ISOLATION AND CHARACTERIZATION OF *pso* MUTANTS SENSITIVE TO PHOTO-ADDITION OF PSORALEN DERIVATIVES IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

We have isolated mutants sensitive to photo-addition of bi-functional and mono-functional derivatives of psoralen in *Saccharomyces cerevisiae*. Three of these *pso* mutants were analyzed in detail. They segregate in meiosis like Mendelian genes and complement each other, as well as existing radiation-sensitive (*rad* and *rev*) mutants. The study of heterozygous diploid strains (*PSO+/pso*) indicates that the three *pso* genes are recessive. The mutant *pso1-1* demonstrates a cross-sensitivity to UV and γ-rays, whereas mutants *pso2-1* and *pso3-1* are specifically sensitive to photo-addition of psoralen derivatives. The comparison of exponentially growing cells to stationary-phase cells demonstrates that for the three mutants the defect in repair capacity of DNA cross-links and monoadducts concerns G1 and early S-phase cells. The *pso2-1* mutant is, however, also defective in G2 repair and loses diploid resistance when it is in the homozygous state.——The block in repair capacity in these novel mutants is discussed in relation to the three other repair pathways known to be involved in the repair of furocoumarins photo-induced lesions in yeast DNA.

THE repair of DNA interstrand cross-links and monoadducts induced by photo-addition of furocoumarins of the psoralen type has been reported in several organisms (for review, see SCOTT, PATHAK and MOHN 1976). In *Escherichia coli*, the excision-resynthesis as well as the post-replicative repair systems are involved in the repair of such DNA lesions (COLE, LEVITON and SINDER 1976; SINDER and COLE 1978; COLE *et al.* 1978).

In *Saccharomyces cerevisiae*, the excision-resynthesis and the mutagenic error-prone pathways (named, respectively, *rad3* and *rad6* types by the prominent loci in each group of genes that govern these two pathways) are implicated in the repair of such furocoumarin photo-induced lesions (AVERBECK and MOUSTACCHI 1975; AVERBECK, MOUSTACCHI and BISAGNI 1978). Mutants blocked in the former pathway are UV-sensitive, whereas mutants blocked in the latter pathway are both UV and X-ray-sensitive (for review, see HAYNES *et al.* 1978). More recently, it was shown that the third major repair pathway, which has been genetically characterized in yeast and governs X-ray sensitive (*rad50* type), is also involved in the repair of psoralen derivative photo-induced damage (HENRIQUES and MOUSTACCHI 1980). Consequently, the overall repair of both

radiation and furocoumarin photo-induced damage share some common step(s). However, given that these treatments induce different DNA lesions and that multiple *rad* mutants are still able to repair a substantial amount of sporalen derivative photo-induced damage, the possibility of an additional pathway uniquely involved in the repair of such lesions may be raised. Moreover, it has been suggested that in Fanconi's anemia, an autosomal recessive disease in man, cells have a specific defect in the repair of DNA interstrand cross-links induced by drugs such as bi-functional mitomycin (SASAKI 1975; FUGIWARA, TATSUMI and SASAKI 1977).

It was the aim of our investigation to look for such a pathway in an organism suitable for genetic analysis by attempting to isolate mutants deficient in it. Mutants were isolated by selecting for photo-sensitivity after treatment with the bi-functional furocoumarin 8-methoxypsoralen (8-MOP) of a population of haploid *Saccharomyces cerevisiae* mutagenized by ethyl methanesulfonate.

The characterization of three of these mutants, called *pso*, is reported here. Genetic analysis indicates that they probably represent three distinct genetic loci.

MATERIALS AND METHODS

Strains: The novel mutants sensitive to 8-MOP photoaddition (or *pso*) were derived from N123 (**a** *his1*). R. C. VON BORSTEL provided *rad1–18*, *rad3–12*, *rad6–1*, *rad9–1*, *rad18–1*, *rad50–1*, *rad51–1*, *rad52–1*, *rad53–1*, *rad54–1*, *rad55–1*, *rad56–1 and rad57–1*, which were all derived from crosses of the original mutants with the wild type *RAD* strain, XV–185–4c (**a** *ade2–1 arg4–17 his1–17 lys1–1 try5–48 hom3–10*). Strains *rad1–3*, *rad2–6*, *rad3–e5* and *rev1*, *rev2* and *rev3* were provided by E. MOUSTACCHI, B. COX, R. WATERS and J. LEMONTT, respectively.

For genetic analysis of meiotic segregation of the *pso* markers, strains 10018 (*α ade1*) and X-508 (*α ade3–59 ura1–1 leu1–171*) provided by G. MAGNI and F. FABRE, respectively, were used.

Media: We used liquid complete glucose medium (YEPD) (0.5% Yeast extract Difco, 2% bactopeptone Difco and 2% glucose) or complete glycerol medium (YEPG) (0.5% yeast extract Difco, 2% bactopeptone Difco, 3% (v/v) glycerol). For prototrophic selection of diploid strains, we used minimal medium (MM) (0.67% Bacto yeast nitrogen base without amino acids, 2% glucose and 4% Bacto Agar Difco). The sporulation media contained 1% potassium acetate, 0.1% Bacto yeast extract Difco, 0.05% glucose and 2% Bacto Agar Difco. Cells were plated on YEPD medium solidified by 2% Bacto Agar Difco.

Furocoumarins: We used a mono-functional furocoumarin 3-Carbethoxypsoralen, 3-CPs (M.W. 258) newly synthetized by E. BISAGNI, Orsay, France (QUEVAL and BISAGNI 1974; AVERBECK, MOUSTACCHI and BISAGNI 1978) and a bi-functional furocoumarin, 8-Methoxypsoralen, 8-MOP (M.W. 216) obtained from Sigma Chemical Company. The final concentration of the furocoumarins in the treated cell suspension was always 5×10^{-5} M, and the solutions were prepared as previously (AVERBECK and MOUSTACCHI 1975; AVERBECK, MOUSTACCHI and BISAGNI 1978).

Isolation of mutants sensitive to 8-MOP photo-addition (pso): Exponential-phase cells of strain N123 (*PSO+*), grown in liquid YEPD, were harvested, washed twice in pH 7.0 phosphate buffer (0.067 M), sonicated 20 sec and resuspended in phosphate buffer at a density of 1×10^7 cells/ml. The cells suspension was exposed to 3% ethyl methanesulfonate solution (v/v) for 50 min at 30° with aeration by shaking. The reaction was stopped by diluting a sample 1:10 in 10% sodium thiosulphate. Mutagenized cells were diluted and plated on YEPD so that there were 50 to 70 colonies per plate (50 min of treatment with EMS resulting 8% survival). After 3 days of incubation at 30°, the plates were replica-plated on YEPD medium containing 500 μg of 8-MOP per plate (approximately 10 μg/cm²) and were submitted to a spray of a solution of

8-MOP at 1 mg/ml. The plates were incubated for 1 hr in the dark and were replica-plated on complete medium (YEPD) for nonirradiated controls. The master plates containing 8-MOP were irradiated for 4 min (4.1 KJm^{-2}) with 365 nm light and, after irradiation, were replica-plated on YEPD without 8-MOP. The dose of 4.1 KJm^{-2} was chosen on the basis of preliminary experiments on normal *PSO+* and *rad1* mutant strains; the *rad1* mutant is known to be sensitive to 8-MOP photo-addition (AVERBECK and MOUSTACCHI 1975). Treatments of 4.1 KJm^{-2} have shown that wild-type irradiated colonies were indistinguishable from controls; whereas, *rad1* irradiated colonies did not grow. All plates were incubated at 30° for 2 to 3 days. The few colonies that did not grow or that grew poorly after irradiation on complete medium plates were considered as putative *pso* mutants. The corresponding colonies were subcloned from control plates for establishment of survival curves. The presumptive *pso* mutants were plated on glycerol medium (YEPG) to select against cytoplasmic "petites" induced by 8-MOP plus 365 nm light; the "petite" mutants are known to be more sensitive to photo-addition of furocoumarins than the respiratory-competent cells (AVERBECK and MOUSTACCHI 1975). Mutants sensitive to 8-MOP without irradiation or to 365 nm light alone were discarded. A total of 36 mutants demonstrating a range of sensitivity to 8-MOP plus 365 nm light was obtained in this manner out of approximately 5073 clones examined. Among the 36 mutants, 11 were sensitive to γ rays and 9 were sensitive to 254 nm UV irradiation. Four mutants belonging to these 2 classes demonstrated a cross-sensitivity to 8-MOP photo-addition, γ rays and UV. The allelism with *rad* mutants and number of complementation groups of *pso* mutants will be described elsewhere. Three of the mutants denominated *pso1-1*, *pso2-1* and *pso3-1* were further analyzed in detail.

Genetic procedures: The 3 *pso* mutants (***a his1***) were mated to a normal *PSO+* strain 10018 (*α ade1*) or X 508 (*α ade3-59 ura1-1 leu1-171*). Diploid strains obtained by prototrophic selection on minimal medium plates were submitted to sporulation. Tetrad analysis by micromanipulation was used to monitor gene segregation among the spore products of meiosis (MORTIMER and HAWTHORNE 1969). The auxotrophic markers and mating type of spores were determined by replica-plating; segregation of sensitivity or resistance to 8-MOP photo-addition was monitored as follows: samples of each spore clone in stationary phase of growth were suspended in saline to obtain about 1×10^6 cells/ml and were treated with 8-MOP at a final concentration of 10 μg/ml for 15 min at 4° in the dark. Drops of treated and untreated suspensions containing about 1 to 2×10^4 cells were spotted on YEPD plates and were exposed to various doses of 365 nm light. After 2 days of incubation at 30°, *PSO+* strains form visible spots of growth on the media, whereas *pso* strains show little or no growth.

Complementation test: The mutants derived from strain N123 (**a** ***his1***) were originally available only in the **a** mating type. Monosporic *pso* clones of the *α* mating type were obtained from tetrads derived from crosses of the *pso* mutants with the normal *PSO+* strains, as described above (see RESULTS for the segregation of the *pso* marker). Complementation tests between the *pso* strains were performed by crossing *pso* strains of opposite mating type and bearing complementary auxotrophic markers on solid complete medium, and the diploid strains were obtained by prototrophic selection. For complementation tests between the 3 *pso* mutants and known radiation-sensitive mutants, the mutants were crossed with *rad* and *rev* mutants of opposite mating types; diploid strains were obtained either by prototrophic selection or by isolation of the individual zygotes by micro-manipulation. Sensitivity to 8-MOP plus 365 nm light of all diploid strains was scored by spot tests, as described above. For these tests, the appropriate homozygous *pso* diploid strains and homozygous diploid strains for a particular *rad* gene were included as controls for every test.

The sensitivity to various doses of UV or γ rays of diploid strains was also checked by spot tests.

Growth conditions for survival curves: Stationary-phase cultures were obtained by inoculating 5×10^6 cells of a liquid YEPD culture in stationary phase in 5 ml of YEPD medium. After 48 hr incubation at 30° with aeration by shaking, the cultures contained 2 to 4×10^8 cells/ml. Exponential-phase cultures were obtained by inoculating 5×10^5 cells of the same YEPD culture in stationary phase in 5 ml of YEPD medium. After 12 hr incubation in the same conditions, the cultures contained 2×10^7 cells/ml. Cells were harvested and washed twice with saline. Clumps were dissociated by sonication of the cell suspension for 30 sec in MSE 60 W ultrasonic desinte-

grator (needle probe No. 21198). The cell number and the proportion of budding cells were determined with a counting chamber. After sonication, all cultures, even in exponential phase of growth, contained less than 2% cell aggregates. For all treatments, cells in either stationary or exponential phase of growth were diluted to a concentration of 3×10^6 cells/ml in saline. In all experiments, aliquots of treated and untreated cells were plated on solid YEPD medium in triplicate. Plates were incubated in the dark at 30° for 3 to 5 days before counting surviving colonies.

Treatment with 8-MOP or 3-CPs plus 365 nm light: Cells were treated in the dark for 15 min at 4° with 8-MOP (10 μg/ml) or 3-CPs (12.8 μg/ml). For irradiation, we used a Philips HPW 125 culot Edison high-pressure mercury lamp, with a maximal output at 365 nm and a dose rate of about 1.1 $KJm^{-2}min^{-1}$ as measured by a Black-ray J 221 long-wave ultraviolet intensity meter (Ultraviolet Products, Inc., San Gabriel, Calif.). A Pyrex glass filter insured that the contribution of wavelength below 340 nm was negligible.

Treatment with UV light: 10 ml cell suspensions at about 3×10^6 cells/ml were irradiated in agitated open petri dishes. The radiation source and its dosimetry are given in MOUSTACCHI (1969).

Treatment with γ rays: 1 ml cell suspensions at about 3×10^6 cells/ml cooled in ice were irradiated with γ rays emitted by a ^{60}Co source. Dosimetry was by ferrous sulfate solution. The dose rate was 0.97 Krads min^{-1}.

RESULTS

Phenotype of the haploid pso *mutants:* The survival curves of the three *pso* mutants of *Saccharomyces cerevisiae* in comparison to that of the original *PSO+* strain after treatment with bi-functional (8-MOP) and mono-functional (3-CPs) furocourmarins plus 365 nm light, 254 nm UV and γ radiations are given in Figures 1, 2, 3 and 4, respectively. Stationary-phase cells were compared to exponential-phase cells in order to determine the influence of the *pso* mutations on the established resistance of growing cells (for furocoumarins, see HENRIQUES *et al.* 1977; for UV, see ELKIND and SUTTON 1959 and CHANET, WILLIAMSON and MOUSTACCHI 1973; for ionizing radiation, see BEAM *et al.* 1954).

For stationary-phase cells, it can be seen that the three mutants are more sensitive than the *PSO+* strain to both 8-MOP and 3-CPs photo-addition. One mutant, *pso1–1*, is also sensitive to UV and γ rays, while the two other mutants have about the same sensitivity as the normal strain to these radiations. For exponential-phase cells, due to the resistance of a fraction of the population, survival curves with complex shapes are observed. In view of this complexity, it is necessary to describe the characteristics of each mutant in comparison to the normal strain.

(1) Normal PSO+ *strain:* As previously shown, in stationary phase the survival curve to 8-MOP plus 365 nm light has a shoulder, followed by a decline and a resistant tail (Figure 1, lower panel) (HENRIQUES *et al.* 1977). As already described for this normal strain (HENRIQUES *et al.* 1977), the exponential-phase cells are clearly more resistant than stationary phase cells and show a survival curve with a complex shape (Figure 1, upper panel).

In accord with previous reports (AVERBACK, MOUSTACCHI and BISAGNI 1978; HENRIQUES and MOUSTACCHI 1980), treatment of cells in stationary phase with 3-CPs plus 365 nm light leads to an exponential survival curve (Figure 2, lower panel). Cells in exponential phase of growth (Figure 2, upper panel) demon-

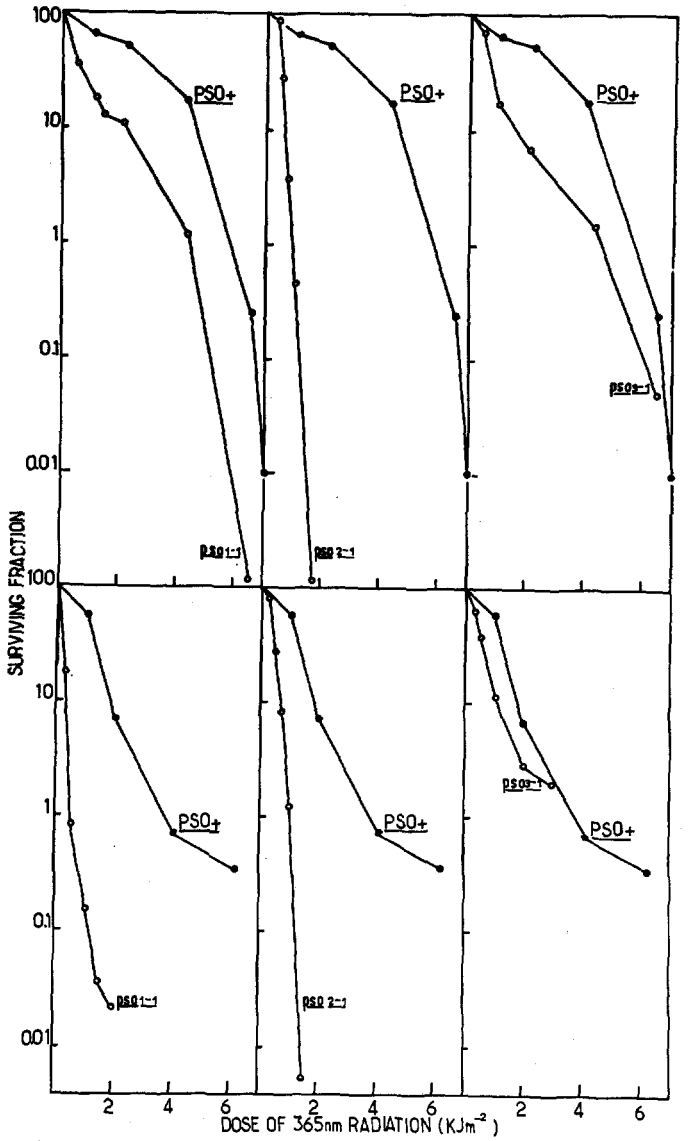

FIGURE 1.—Survival to 8-MOP plus 365 nm light treatment of the normal *PSO*+ strain (●) and *pso* mutants (○). Cells were treated either in exponential phase of growth (upper panel) or in stationary phase (lower panel).

strate a resistant component, as already described (HENRIQUES and MOUSTACCHI 1980).

After UV treatment, a slight inflexion in survival curves is systematically observed for exponential-phase cells (ELKIND and SUTTON 1959). In synchronized populations, cells at the end of S-G2 are about twice as resistant to UV treatment as cells in G1 (CHANET, WILLIAMSON and MOUSTACCHI 1973). How-

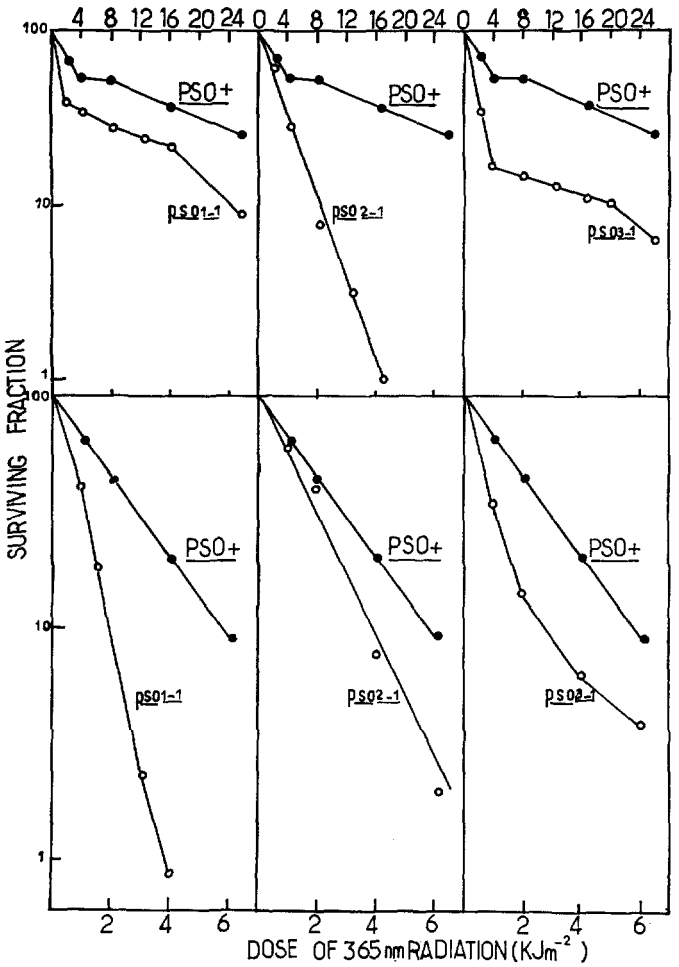

FIGURE 2.—Survival to 3-CPs plus 365 nm light treatment of the normal *PSO+* strain (●) and *pso* mutants (○). Cells were treated either in exponential phase of growth (upper panel) or in stationary phase (lower panel).

ever, in random cultures the difference in sensitivity of cells at different stages in the cycle is never as pronounced (Figure 3). All the points to define this inflexion are not shown in Figure 3, but this phenomenon has been seen systematically by us in other experiments using the same normal strain (N123) (MOUSTACCHI and ENTERIC 1970).

After γ-ray treatment, the resistant tail is clearly seen for exponentially growing cells (Figure 4, upper panel) (BEAM *et al.* 1954).

(2) Mutant pso2–1: is specifically sensitive to the photo-addition of mono- and bi-functional furocoumarins in both exponential and stationary phase (Figures 1 and 2). After 8-MOP photo-addition, the shoulder of the first part of the survival curves is maintained, but with a reduced magnitude compared to that of

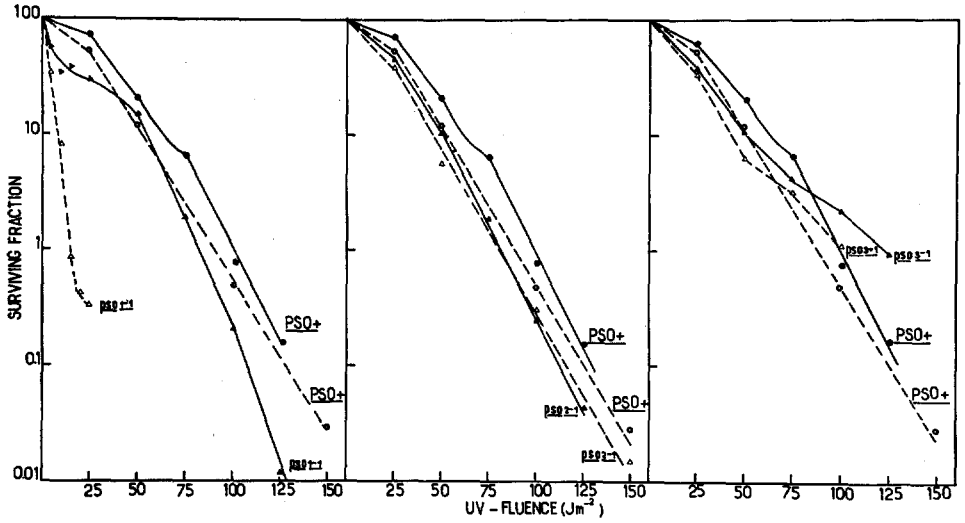

FIGURE 3.—Survival after 254 nm ultraviolet irradiation of the normal *PSO+* strain (●,○) and *pso* mtuants (▲,△). Full lines (—) correspond to cells treated in exponential phase of growth; dotted lines (- - -) correspond to cells treated in stationary phase.

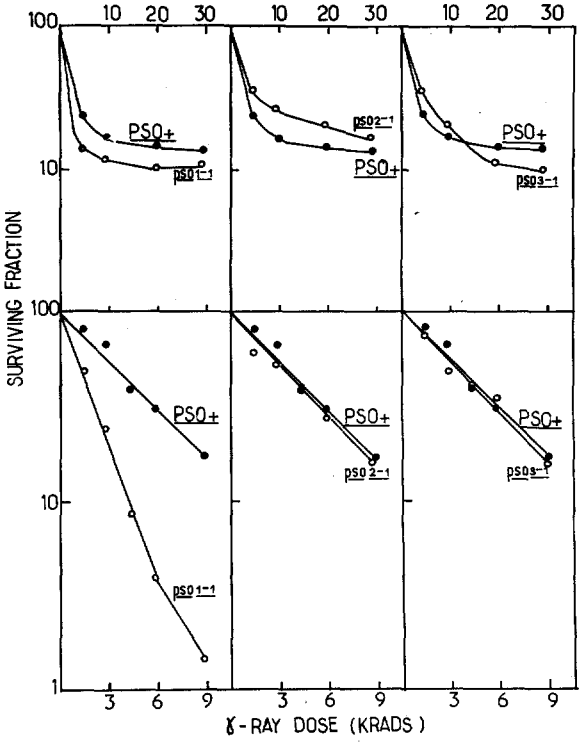

FIGURE 4.—Survival to γ-rays (^{60}Co) of the normal *PSO+* strain (●) and *pso* mutants (○). As in Figures 1 and 2, the upper panel refers to cells treated in exponential phase of growth and the lower panel to stationary phase cells.

the normal strain. The resistant component is abolished (Figure 1, upper panel). In contrast to *PSO*$^+$ strains, exponential-phase cells have the same sensitivity as stationary-phase cells.

After 3-CPs photo-addition, the survival curves are exponential in both growth phases, and the slope is steeper than that of the normal strain. Exponential-phase cells are more resistant than stationary-phase cells, but in *pso2–1*, as after 8-MOP photo-addition, there is no resistant component in the survival curve (Figure 2). In stationary-phase cells, the *pso2–1* mutant is about 2.5 times more sensitive than the *PSO*$^+$ strain to 8-MOP photo-addition (ratio LD_{10} mutant over *PSO*$^+$), and it is 1.6 times more sentitive to 3-CPs photo-addition (Figures 1 and 2; Table 1).

In stationary phase, the sensitivity to 254 nm UV and γ rays is normal (Figures 3 and 4; Table 1). It should be noted that *pso2–1* mutant, when treated in exponential phase of growth with γ rays, maintains, like *PSO*$^+$ cells, the resistant component related to the proportion of budding cells (Figure 4). This is in contrast to the response to furocoumarin photo-addition. In order to determine if after UV treatment a slightly resistant fraction is still present in *pso2–1*, experiments on synchronized populations remain to be done.

(3) Mutant pso3–1: is also specificially sensitive to the photo-addition of mono- and bifunctional furocoumarins (Figures 1 and 2). After 8-MOP photo-addition, the shoulder present in the first part of the survival curves of normal *PSO*$^+$ cells is abolished for stationary-phase cells, and then the slope of the linear part is similar to that of the *PSO*$^+$ strain (Figure 1, lower panel). The survival curve of exponential-phase cells has a complex shape (Figure 1, upper panel). However, the shoulder related to the resistant fraction in the growing population is less pronounced than that of the parental *PSO*$^+$ strain.

After 3-CPs photo-addition, the first exponential part of the survival curve has a steeper slope than that of the *PSO*$^+$ strain; the second resistant component is related to budding cells that were still present in *pso3–1* even in the stationary-phase culture treated (Figure 2, lower panel). Indeed, for some unknown reason, even after several days of incubation in complete growth medium, the *pso3–1* mutant still contains an appreciable number of budding cells. This may be due

TABLE 1

Survival characteristics of the pso *mutants after treatment in stationary phase of growth with 8-MOP or 3-CPs plus 365 nm light, UV radiation (254 nm) and γ-rays*

Strain	8-MOP + 365 nm light LD_{10} (KJm^{-2})	LD_{10} ratio *PSO*$^+$/*pso*	3-CPs + 365 nm light LD_{10} (KJm^{-2})	LD_{10} ratio *PSO*$^+$/*pso*	3-CPs LD_{10} / 8-MOP LD_{10}	UV LD_{10} (Jm^{-2})	UV LD_{10} ratio *PSO*$^+$/*pso*	γ-rays LD_{37} (Krads)	LD_{10} ratio *PSO*$^+$/*pso*
N123	1.9	1.0	5.7	1.0	3.0	52.5	1.0	4.5	1.0
pso 2–1	0.75	2.5	3.65	1.6	4.9	42.5	1.2	4.5	1.0
pso 3–1	1.1	1.7	2.75	2.1	2.5	45	1.2	4.5	1.0
pso 1–1	0.3	6.3	1.9	3.0	6.3	8.75	6.0	1.6	2.8

either to a block in a late step of nuclear division associated to the *pso3–1* mutation or to independent cells held together by cell-wall material. In exponential phase of growth, the resistant component of the *pso3–1* population has the same sensitivity as that in the *PSO*+ population (Figure 2, upper panel).

The sensitivity of *pso3–1* to UV and γ rays is in the same range as that of the *PSO*+ strain in both growth phases (Figures 3 and 4; Table 1).

(4) Mutant pso1–1: exhibits in stationary-phase of growth a pronounced sensitivity to all four agents: 8-MOP, 3-CPs, UV and γ rays (Figures 1, 2, 3 and 4; Table 1). After treatment with 8-MOP plus 365 nm light of stationary-phase cells, the shoulder in the initial part of the survival curves is abolished, and the slope of the linear part is steeper than that of the *PSO*+ strain. Cells of the *pso1–1* mutant treated in exponential phase are much more resistant than stationary-phase cells (Figure 1), but still maintain the sensitivity compared to *PSO*+ cells. In this condition of growth, the survival curve has a complex shape. The first part of the curve is exponential and is followed by a resistant fraction related to the proportion of budding cells.

After 3-CPs photo-addition, stationary-phase cells are clearly more sensitive than *PSO*+ cells (Figure 2, lower panel). In exponential-phase cells, the resistant component is present, and the response of this fraction of the population is about the same as that in the *PSO*+ strain (Figure 2, upper panel).

After UV treatment, *pso1–1* in stationary phase is very sensitive and demonstrates an exponential survival curve. The slight inflexion in UV survival curves observed for *PSO*+ exponential-phase cells is persistant in the *pso1–1* mutant in the same phase of growth. Due to the high sensitivity of cells in G1, the tail in the survival curve due to budding cells is more pronounced than that in *PSO*+ strain (Figure 3).

Interestingly enough, in exponential phase of growth this mutant has a normal sensitivity to γ rays, whereas in stationary phase (G1 cells) the mutant is more radiosensitive than the *PSO*+ strain (Figure 4; Table 1).

Genetic analysis of the pso *mutations:* Segregation of the mutations for sensitivity to 8-MOP photo-addition was analyzed in crosses between the three *pso* mutants and a *PSO*+ strain of opposite mating type. Table 2 shows a regular 2:2 segregation of the resistant and sensitive characters for the *pso* genes studied. With a few exceptions that always occur in yeast tetrads, segregation of the nutritional markers was normal. We found that no ascospore clone was affected by 365 nm light alone or by 8-MOP treatment without irradiation.

Response of homozygote and heterozygote diploid strains to 8-MOP photo-addition: The dominance relationship of the *PSO*+ character was tested on homozygotes diploid cells in stationary phase. Haploid *PSO*+ cells are known to be slightly more sensitive to 8-MOP photo-addition than are homozygote *PSO*+ diploid cells (HENRIQUES *et al.* 1977). As seen in Figure 5, this difference in sensitivity is manifested in a reduction in magnitude of the shoulder in haploid survival curves, the final slope being about the same in haploid and diploid cells.

Diploid cells homozygous for *pso2–1* have about the same response to 8-MOP photo-addition as haploid *pso2–1* mutant cells (Figure 5). In other words, the

TABLE 2

Segregation of the pso *mutation (sensitivity to 8-MOP photo-addition)*

Crosses	Number of asci analyzed	Segregation of *his1* 3+:1—	2+:2—	Segregation of *ser1–171* 2+:2—	Segregation of *pso* 3 *PSO*+: 1 *pso*	2 *PSO*+: 2 *pso*
pso 2–1 × 10018	6		6	–		6
pso 3–1 × 10018	4	1	3	–		4
pso 1–1 × 10018	9		9	–		9
pso 2–1 × X 508	4		–	4	1	3
pso 3–1 × X 508	4		–	4		4
pso 1–1 × X 508	5		–	5		5

A qualitative test for the sensitivity to 8-MOP photo-addition of the monosporic clones was used as follows: samples of each spore clone were suspended in saline (about 1×10^6 cells/ml) were treated in the dark for 15 min at 4° with 10 μg/ml of 8-MOP. Aliquots containing approximately 1 to 2×10^4 cells were spotted on YEPD plates and were exposed to various doses of 365 nm light. After two days incubation, the cellular density of the spots was examined. Genotypes of the hybrids (*pso* × 10018) **a** *his1 pso* × α *ade1*; (*pso* × X 508) **a** *his1 pso* × α *ade3–59 ura1 ser1–171*.

The segregation of only *his1* and *ser1–171* markers are presented to illustrate normal meiotic events ; the other markers were also tested and all asci demonstrated a 2:2 segregation.

PSO+ refers to the wild-type response and *pso* to sensitivity to 8-MOP photo-addition.

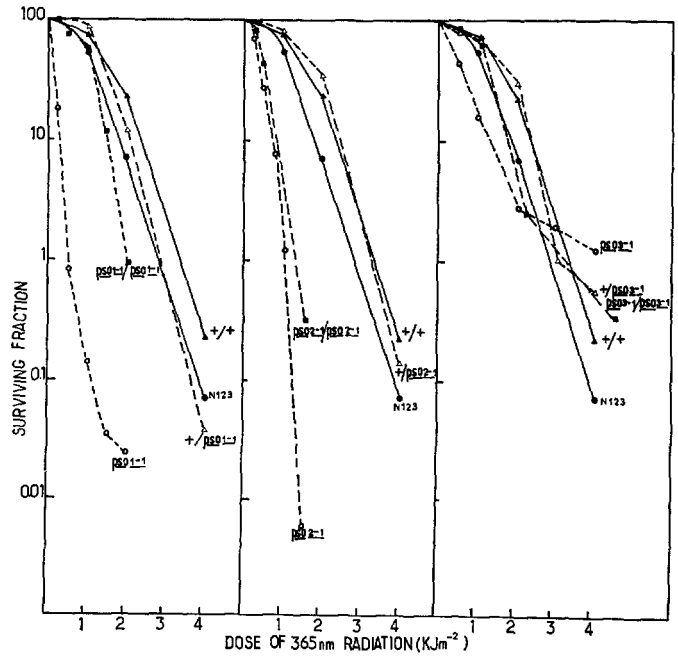

FIGURE 5.—Survival to 8-MOP plus 365 nm light treatment of haploid *PSO*+ strain N123 (●) and *pso* mutants (○) in comparison to diploid homozygous *PSO*+ strains (▲, +/+) and *pso* (■, *pso/pso*) as well as heterozygous (△) diploid strains (+/*pso*).

"diploid resistance" appears to abolished by this mutation. The heterozygous diploid cells obtained by a cross between *pso2–1* and the *PSO*+ strain demonstrate about the same response as a homozygous *PSO*+ diploid strain; consequently, the *pso2–1* gene is recessive.

Diploid cells homozygous for *pso1–1* are more resistant than the *pso1–1* haploid mutant; the "diploid resistance" is maintained. However, this strain is more sensitive than the *PSO*+ homozygous diploid strain, and both a reduction in the shoulder and a steeper slope of the linear part are observed.

The survival curve of the heterozygous diploid *pso1–1*/+ demonstrates the same shoulder as the homozygous *PSO*+ diploid strain and, for higher doses, leads to an intermediary response between a *PSO*+ haploid and diploid strain. Consequently, the *PSO*+ gene is either dominant or perhaps semi-dominant over the *pso1–1* gene.

The *pso3–1* homozygous and heterozygous diploid strains show the same pattern as *pso1–1* in comparison to the *PSO*+ strain. However, the difference in sensitivity between the strains is less pronounced, and the *pso3–1* gene is clearly recessive.

For the three mutations, homozygous diploid strains are more sensitive than heterozygous diploid strains. Consequently, the complementation test should permit the classification of the genes.

Complementation test: Complementation for the response to 8-MOP photo-addition was checked systematically. The response to UV or γ rays was also examined in most of the crosses with *rad* and *rev* mutants.

The mutants *pso2–1*, *pso3–1* and *pso1–1* all complement each other, indicating that they are in different complementation groups.

The three *pso* mutants complement *rad* mutants of the excision-resynthesis repair pathway (*rad1–18*, *rad1–3*, *rad2–6*, *rad 3–e5*), as well as with mutants belonging to the mutagenic error-prone repair pathway (*rad6–1*, *rad9–1*, *rad18–1*, *rev1–1*, *rev2–1* and *rev3–1*). The *pso* mutants also complement eight X-ray-sensitive *rad* mutants (*rad50–1*, *rad51–1*, *rad52–1*, *rad53–1*, *rad54–1*, *rad55–1*, *rad56–1* and *rad57–1*). Consequently, it is likely that the three *pso* mutants represent loci that are distinct from the *rad* and *rev* loci tested. However, the phenomenon of intragenic complementation being well established in yeast, before recombinational analysis is performed, complementation should be taken as only presumptive evidence that the *pso* mutants represent different loci.

Effect of the pso *genes on meiosis:* The effect of the *pso* mutations on sporulation ability was measured, since it is well established that DNA repair and recombination share some common steps. For instance, several *rad* mutants demonstrate alterations in sporulation capacity, accompanied by a deficiency in recombination ability (GAME and MORTIMER 1974; SAEKI, MACHIDA and NAKAI 1980). The sporulation efficiency of diploids homozygous for the *pso* genes was compared to that of the *PSO*+ diploid strain N123 × 10018 from which the **a** and α *pso* mutants were derived. Results are given in Table 3. Sporulation was very low in homozygous *pso3–1* diploid cells, and of the few asci observed contained only two spores. Sporulation was reduced in *pso1–1*, but was normal

TABLE 3

Effect of the pso *genes on sporulation*

Strain	% sporulation
N123 × 10018 (wild type)	49.5
pso1–1/pso1–1	24.0
pso2–1/pso2–1	43.8
pso3–1/pso3–1	3.8

The total number of cells counted in each sporulation culture was about 500.

TABLE 4

Summary of the phenotypic characteristic of the pso *mutants*

Treatment	8-MOP plus 365 nm light		3-CPs plus 365 nm light		UV (254 nm)		γ-rays	
Strain	Stationary phase	Resistant fraction in exponential phase	Stationary phase	Resistant fraction in exponential phase	Stationary phase	Resistant fraction in exponential phase	Stationary phase	Resistant fraction in exponential phase
N123 (*PSO*+)	N	+++	N	+++	N	+	N	+++
pso1–1	SSS	++	SSS	+++	SSS	+	SS	+++
pso2–1	SS	—	S	—	N	?	N	+++
pso3–1	S	+	S	+++	N	+	N	+++

N refers to the wild type response.
SSS, SS, S refer to decreasing degrees of sensitivity to the different treatments.
+++, ++, + refer to the presence of a resistant fraction in the exponential-phase population with a decreasing sensitivity to the different treatments.
— refers to the absence of a resistant fraction.

in *pso2–1* homozygous diploid strains. Differences of the same order were seen in diploid strains with different genetic backgrounds, indicating that the alterations observed for *pso3–1* and *pso1–1* are truly related to the *pso* homozygous state. The analysis of spore viability in connection with the study of the effect of the *pso* genes on meiotic recombination is presently being undertaken.

DISCUSSION

Table 4 summarizes the phenotypic characteristics of the three *pso* mutants as compared with the isogenic normal strain.

In order to test if some of the *pso* mutants were sensitive to furocoumarins due to differences in uptake or photo-addition of DNA, we determined these parameters using ^{3}H- or ^{14}C-labelled 8-MOP. This possibility was ruled out. Indeed, at equimolar concentration and equal doses of 365 nm UV, the uptake and fixation of 8-MOP on DNA per cell were the same in the three *pso* mutants and in strain N123 (data to be published elsewhere).

The mutant *pso1–1* demonstrates a cross-sensitivity of furocoumarin photo-addition and to UV or γ rays, while the two other mutants, *pso2–1* and *pso3–1*, are specifically sensitive to photo-addition of psoralen derivatives. From the meiotic analysis, it is clear than such sensitivity segregates as a Mendelian factor

in crosses of each of the mutants with a *PSO*$^+$ strain (Table 2). The three mutants complement each other and all the *rad* and *rev* mutants tested.

In terms of survival to the four types of treatments studied (Figures 1 through 4), the mutant *pso1–1* has qualitatively the same phenotype as that of mutants defective in the mutagenic pathway, *i.e.*, the *rad6* type (LAWRENCE and CHRISTENSEN 1976; LEMONTT 1971; PRAKASH 1976). Moreover, recent data obtained by us show that, like the *rad6* type of mutants, *pso1–1* demonstrates a considerable reduction in UV and 8-MOP plus 365 nm light-induced mutation frequencies as compared with *PSO*$^+$ strains (in preparation). Consequently, *pso1–1* appears to be blocked in the mutagenic pathway. However, this mutant is likely to represent a new locus since it complements with the mutants blocked in this pathway (*rad6*, *rad9*, *rad18*, *rev1*, *rev2* and *rev3*). This study of the *pso1–1 rad6* type of double mutants is presently undertaken in order to determine if *pso1–1* belongs to the *rad6* type epistatic group of genes.

The block in repair capacity in the *pso1–1* mutant essentially affects cells in the G1 phase of the cycle since the resistance of budding cells (S and G2 phase cells) to the four types of treatments is maintained (Figures 1 through 4, exponential phase of growth). This observation, taken together with the persistance of the "diploid resistance" in the *pso1–1* homozygous diploid strain (Figure 5) compared with the haploid mutant strain, indicates that a repair function(s) of the recombinational type is still present in the *pso1–1* mutant. The slight sensitivity of the *pso1–1/PSO*$^+$ heterozygous diploid in comparison to the homozygous *PSO*$^+$/*PSO*$^+$ diploid strain (Figure 5) and the reduction in sporulation ability of the *pso1–1* homozygous diploid strain (Table 3) may indicate that that this function is, however, slightly perturbed.

The mono-functional agent 3-CPs induces only mono-adducts in DNA after photo-addition, whereas the bi-functional derivative 8-MOP induces both DNA interstrand cross-links and mono-adducts (AVERBECK, MOUSTACCHI and BISAGNI 1978). From Figures 1 and 2, it can be seen that the *pso1–1* mutant is about 6.3 times more sensitive than the *PSO*$^+$ strain to 8-MOP and is three times more sensitive to 3-CPs after 365 nm irradiation (Table 1). This indicates that the *pso1–1* mutant is defective in the required enzymatic step(s) for the repair of both mono-adducts and cross-links. However, the lesions that seem to be predominantly lethal in *pso1–1* appear to be the cross-links, since the survival curve to the bi-functional derivative is exponential (Figure 1), in contrast to the shouldered survival curve observed for the *PSO*$^+$ strain. Moreover, the wild type is three times more resistant to the mono-functional agent 3-CPs than to 8-MOP whereas *pso1–1* demonstrates a six-fold higher resistance to 3-CPs than to 8-MOP (Table 1).

The *pso2–1* mutant is sensitive to psoralen-derivative photo-addition. The response to γ rays equals that of the normal strain in both exponential and stationary phases of growth (Figure 4). After UV treatment, the response of *pso2–1* is almost normal (Figure 3). The survival curve of stationary-phase cells demonstrates a reduced shoulder in comparison to that of the normal strain after 8-MOP photo-addition, and the dose-modifying factor at the 10% survival level

equals 2.5 (Figure 1 and Table 1). This indicates that cells in the G1 phase are affected in their repair capacity. However, the main feature of the *pso2–1* mutant consists in the loss of the resistant component in survival curves of haploid cells after 8-MOP or 3-CPs photo-addition (Figures 1 and 2). This is accompanied in homozygous diploid *pso2–1/pso2–1* cells by the disappearance of the "diploid resistance" characteristic of normal diploid cells. In other words, this strain appears to be defective in the repair process of the recombinational type that takes place in diploid cells and by the end of S and G2 phases of the cell cycle in normal haploid strains. It should be noticed, however, that such a deficiency does not cause a reduced sporulation (Table 4), suggesting that the *pso2–1* mutation controls a step specific for mitotic G2 repair. A similar concomitant loss of resistance to γ rays of budding cells and of diploids was found in certain *rad50* type of mutants; this was accompanied by a reduction in induced mitotic recombination (SAEKI, MACHIDA and NAKAI 1980) and ability to undergo almost normal meiosis (GAME and MORTIMER 1974).

The mutant *pso2–1* is about 2.5 and 1.6 times more sensitive than the normal strain, respectively, to 8-MOP and 3-CPs photo-addition. Like the *pso1–1* mutant, the *pso2–1* strain is defective in the repair of both cross-links and mono-addition. However, a certain repair capacity persists in this strain since it has an intermediary response when compared to *pso1–1* and *PSO*+ (for instance, see LD_{10} in Table 1). Moreover, it appears to repair the mono-functional lesions more efficiently than the cross-links, as seen from LD_{10} ratio for 3-CPs over 8-MOP photo-addition (LD_{10} 3-CPs/8-MOP equals 3.0 for the *PSO*+ and 4.9 for *pso2*).

The third mutant, *pso3–1*, is also specifically sensitive to 8-MOP and 3-CPs photo-addition. The response to UV and γ rays is normal. The resistance of the budding fraction in the haploid population, as well as that of homozygous *pso3–1/pso3–1* diploid cells, is maintained. In other words, as in *pso1–1*, the defect in repair capacity essentially concerns G1 and early S phase cells. The nature of the block is, however, different from that of *pso1–1* since not only did *pso3–1* not demonstrate a cross-sensitivity to UV and γ rays, but also recent experiments on induced mutagenesis demonstrate that it behaves differently from *pso1–1*. Compared to *pso1–1* and *pso2–1*, the mutant *pso3–1* is less sensitive to 8-MOP photo-addition (Table 1 and Figure 1), but is more sensitive than *pso2–1* to 3-CPs photo-addition (Table 1 and Figure 2). Like the two other mutants, *pso3–1* appears to be deficient in the repair of both cross-links and mono-addition.

A number, but not all, X-ray-sensitive mutants (*rad50* type) are also sensitive to furocoumarin photo-addition (HENRIQUES and MOUSTACCHI 1980). This suggests that the repair of X-ray damage and furocoumarin photo-induced damage may involve some common steps. However, since at least two mutants, *pso2–1* and *pso3–1*, confer sensitivity only to psoralen-derivative photo-addition, it indicates that some steps may be unique to furocoumarin photo-induced damage.

The mutation *pso1–1*, which shows UV, γ-ray and furocoumarin photo-addition sensitivity, may participate in the *rad6* error-prone pathway since mutants in this pathway are also sensitive to all three treatments. However, until further work is done with the *pso* mutants we cannot be certain of the role they actually

play in the repair of damaged DNA. In particular, a study of double mutants with genes belonging to each epistatic group along the three known pathways for dark repair of DNA in yeast and the direct biochemical analysis of repair in *pso* mutants, which is presently being undertaken, should shed some light on this matter.

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