSTACCHI 1975; HENRIQUES and MOUSTACCHI 1980a). A novel class of yeast mutants sensitive to photoaddition of bifunctional and monofunctional derivatives of psoralen was recently isolated (HENRIQUES and MOUSTACCHI 1980b). Three of these *pso* mutants were analyzed in detail. It was shown that in each strain the *pso* character is determined by one nuclear gene and is recessive. The three mutants complement each other, as well as the existing radiation-sensitive *rad* and *rev* mutants; consequently, they define three distinct complementation groups.

Different DNA lesions may be repaired either by separate mutagenic pathways or by a common pathway (*rad6*). Furthermore, since the *pso* genes are not allelic with the already known mutationally defective *rad6* type of genes, it seemed of interest to determine whether they belong to an error-prone pathway and to examine their mutagenic response to both photoaddition of psoralen derivatives and UV (254 nm). The mutagenic effect on both reversions and forward mutations of these two types of agents was examined here. The haploid and homozygous diploid mutant strains were compared to the normal *PSO*<sup>+</sup> strain. The mutagenic effect of photoaddition of a bifunctional furocoumarin, 8-methoxypsoralen (8-MOP), was compared with that of a monofunctional furocoumarin 3-carbethoxypsoralen (3-CPs) (AVERBECK, MOUSTACCHI and BISAGNI 1978).

#### MATERIALS AND METHODS

*Strains*: The haploid normal strain N123 (*PSO*<sup>+</sup>) and the derived mutants *pso1-1*, *pso2-1* and *pso3-1* have been described previously (HENRIQUES and MOUSTACCHI 1980b). All of these strains are a *his1* and are sensitive to canavanine (*CAN1*) and to cycloheximide. The *his1* allele used in this study, although not characterized, is likely to be of the missense type, according to the study of SNOW (1978). Mutants resistant to the toxic effect of the arginine analogue, canavanine, arise by forward mutation at a single recessive gene, *can1*, responsible for an arginine-specific permease activity (GRENSON *et al.* 1966). Resistance to cycloheximide, a protein synthesis inhibitor, results from forward mutations at a multigenic system (WILKIE and LEE 1965).

The homozygous diploid strains of the normal (*PSO*<sup>+</sup>/*PSO*<sup>+</sup>) and of the mutant (*pso/pso*) genotypes were obtained by prototrophic selection as described elsewhere (HENRIQUES and MOUSTACCHI 1980b). In terms of auxotrophic markers, the genotype of the diploid strains was originally *ade1*/<sup>+</sup> *his*/<sup>+</sup>. In order to study the reversion at the *his1* locus in diploid cells, strains homozygous for the *his1* allele were required. Mitotic recombinants were obtained by irradiating the diploid strains with 6 kr of  $\gamma$  rays from a Cobalt-60 source. After plating on complete medium, the surviving colonies were replica-plated on minimal medium and on minimal medium supplemented with histidine. The putative *his*<sup>-</sup> clones were isolated and their phenotype further established. The dose of  $\gamma$  rays used resulted in 90% survival and 1 to 2% (*his*<sup>-</sup>) recombinants.

*PSO*<sup>+</sup> *lys1-1* and *pso1-1 lys1-1* strains (see Table 1) were obtained by a cross of the original mutant *pso1-1* to strain XV423-2A ( $\alpha$  *ade2-1*, *lys1-1*, *trp5-48*, *his1-7* *hom3-10*, *rad3-12*) kindly provided by R. C. VON BORSTEL. After tetrad analysis, two monosporic clones from the same ascus carrying the required genotypes were isolated. The *lys1-1* allele is an ochre-suppressible mutation.

*Media*: The complete medium (YEP) contained 0.5% Yeast Extract Difco, 2% Bacto-peptone Difco and 2% glucose. The minimal medium (MM) contained 0.67% Bacto-Yeast Nitrogen Base without amino acids Difco and 2% glucose. Media for plates was solidified with 2% agar.

Canavanine sulfate (Sigma) at 40  $\mu$ g/ml was added to the MM supplemented with the required amino acids. Cycloheximide (Calbiochem) at 0.5  $\mu$ g/ml was added to the YEP.

*Furocoumarins*: Chromatographically pure 3-carbethoxypsoralen was kindly provided by E. BISAGNI. 8-methoxypsoralen was purchased from Sigma Co. The final concentration of the furocoumarins in the treated cell suspension was always  $5 \times 10^{-5}$  M, and the stock solutions were prepared as previously described (AVERBECK and MOUSTACCHI 1975).

*Culture conditions, measurement of survival and mutation frequency after treatment with 8-MOP, 3-CPs and UV*: Cells were routinely cultured in YEP from single-colony isolates. In each independent experiment,  $2 \times 10^6$  cells per ml were seeded and shaken vigorously at 30° for 3 days. These stationary phase cells were washed three times with saline (9% NaCl in distilled water) and the cell suspension sonicated for 15 sec in a MSE 150-watt ultrasonic disintegrator Mk2 (needle probe 38121-108). The number of cells and proportion of buds was measured in a

counting chamber. A cell suspension at  $2 \times 10^7$  cells per ml was then incubated for 20 min at  $4^\circ$  with  $5 \times 10^{-5}$  M 8-MOP or 3-CPs and irradiated with a 365 nm light source, as described previously (AVERBECK, MOUSTACCHI and BISAGNI 1978).

After irradiation, cells were concentrated by centrifugation to a density of  $2 \times 10^8$  cells per ml. For each dose, cells were spread onto 6 plates of omission medium (MM) for detection of *HIS*<sup>+</sup> revertants. Part of the irradiated suspension was appropriately diluted and plated on complete medium for cell viability (3 plates) or on MM plus canavanine supplemented with histidine (6 plates) or on YEP plus cycloheximide (6 plates) for detection of forward mutants.

Treatments with UV (254 nm) radiation were performed as previously described (MOUSTACCHI 1969).

The *HIS*<sup>+</sup> revertants were scored after 5 days of incubation; whereas, the *can1* and *cyh*-resistant mutants were scored after 4 days of incubation at  $30^\circ$ .

We determined that the proportion of mutants remained unchanged within a 50-fold difference in cell densities of plated cell suspensions. Each strain was examined on at least three separate occasions, and the *PSO*<sup>+</sup> strain was included in each experiment.

*Spontaneous rate of mutants:* By using a fluctuation test (VON BORSTEL (1978) adapted to the N123 haploid strain for the limiting concentration of histidine (CHANET and VON BORSTEL 1979), the effect of the *pso* genes on spontaneous rates of reversion was determined.

*Induction of HIS<sup>+</sup> revertants by 6-N-hydroxyaminopurine (6-N-HAP):* A stock solution of 6-N-HAP (Nutritional Biochemical Corporation) at a concentration of 4.4 mg per ml in dimethylsulfoxide was prepared. 6-N-HAP at concentrations varying from 2 to 100  $\mu$ g/ml was then added to YEP seeded with  $6.10^8$  cells per ml. After incubation with shaking at  $30^\circ$ , cells in stationary phase were plated on MM or on supplemented MM.

## RESULTS

*Induction of reversion of his1 and of forward mutation in psol-1:* As shown in Figures 1 and 2, the *psol-1* strain fails to show any induced reversion of the *his1* allele after photoaddition of 8-MOP or 3-CPs. Figure 3 shows that forward mutability to *can1* is clearly depressed in *psol-1* compared with the *PSO*<sup>+</sup> normal strain after 8-MOP photoaddition. The same is true for forward mutation induction at the *CYH* loci (data not shown). This block in induction of revertants and forward mutants in *psol-1* is observed at equal doses, as well as at equal levels of survival, in comparison to the normal *PSO*<sup>+</sup> (Figure 3 as an example). It is worth noticing that, in two *rad6-1* mutant strains, 8-MOP photoaddition did not enhance the frequency of revertants at the *his1-7*, *lys1-1* and *hom3-10* loci; whereas, normal induction was found in the corresponding wild type (data not shown).

As already described (HENRIQUES and MOUSTACCHI 1980b), *psol-1* is also sensitive to the lethal effect of 254 nm UV radiation. Fig. 4 shows that the induction of reversions of *his1*, as well as of forward mutations of *CAN1*, by UV is almost completely blocked in *psol-1* at different UV doses and levels of survival.

The homozygous diploid *psol-1/psol-1*, although more sensitive than the normal *PSO*<sup>+</sup>/*PSO*<sup>+</sup> strain, is clearly more resistant than the haploid *psol-1* mutant to inactivation by 8-MOP photoaddition (HENRIQUES and MOUSTACCHI 1980b and Figure 5). However, this diploid strain also fails to demonstrate any induced *HIS*<sup>+</sup> reversion by such treatment (Fig. 5). The same conclusions hold for treatments with the monofunctional agent, 3-CPs (Figure 6) and with 254 nm UV radiation (Figure 7).

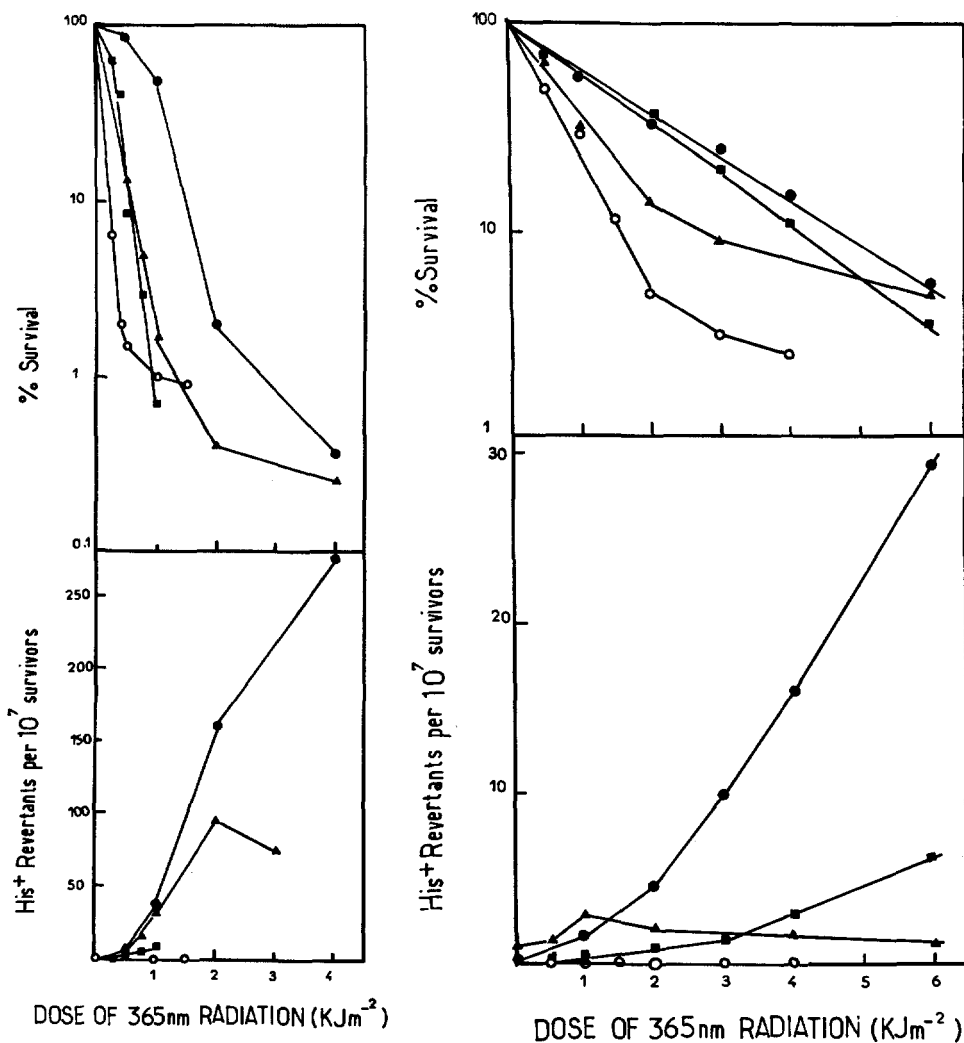


FIGURE 1.—Survival and induction of *HIS*<sup>+</sup> revertants in normal *PSO*<sup>+</sup> (●), *pso1-1* (○), *pso2-1* (■) and *pso3-1* (▲) strains after treatment with 8-MOP plus 365 nm irradiation as a function of irradiation dose.

FIGURE 2.—Survival and induction of *HIS*<sup>+</sup> revertants in normal *PSO*<sup>+</sup> (●), *pso1-1* (○), *pso2-1* (■) and *pso3-1* (▲) strains after treatment with 3-CPs plus 365 nm irradiation as a function of irradiation dose.

In summary, the induction of revertants and forward mutants is blocked in *pso1-1* after the three types of inducing treatments applied. This is true in both the haploid and diploid states.

*Induction of reversion of his1 and of forward mutation in pso2-1:* Figures 1 and 2 show that the induction of *HIS*<sup>+</sup> revertants is detectable in the haploid *pso2-1* mutant after photoaddition of 8-MOP or 3-CPs. However, the frequencies are extremely low in comparison to *PSO*<sup>+</sup>. This is also true in the case of the

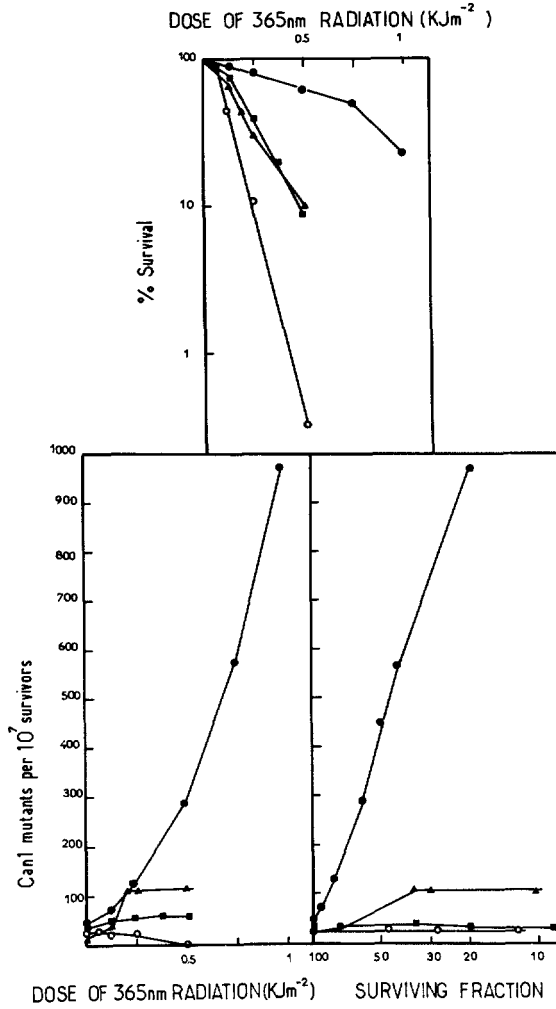


FIGURE 3.—Induction of *can1* forward mutants in normal *PSO*<sup>+</sup> (●), *pso1-1* (○), *pso2-1* (■) and *pso3-1* (▲) strains after treatment with 8-MOP plus 365 nm irradiation as a function of irradiation dose (left panel) or of survival level (right panel). Survival as a function of dose is given in the top panel.

induction of forward mutation at the *CAN1* locus (Figure 3), as well as at the *CYH* loci (data not shown) by 8-MOP photoaddition.

The mutant *pso2-1* is specifically sensitive to the killing effect of furocoumarin photoaddition (HENRIQUES and MOUSTACCHI 1980b). The survival after 254 nm UV radiation equals that of the normal. As seen in Figure 4, the frequency of induced *HIS*<sup>+</sup> revertants is reduced in *pso2-1* in comparison to the normal strain. The induction of forward mutants by UV is close to that obtained in the normal strain.

In the case of the homozygous diploid, *pso2-1/pso2-1*, after photoaddition of mono- and bifunctional furocoumarins, the induction of *HIS*<sup>+</sup> revertants is either reduced (Figure 6) or blocked (Figure 5). It should be noticed that, as

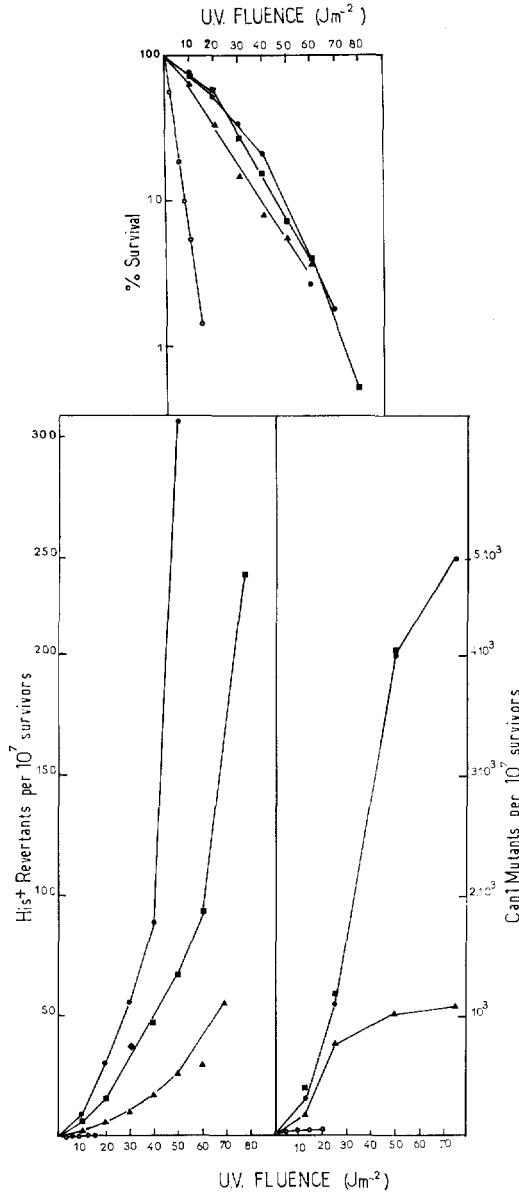


FIGURE 4.—Survival and induction of *HIS*<sup>+</sup> revertants (left lower panel) and *can1* forward mutants of (right lower panel) in normal *PSO*<sup>+</sup> (●), *pso1-1 pso2-1* (■) and *pso3-1* (▲) strains as a function of UV dose.

already described (HENRIQUES and MOUSTACCHI 1980b), the *pso2-1* mutation abolishes the resistance of diploid cells to 8-MOP photoaddition-induced killing (Figure 5), whereas the response to 3-CPs photoaddition is similar to that of the normal diploid strain (Figure 6).

After UV treatment, as in haploids, the frequency of *HIS*<sup>+</sup> revertants is only

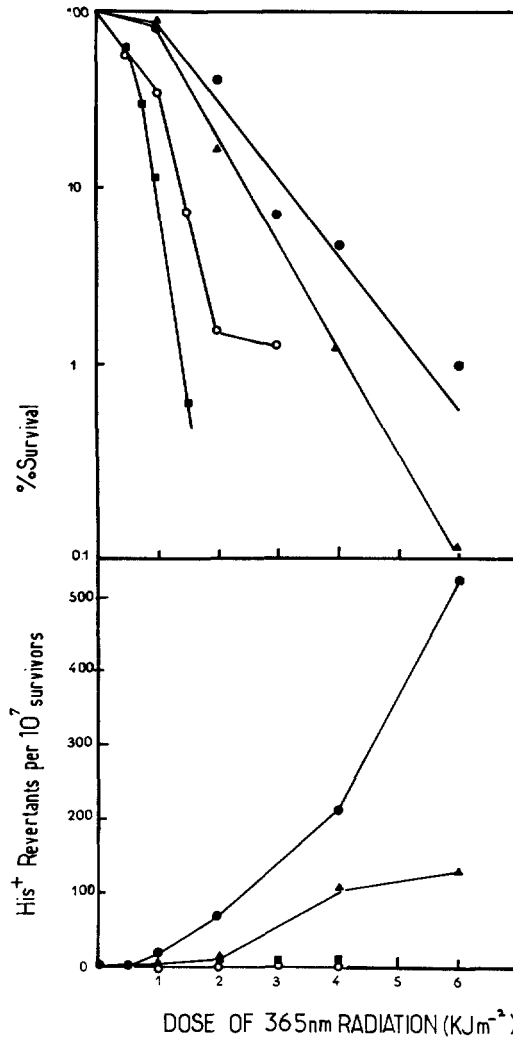


FIGURE 5.—Survival and induction of *HIS*<sup>+</sup> revertants in *PSO*<sup>+</sup>/*PSO*<sup>+</sup> (●), *psol-1/psol-1* (○), *psol-1/psol-1* (■) and *psol-1/psol-1* (▲) diploid strains after treatment with 8-MOP plus 365 nm irradiation as a function of irradiation dose.

slightly reduced (Figure 7) in comparison to the normal *PSO*<sup>+</sup>/*PSO*<sup>+</sup> diploid strain.

In other words, the induction of revertants and forward mutants by furocoumarin photoaddition is either blocked (Figure 5) or considerably reduced (Figure 6) in both haploid and diploid *psol-1* mutants. The mutability after UV treatment is, however, either close to normal or reduced (Figures 4 and 7).

*Induction of reversion of his1 and forward mutation in psol-1:* As seen in Figures 1, 2 and 3, mutants are induced in *psol-1* by photoaddition of furocoumarins; however, the mutagenic response for both reversion and forward



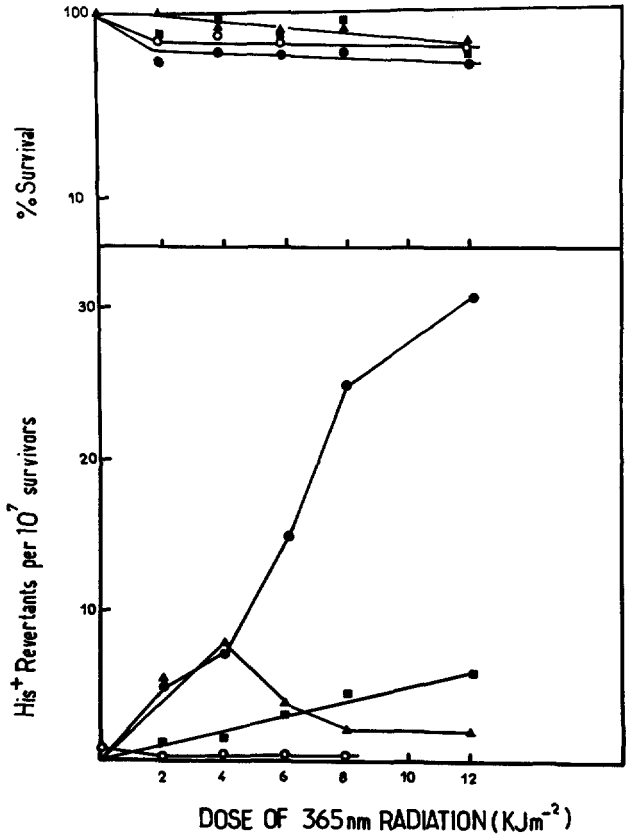


FIGURE 6.—Same as for Figure 5, but after treatment with 3-CPs plus 365 nm irradiation.

mutation appears to be dose dependent when the *pso3-1* mutant is treated with either 8-MOP or 3-CPs plus 365 nm radiation. In the low-dose range, the frequency of induced mutants follows about the same kinetics as the normal haploid *PSO+* strain; whereas, at high dose levels, the average mutation frequencies remain constant and are significantly lower than in the normal *PSO+* type.

Similarly to *pso2-1*, *pso3-1* demonstrates a sensitivity to the lethal effect of UV close to that of the normal *PSO+* type (HENRIQUES and MOUSTACCHI 1980b and Figure 4). However, the mutagenic response to UV is reduced for induction of revertants and for forward mutations (Figure 4). As with the photoaddition of furocoumarins, a dose dependence is observed.

As in the haploid mutant, the homozygous diploid strain *pso3-1/pso3-1* demonstrates complex induction kinetics of *HIS+* revertants after 8-MOP or 3-CPs photoaddition (Figures 5 and 6). Indeed, after an increase in the frequency of mutants in the low-dose range, a plateau is reached at higher doses and the magnitude is clearly reduced in comparison to the *PSO+* diploid strain. Figure 7 shows that, as in haploids, after a UV treatment the induction of revertants is

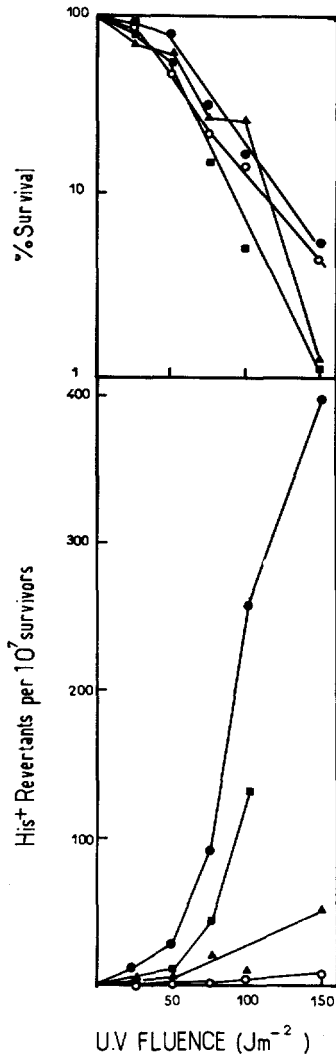


FIGURE 7.—Same as for Figure 5, but after treatment with UV irradiation.

reduced in the diploid *pso3-1* strain. In summary, the *pso3-1* gene also has an influence on induced mutagenesis.

It should be noted that the three *pso* strains, when tested in exponential phase of growth (more than 20% budding cells) with 8-MOP plus 365 nm irradiation, demonstrate the same difference in mutagenic response when compared to the normal type (data not shown) as observed for stationary-phase treated cells.

*Spontaneous mutation rate in pso mutants:* Since the three *pso* mutants appeared to be less mutable than the wild-type strain after mutagenic treatments, we examined the effect of the *pso* genes on spontaneous mutation rates. As seen in Table 1, the spontaneous reversion rate of *his1* in *pso2-1* and *pso3-1* is very

TABLE 1  
Fluctuation test as adapted by CHANET and VON BORSTEL (1979)

Strain	Number of positive cases Number of compartments examined	Cells/ml ( $\times 10^6$ )	Mutation rate ( $10^{-8}$ revertants cell/division)
<i>PSO+</i> ( <i>his1</i> → <i>HIS+</i> )	14/250	15.7	1.8
( <i>lys1-1</i> → <i>LYS+</i> )	95/563	1.09	0.8
<i>pso1-1</i> ( <i>his1</i> → <i>HIS+</i> )	6/1025	3.48	0.4
( <i>lys1-1</i> → <i>LYS+</i> )	16/500	0.87	0.18
<i>pso2-1</i> ( <i>his1</i> → <i>HIS+</i> )	11/225	11.42	2.1
<i>pso3-1</i> ( <i>his1</i> → <i>HIS+</i> )	10/231	15.51	1.4

Growth is restricted by limiting the concentration of the relevant requirement factor (0.2  $\mu\text{g/ml}$  for histidine and 1  $\mu\text{g/ml}$  for lysine).

close to that of the normal *PSO+* strain from which they were derived. In contrast, the rate of reversion in *pso1-1* is reduced by a factor of about 4. The same is true for the spontaneous rate of reversion of the *lys1-1* allele. In other words, *pso1-1* behaves like an antimutator gene.

*Induction of reversion in psu and rad6-1 mutants by 6-N-HAP:* The induction of reversion at the *his1* locus by various concentrations of 6-N-HAP, an analogue of adenine that seems to act as a direct mutagen (for review see RONEN 1979), was compared in the normal *PSO+* and mutant *pso1-1* and *pso2-1* strains. The mutation rate was calculated according to GLICKMAN, VAN DEN ELSEN and RADMAN (1978). In Table 2, it can be seen that although growth is inhibited to various extents by increasing the concentration of 6-N-HAP, reversions are induced by this agent in the *psu* mutants, as well as in the normal. When compared to the corresponding wild type, we observed that in two *rad6-1* (kindly provided by R. C. VON BORSTEL) mutant strains, 6-N-HAP clearly induces revertants in the *his1-7*, *lys1-1* and *hom3-10* loci (data not shown). In other words, both nonsense and frameshift mutations are induced by this agent. These experiments essentially show that the mutagenic repair pathway(s) governed by the *PSO1*, *PSO2* and *RAD6* genes are not absolutely required for the

TABLE 2  
Induction of *HIS+* revertants by various concentrations of 6-N-HAP

Concentration of 6-N-HAP ( $\mu\text{g/ml}$ )	<i>N</i> ( $\times 10^8$ )			<i>M</i>			<i>g</i>			<i>a</i> ( $\times 10^{-8}$ )		
	<i>PSO+</i>	<i>psu1-1</i>	<i>psu2-1</i>	<i>PSO+</i>	<i>psu1-1</i>	<i>psu2-1</i>	<i>PSO+</i>	<i>psu1-1</i>	<i>psu2-1</i>	<i>PSO+</i>	<i>psu1-1</i>	<i>psu2-1</i>
0	8.5	4.7	3.3	0	0	0	17.4	16.5	16	—	—	—
10	5.6	3.9	2.5	125	125	293	16.8	16.2	15.6	1.3	2	7.5
20	2.9	3.3	1.4	397	271	318	15.8	16.0	14.8	8.6	5.1	15.3
30	3.8	3.1	0.64	782	453	255	16.2	15.9	13.6	12.7	9.1	29.3
40	0.79	1.6	0.37	361	401	258	13.9	14.9	12.8	32.8	16.8	54.4
50	0.96	0.95	0.27	720	422	383	14.2	14.2	12.4	52.8	31.3	114.4

The mutation rate *a* is calculated according to GLICKMAN, VAN DEN ELSEN and RADMAN (1978). *N* is the population size, *M* the number of induced mutants and *g* the number of generations in the presence of 6-N-HAP. The mutation rate *a* equals *M/gN* (STAHL 1969).

induction of mutants by 6-N-HAP. We suggest that, as in *E. coli* (JANION 1978), this drug may act as a direct mutagen in *S. cerevisiae*. The differences in response of the mutants to 6-N-HAP, in comparison to the wild type, merit further analysis.

#### DISCUSSION

After treatment with either psoralen derivatives plus 365 nm irradiation or UV (254 nm), the three *pso* mutants analyzed turn out to be mutationally defective to various degrees for both induced reversions and forward mutations.

Analysis of the genetic control of UV-induced mutagenesis in *S. cerevisiae* led to the identification of more than ten loci (of the *rad6* type) that are active in the production of induced mutations. Such loci represent a single epistatic group with respect to UV survival (for review, see LEMONTT 1980). The analysis of survival after 8-MOP photoaddition, UV and  $\gamma$  rays of heterozygous diploid strains obtained from crosses between the *pso* mutants and *rad6*, *rad9*, *rad18*, *rev1*, *rev2* and *rev3* compared to corresponding homozygous strains showed that the *pso* mutations complement each other, as well as the *rad6* mutations (HENRIQUES and MOUSTACCHI 1980a). They consequently define complementation groups different from the *rad6* type genes, which are known to play an essential role in the mutagenic repair pathway. Moreover further genetic studies, including tetrad analysis of monosporic clones derived from crosses between *pso* and *rad6* mutants, show a clear-cut segregation of the *pso* and *rad6* phenotypes (HENRIQUES and MOUSTACCHI, in preparation).

The *pso1-1* mutant demonstrates a cross-sensitivity to furocoumarin photoaddition, UV and ionizing radiations (HENRIQUES and MOUSTACCHI 1980b). We show here that the spontaneous rate of reversion of *his1* and *lys1-1* is reduced in *pso1-1* by comparison with the isogenic *PSO*<sup>+</sup> wild type. Consequently, the *pso1-1* mutant has a mutational phenotype different from that of *rad6* and *rad18*. Indeed, these latter two mutants possess a mutator activity (HASTINGS, QUAH and VON BORSTEL 1976), whereas *pso1-1* behaves like an antimutator (Table 1). Different *rev3* alleles have antimutator activity (QUAH and HASTINGS, personal communication) and are blocked in induced mutagenesis (LAWRENCE and CHRISTENSEN 1979), similar responses being observed with *pso1-1*. However, *pso1-1* complements *rev3* for survival after 8-MOP photoaddition and UV, and the phenotypes of these strains differ in several aspects (J.A.P. HENRIQUES, in preparation).

The reversion frequencies of the *his1* locus, as well as forward mutations of *CAN1* and *CYH* induced by mono- and bifunctional furocoumarin photoaddition or UV, are considerably reduced in both haploid and homozygous diploid *pso1-1* strains. This block in induced mutagenesis in *pso1-1* is observed as a function of dose, as well as a function of survival level. Consequently, it appears that the *PSO1-1* locus plays an essential role in spontaneous and induced mutagenesis.

The mutant *pso2-1* is specifically sensitive to furocoumarin photoaddition (HENRIQUES and MOUSTACCHI 1980b), and we show that the induction of re-

vertants and forward mutants, although not entirely abolished as in *pso1-1*, is greatly reduced in the *pso2-1* mutant after treatment with 3-CPs or 8-MOP plus 365 nm irradiation. This reduction in induced mutagenesis is observed as a function of dose or of survival level. The *pso2-1* mutation in the homozygous state abolishes the resistance to 8-MOP photoaddition characteristic of diploid cells, as well as the resistance of G2 haploid cells (HENRIQUES and MOUSTACCHI 1980b). It may be noticed that the frequency of induced revertants is lower (almost undetectable) in the homozygous diploid *pso2-1* strain as compared to the haploid strain.

After UV treatment, although the survival is close to normal, the mutagenic response is slightly reduced for both haploid and homozygous diploid *pso2-1* strains. However, the defect in mutagenic repair in the *pso2-1* mutant is predominantly concerned with furocoumarin photoinduced lesions.

Like the *pso2-1* mutant, *pso3-1* is specifically sensitive to the lethal effect of furocoumarin photoaddition (HENRIQUES and MOUSTACCHI 1980b). The frequency of induced revertants and forward mutants is comparable to that of the normal *PSO*<sup>+</sup> strain in the low dose range; whereas, it remains at a low level for high doses. Similar situations are encountered with *rad6* (PRAKASH 1974) after certain mutagenic treatments. It is possible that the residual induced mutagenesis in *pso3-1* is due either to leakiness of the *pso3-1* mutation or to the action of the *RAD6* pathway. For a greater proportion of mono- and bifunctional lesions, however, this channelling of mutagenic damage would not be possible. The mutagenic response to UV treatment is notably reduced in both haploid and homozygous diploid *pso3-1* strains, while the survival is almost normal.

The nature of the mutagenic lesions can be inferred from the comparison of the mutagenic efficiency of bifunctional (8-MOP) and monofunctional (3-CPs) photoaddition. After such comparisons, it was concluded that in yeast cross-links as well as monoadducts are mutagenic (AVERBECK and MOUSTACCHI 1979, 1980; GRANT, VON BORSTEL and ASHWOOD-SMITH 1979). In particular, this conclusion was drawn from the comparison of slopes of induced mutants *versus* dose in a double-log plot (GRANT, VON BORSTEL and ASHWOOD-SMITH 1979). Our data are in accord with these observations for the haploid and diploid *PSO*<sup>+</sup> strains. Indeed, after 8-MOP treatment, the mutagenic effect follows two-hit kinetics; whereas, after 3-CPs, one-hit kinetics is observed (the data presented in Figures 1 and 5 lead to a slope close to two for 8-MOP and close to one for 3-CPs on a log-log scale). This is also true for *pso3-1* and *pso3-1/pso3-1* in the low-dose range. On the other hand, the *pso2-1* strain shows one-hit kinetics after 8-MOP, as well as 3-CPs photoaddition. This response is similar to that observed for an excision-defective strain treated with 8-MOP or angelicin plus 365 nm irradiation (GRANT, VON BORSTEL and ASHWOOD-SMITH 1979). It is thus possible to suggest that monoadditions are mutagenic in *pso2-1* and in excision-defective strains, although to a much lesser extent than in wild type. In repair-defective strains, cross-links may exist as nonpremutagenic lesions that may essentially lead to lethality.

It should be noticed that, although survival to UV is close to normal in *pso2-1* and *pso3-1*, there is a slight effect of *pso2-1* and a more pronounced effect of *pso3-1* on UV-induced mutagenesis. Such a defect in UV mutagenic repair may concern some UV-induced photolesion(s), which have some characteristics in common with furocoumarin-photoinduced lesions.

In all strains studied here, although survival is enhanced in diploid cells compared to haploid cells, the mutagenic effect of 8-MOP and 3-CPs photoaddition is weaker in diploid than in haploid cells at equal doses. For example, the *pso1-1* mutation blocks the mutagenic repair of cross-links, monoadditions and UV-induced pyrimidine dimers. Such a block is also seen in the homozygous diploid strain for which survival is enhanced in comparison with the haploid strain. An underestimation of prototrophs in diploid compared to haploid strains due to second-site revertants occurring in haploid cells and being recessive in diploid cells seems unlikely. Indeed, an opposite effect has been described (LAX and FOGEL 1978). Moreover, in *pso2* diploids, for instance, no revertants at all are detected. Therefore, we suggest that the recombinational events taking place in the diploid state favor error-free repair.

The repair model of cross-links proposed by COLE, LEVITAN and SINDEN (1976) for *E. coli* implies recombinational events of the prereplicative type. In the experiments presented here, haploid cells were treated in stationary phase, *i.e.*, a stage during which recombinational processes do not take place and no difference is observed with the mutagenic response of cells treated during the exponential phase of growth (data not shown). The lesions induced in G1 may be carried over into S and initiate recombinational events resolved in S-G2. However, cross-links must be modified in G1 in a way that allows DNA replication since for the wild type such lesions are mutagenic in cells treated at this stage. Consequently, certain steps of the mutagenic repair of cross-links induced in yeast may be different from those in the model postulated by COLE, LEVITAN and SINDEN (1976). Further experiments will be undertaken to test this possibility.

The major conclusion of this work is that the three *pso* mutants studied have a diminished mutagenic response compared to *PSO*<sup>+</sup>. It is striking that, in such a small number of mutants tested for their mutagenic responses, all three demonstrated the same feature. It may be asked why they escaped detection in radiation and chemical mutagenesis experiments performed in different laboratories. One reason may reside in the fact that the *pso* mutants were specifically selected for sensitivity to 8-MOP photoaddition. Now, at equal survival levels, this agent is 10 to a 100 times more efficient in mutation induction than is UV or  $\gamma$  rays (AVERBECK and MOUSTACCHI 1979), the two agents most commonly used for the selection of repair-defective mutants. Consequently, it may be that a defect in psoralen-photoinduced lesions, revealed by an enhanced sensitivity to cell killing, is associated with a defect in mutagenic repair. Examination of the mutagenic responses of other *pso* mutants in our collection (HENRIQUES and MOUSTACCHI 1980b) will determine whether this hypothesis is valid.

Finally, it is clear that, in contrast to 8-MOP photoaddition, in the *pso* and *rad6* mutants examined 6-N-HAP induces reversion. It is therefore likely that this analogue acts as a direct mutagen in yeast, as well as in *E. coli* (JANION 1978).

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