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Interactions Between Mutations for Sensitivity to Psoralen Photoaddition (*pso*) and to Radiation (*rad*) in *Saccharomyces cerevisiae*

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The mode of interaction in haploid *Saccharomyces cerevisiae* of two *pso* mutations with each other and with *rad* mutations affected in their excision-resynthesis (*rad3*), error-prone (*rad6*), and deoxyribonucleic acid double-strand break (*rad52*) repair pathways was determined for various double mutant combinations. Survival data for 8-methoxypsoralen photoaddition, 254-nm ultraviolet light and gamma rays are presented. For 8-methoxypsoralen photoaddition, which induces both deoxyribonucleic acid interstrand cross-links and monoadditions, the *pso1* mutation is epistatic to the *rad6*, *rad52*, and *pso2* mutations, whereas it is synergistic to *rad3*. The *pso2* mutation, which is specifically sensitive to photoaddition of psoralens, is epistatic to *rad3* and demonstrates a nonepistatic interaction with *rad6* and *rad52*. *rad3* and *rad6*, as well as *rad6* and *rad52*, show synergistic interactions with each other, whereas *rad3* is epistatic to *rad52*. Consequently, it is proposed that *PSO1* and *RAD3* genes govern steps in two independent pathways, the *PSO1* activity leading to an intermediate which is repaired via the three independent pathways controlled by *RAD6*, *RAD52*, and *PSO2* genes. Since *pso1* interacts synergistically with *rad3* and *rad52* and epistatically with *rad6* after UV radiation, the *PSO1* gene appears to belong to the *RAD6* group. For gamma ray sensitivity, *pso1* is epistatic to *rad6* and *rad52*, which suggests that this gene controls a step which is common to the two other independent pathways.

The resistance of the yeast *Saccharomyces cerevisiae* to radiation is affected by mutations (*rad*) in a large number of different loci (12, 23). Double mutants containing various pairwise combinations of alleles have been used for comparison with single mutants to determine whether the interaction among the genes is epistatic, additive, or synergistic (4, 6, 8, 10, 11, 15, 17, 19, 24, 26, 27). It has been argued that epistasis with respect to UV light, X-ray, or chemicals means that the two loci control steps in the same repair pathway, whereas synergism indicates that the loci control steps in different repair pathways that affect the same lesions. Additivity would result if two mutations conferred sensitivity by independent mechanisms acting on different substrates (4, 6). By this genetic method, the *rad* mutants have been classified into three epistatic groups of loci. Genes in these groups affect three distinct modes of DNA repair named for a prominent locus in each. Loci of the *RAD3*, *RAD6*, and *RAD52* groups are known to govern, respectively, excision of UV radiation-induced pyrimidine dimers, error-prone repair, and DNA double-strand break repair. Loci of the *RAD52* group also affect recombination (29).

Mutants blocked in any of the three major *rad* repair pathways demonstrate a higher sensitivity than does the corresponding wild type to psoralen derivatives plus 365-nm irradiation (2, 13). Recently, a new class of *S. cerevisiae* mutants sensitive to photoaddition of psoralen derivatives (*pso*) has been isolated (14). The three *pso* mutations analyzed segregate in meiosis like Mendelian genes, they are recessive, and they complement each other as well as *rad* mutations. They consequently define new complementation groups.

A question that arises is whether the *pso* loci control steps in any of the already characterized *rad* repair pathways or whether they define an independent set(s) of metabolic stages in repair. In this paper, the interaction of two *pso* genes with each other as well as with the prominent *rad* loci in each of the three epistatic groups is

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described. The sensitivities to 8-methoxypsoralen (8-MOP) photoaddition, 254-nm UV light, or gamma rays of different double mutant combinations are compared with those of single mutants obtained from the same cross. In combination with 365-nm radiation, 8-MOP, a bifunctional psoralen derivative, is known to induce both DNA interstrand cross-links and monoaddition on pyrimidine bases (33).

MATERIALS AND METHODS

Media. The medium used contained: 0.5% yeast extract (Difco Laboratories), 2% peptone (Difco), and 2% dextrose. For plates, the medium was solidified with 2% agar (Difco).

Strains. Strains carrying the mutant alleles *pso1-1* and *pso2-1* were isolated in our laboratory as derivatives of strain N123 (a *his1*) or as monosporic clones from crosses between strains carrying the *pso* original mutants and strain 10018 (α *ade1*) (14). The strains carrying the *rad3-12* and *rad6-1* alleles were provided by R. C. Von Borstel (Von Borstel strain XV423-2a and Y014-21C), the strain carrying the *rad2-6* mutation was provided by B. Cox (Cox and Parry *uvs2*, mutant derivative of 197/2D), the strain with the *rad52-1* genotype was provided by F. Fabre (Fabre 1152, Berkeley Stock Center, originally isolated by M. Resnick).

The haploid segregants described below derive from sporulation and tetrad analyses from diploids of the genotypes described in Table 1.

Genetic procedures. The haploid strains were prepared by inducing sporulation of diploids that were heterozygous for *pso* and *rad* mutations and dissecting the tetrads.

Since sensitivity of mutants with *rad pso* mutations can vary in different strains owing to differences in genetic background, all comparisons were performed with haploid segregants of tetrads from the same cross. In each cross, 6 to 12 four-spored asci were dissected, and the phenotypes of all monosporic clones were determined by spot tests for sensitivity to 8-MOP photoaddition, 254-nm UV radiation, and gamma rays. Asci with tetratypes were chosen for the following analysis. Strains of spores carrying single and double mutations were classified by crossing each spore clone against strains of both mating types carrying single mutations and scoring for the presence or absence of complementation.

8-MOP treatment and irradiation. The 365-nm and 254-nm UV light sources, as well as the Cobalt 60 source for the gamma rays and the procedure for the determination of survival curves, were as described previously (14). Unless specified, stationary-phase cells (less than 2% budding cells) were used throughout this work. Before treatment either with 8-MOP (10 μg/ml) plus 365-nm light or with 254-nm UV light, the cells were washed and kept for 2 h in buffer at 4°C. This starvation procedure led to better reproducibility of survival curves. In most cases, for each cross several tetrads demonstrating a tetratype from the spot test were examined for survival curves, and the data reported for a given tetrad were obtained at least twice by using the same set of strains.

RESULTS

The survival of original strains with *rad* and *pso* mutations was compared with that of the haploid segregants analyzed below. Since there were negligible differences between the sensitivity of the *rad* and *pso* mutants and that of the single mutants classified according to complementation tests, the survival curves of the original mutants are not shown in the figures. For the different combinations of mutants, typical results for one ascus are presented. However, for each cross at least two different asci with tetratypes were examined, and each set of experiments for a given ascus was repeated at least twice.

Interaction between *pso1-1* and *pso2-1*. The data for survival after 8-MOP photoaddition for one tetrad derived from diploid 100 (*pso1-1* × *pso2-1*) are presented in Fig. 1. The double mutant carrying the *pso1-1 pso2-1* mutation had a sensitivity similar to that of a strain containing only the *pso1-1* mutation. In other words, an epistatic interaction was seen between these two loci.

TABLE 1. *Genotypes from which haploid segregants were derived*

Cross no.	Genotype
100	a *pso1-1* + *his1* + / α + *pso2-1* + *ade1*
200 and 201	α + / a *pso1-1* or *pso2-1* ; *rad3-12* / + ; *his1-7* / + ; *hom3-10* / + ; *ade2-1* / + ; *lys1-1* / + ; *trp5-48* / + ; + / *his1*
205 and 206	α + / a *pso1-1* or *pso2-1* ; *rad2-6* / + ; *ade2-1* / + ; + / *his1-1*
300 and 301	α + / a *pso1-1* or *pso2-1* ; *rad6-1* / + ; *ade2-1* / + ; *his1-7* / + ; *hom3-10* / + ; *leu1-12* / + ; *lys1-1* / + ; *trp5-48* / + ; + / *his1*
400 and 401	α + / a *pso1-1* or *pso2-1* ; *rad52-1* / + ; *his5* / + ; *lys1-1* / + ; + / *his1*

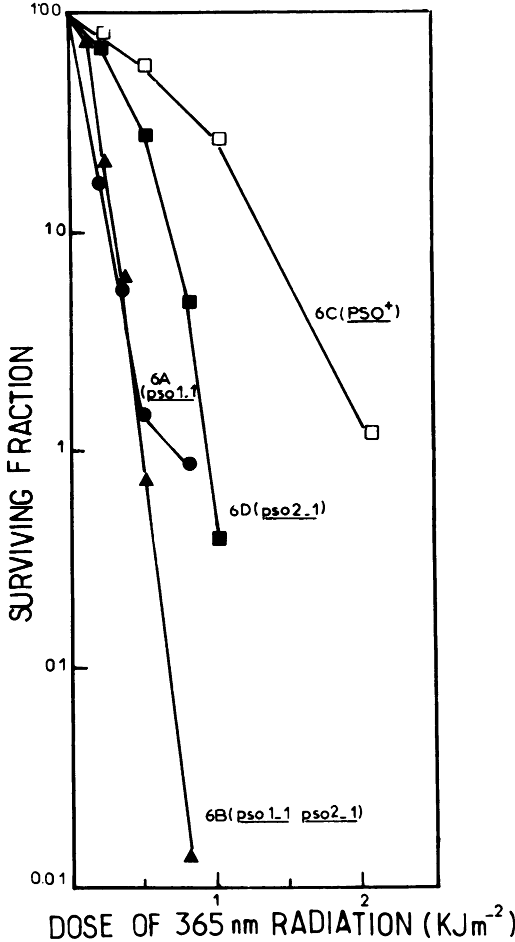

FIG. 1. *Interaction between pso1-1 and pso2-1 mutations. Survival after 8-MOP (10 μg/ml) photoaddition of PSO⁺ (□), pso1-1 (●), pso2-1 (■), and pso1-1 pso2-1 (▲) haploid segregants derived from cross 100. (The nomenclature of haploid strains includes the number of the diploid strains from which each is derived. Thus, 100/4C is spore C of the fourth tetrad dissected from diploid 100.)*

Interaction between *pso1-1* and *rad* mutations. As previously shown, the *pso1-1* mutation is sensitive not only to the photoaddition of psoralen derivatives but also to 254-nm UV and ionizing radiations (14). The interaction of *pso1-1* with *rad3*, *rad6*, and *rad52* mutations, which are representative of the three major repair pathways, is presented in Fig. 2 and 3 and Table 2.

(i) Survival after 8-MOP photoaddition. The sensitivity of the double mutant containing *pso1-1 rad3-12* was clearly higher than that of the most sensitive single mutant, that is, the excision-defective *rad3-12* mutation (Fig. 2A). This was confirmed with another mutant, *rad2-6*, also located on the excision pathway. This strain was more resistant than was the mutant carrying *rad3-12* to 8-MOP photoaddition but was clearly more sensitive than was the strain containing *rad3-12* to 254-nm UV radiation (data not shown).

On the other hand, the double mutants with *pso1-1 rad6-1* or *pso1-1 rad52-1* demonstrated the same sensitivity to 8-MOP photoaddition as did the most sensitive single mutants (Fig. 2B and C). The strain containing the *rad6-1* mutation, blocked in the error-prone pathway, had the same sensitivity as did the one carrying *pso1-1* (Fig. 2B), whereas the *rad52-1* mutation, leading to a block in the repair of induced DNA double-strand breaks, had a higher resistance than did *pso1-1* (Fig. 2C).

In summary, a synergistic interaction was found between the mutation *pso1-1* and mutations blocked in the excision repair pathway (*rad3-12* and *rad2-6*), whereas the *pso1-1* mutation showed an epistatic interaction with mutations that result in blocks either in the mutagenic (*rad6-1*) or in the recombinational (*rad52-1*) repair pathways.

(ii) Survival after 254-nm UV radiation. Double mutants carrying *pso1-1 rad3-12*, *pso1-1 rad2-6* (data not shown), and *pso1-1 rad52-1* were all more sensitive to UV radiation than were strains carrying the corresponding single mutants (Fig. 3A and C). This indicates that for 254-nm UV-induced lesions, *pso1-1* was involved in an epistatic group which was different from the *rad3* or *rad52* group.

The data for survival after UV radiation for one tetrad derived from diploid 300 (*pso1-1* × *rad6-1*) are shown in Fig. 3B. If the haploid segregants were treated in the stationary phase, as was done throughout this work, the double mutant *pso1-1 rad6-1* had a higher sensitivity than did the single mutants (Fig. 3B). However, since the reductions in survival brought about by UV radiation for both the haploid segregant carrying *rad6-1* (spore 5A) and for the parental strain carrying *rad6-1* were, in such a condition, much less than would be expected from published data, we suspected that this mutation was suppressed. Indeed, the *rad6-1* allele is known to be susceptible to different types of suppression (16, 18, 34). Interestingly enough, when the comparison was performed by treating exponential-phase cells with UV radiation, the haploid segregant 5A as well as the parental strain carrying *rad6-1* demonstrated the high sensitivity expected from the literature. Moreover, the double mutant containing the *pso1-1 rad6-1* mutations showed the same sensitivity as the single mutant with *rad6-1*. Therefore, since in unsuppressed conditions *pso1-1* and *rad6-1* demon-

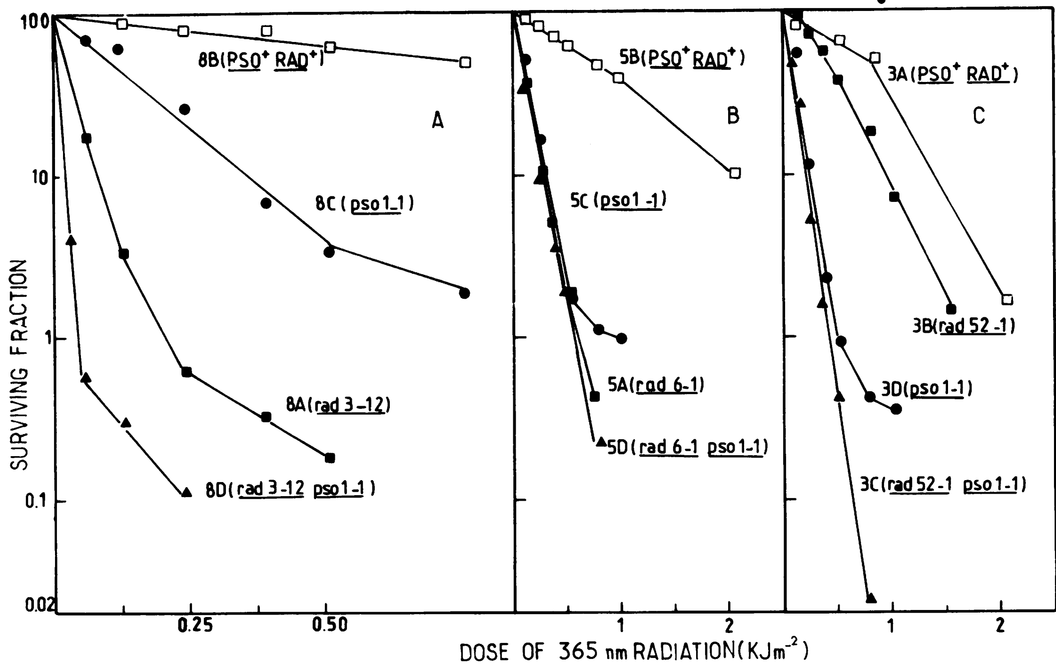

FIG. 2. *Interaction between pso1-1 and rad mutations blocked in the three major pathways. Survival after 8-MOP photoaddition of the haploid segregants derived: (A) from cross 200, PSO⁺ RAD⁺ (□), pso1-1 (●), rad3-12 (■), and the double mutant rad3-12 pso1-1 (▲); (B) from cross 300, PSO⁺ RAD⁺ (□), pso1-1 (●), rad6-1 (■), and the double mutant rad6-1 pso1-1 (▲); (C) from cross 400, PSO⁺ RAD⁺ (□), pso1-1 (●), rad52-1 (■), and the double mutant rad52-1 pso1-1 (▲).*

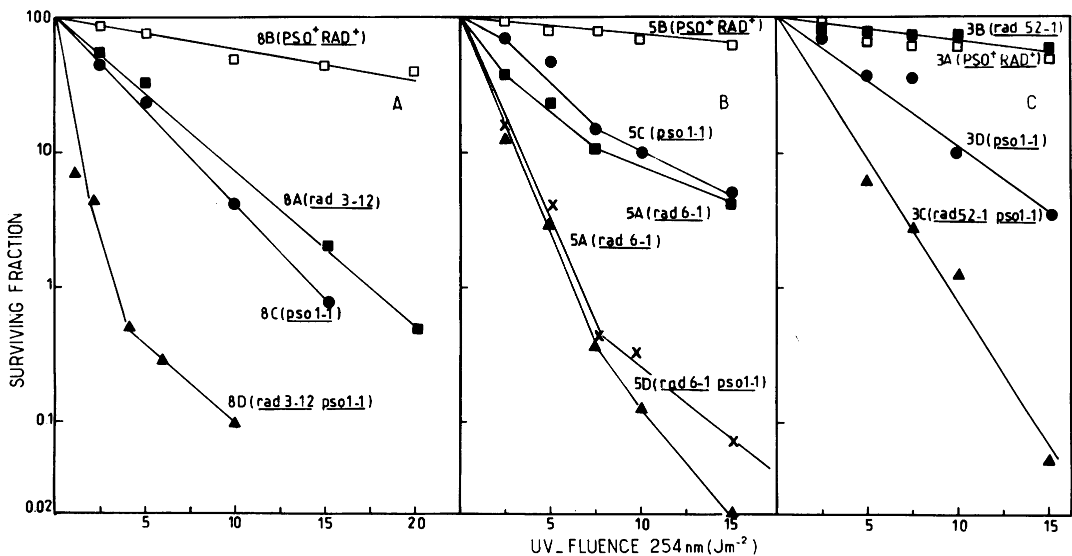

FIG. 3. *Interaction between pso1-1 and rad mutations blocked in the three major pathways. Survival to 254-nm UV radiation. The same tetrads as in Fig. 2. Symbols as in Fig. 2. In panel B, ■ refers, as in Fig. 2, to rad6-1 (monosporic clone 5A [spore A of the fifth tetrad]) treated in the stationary phase and × to the same strain treated in the exponential phase of growth.*

strated an epistatic interaction for the repair of UV-induced lesions, it is concluded that, as seen for 8-MOP photoaddition, the two genes are associated with the same pathway.

(iii) Survival after treatment with gamma rays. As a function of dose, exponential survival curves (one-hit) were observed for *RAD*$^+$, as well as for single and double mutant monosporic clones (data not shown). The Do values given in Table 2 demonstrate that none of the two double mutants was significantly more sensitive than the *pso1-1* single mutant taken from the same pedigree, although variation existed in the sensitivity of various monosporic clones containing RAD$^+$ or *pso1-1*. In other words, for gamma-ray-induced lesions, *pso1-1* demonstrated an epistatic interaction with *rad6-1* and *rad52-1*.

Interactions between mutants containing *pso2-1* and *rad*. The *pso2-1* mutation was specifically sensitive to photoaddition of psoralen derivatives; the responses to 254-nm UV and gamma rays of this mutant were similar to those of the isogenic wild type (14). Consequently, we studied the response of the double mutant containing *pso2-1* and *rad* mutations to only the photoaddition of 8-MOP.

TABLE 2. *Do[a] values in kilorads for haploid segregants from tetrads derived from diploids 300 (pso1-1/+ +/rad6-1) and 400 (pso1-1/+ +/rad52-1)*

Haploid strain	Do
5B (*RAD*$^+$)	4.05
5C (*pso1-1*)	1.80
5A (*rad6-1*)	2.25
5D (*pso1-1 rad6-1*)	1.65
3A (*RAD*$^+$)	6.90
3C (*pso1-1*)	1.80
3B (*rad52-1*)	4.80
3D (*pso1-1 rad52-1*)	1.65

[a] Do, Dose which leaves 37% survivors; i.e., corresponds to a mean of one lethal hit per cell.

The double mutant containing *pso2-1* and *rad3-12* was as sensitive as the most sensitive single mutant with *rad3-12* alone (Fig. 4A). The same was true in the *pso2-1 rad2-6* combination (data not shown). However, double mutants with the *pso2-1 rad6-1* (Fig. 4B) and *pso2-1 rad52-1* (Fig. 4C) mutations were more sensitive than were the corresponding most sensitive single mutants. Therefore, an epistatic interaction took place between *pso2-1* and a mutant blocked in the excision pathway. However, a nonepistatic interaction was observed between *pso2-1* and *rad6-1* or *rad52-1*.

Interaction between *rad* mutations with respect to 8-MOP photoaddition. A nonepistatic interaction between mutants blocked in the excision and the error-prone pathways was demonstrated for 254-nm UV radiation (16) and for photoaddition of psoralen derivatives (2, 3). The same was true for the *rad6-1* and *rad52-1* mutations (Fig. 5). However, an epistatic interaction was found between *rad3-12* and *rad52-1* after 8-MOP photoaddition (R. Chanet, personal communication).

DISCUSSION

Table 3 summarizes the interactions observed between two *pso* mutations as well as between *pso* and *rad* mutations which lead to blocks in

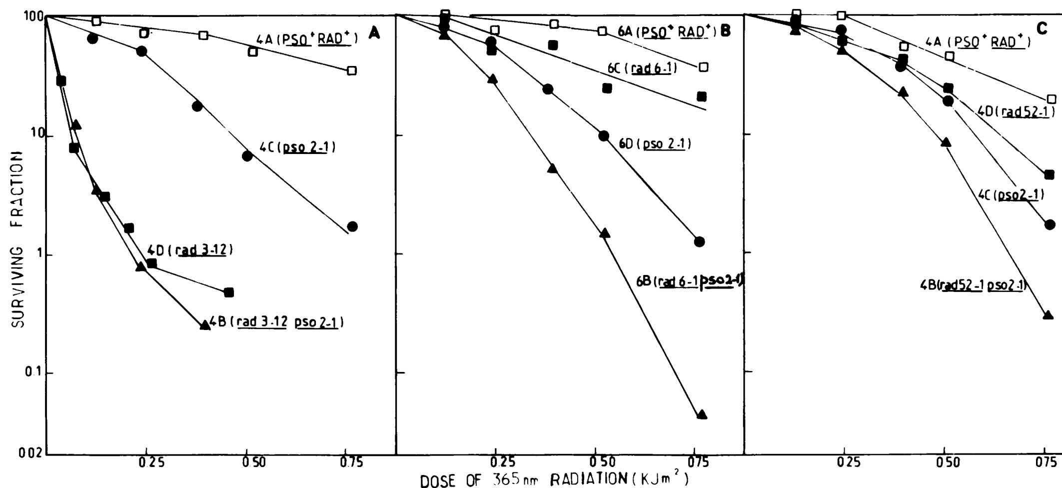

FIG. 4. *Interaction between pso2-1 and rad mutations blocked in the three major repair pathways. Survival after 8-MOP photoaddition of the haploid segregants derived: (A) from cross 201; (B) from cross 301, and (C) from cross 401. Symbols: wild type, □; pso2-1, ●; single rad mutants, ■; and double mutants rad pso2-1, ▲.*

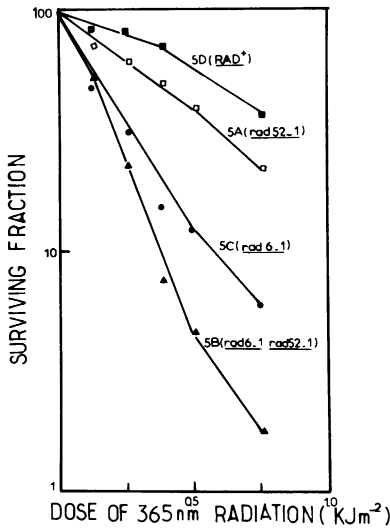

FIG. 5. *Interaction between rad6-1 and rad52-1 mutations. Survival after 8-MOP photoaddition of the haploid segregants derived from tetrad analysis of the diploid Y014-21C (rad6-1)/1152 (rad52-1). Symbols: RAD+, ■, rad52-1, □; rad6-1, ●; and the double mutant rad6-1 rad52-1, (▲).*

TABLE 3. *Qualitative nature of the interaction between various gene pairs with respect to sensitization of the double mutants to inactivation by 8-MOP photoaddition, 254-nm UV radiation, and ionizing radiation*[a]

Pairwise combination of mutants	Interaction for:		
	8-MOP photoaddition	UV (254 nm) radiation	X or gamma rays
pso1 pso2	E	—[b]	—
pso1 rad3	S	S	E
pso1 rad6	E	E	E
pso1 rad52	E	S	E
pso2 rad3	E	—	—
pso2 rad6	S	—	—
pso2 rad52	NE	—	—
rad3 rad6 types	NE (2)	NE or S (6–16)	E (4)
rad6 rad52 types	NE	S (10)	NE (21)
rad3 rad52 types	E[c]	E or S (10)	E (21)

[a] E, S, and NE: Epistasis, synergism, and nonepistasis (additivity or generally slightly higher sensitivity than strict additivity), respectively. For data from the literature (references in parentheses) the pairwise combinations of alleles used in the different studies belong to the *rad3*, *rad6*, or *rad52* classes of mutants blocked in the three major repair processes.

[b] —, Not tested.

[c] R. Chanet, personal communication.

the three major pathways that have been described in *Saccharomyces*. It also includes the interactions between repair-defective mutations that have been reported in the literature.

Pathways analysis based on the phenotypes of double and single mutants provides useful information. As mentioned earlier, such an analysis led to the identification of three major repair pathways in *S. cerevisiae* for radiation-induced lesions. However, before we discuss the information included in the actual work, it is worth recalling that several practical and theoretical difficulties in interpretation are encountered in this approach (6, 10, 17). These include variation among strains with the same *rad* allele, probably owing to modifier or suppressor genes, leakiness of the mutant alleles used, unusual substrates in repair-defective mutants leading to interactions which may not be present in wild-type cells, disturbance of the regulation of enzymes in radiation-defective mutants, etc. In particular, it should be stressed that the interactions of the epistatic type (Table 3) must be considered with caution at the moment. Indeed, it is conceivable that the accumulation of the initial substrate (damaged DNA) or of a repair intermediate, as a result of one mutation, completely inhibits a reaction in a separate pathway. Consequently, that mutation will be apparently epistatic to another locus which governs the inhibited reaction and those following it. Indeed, the three major epistatic groups are not entirely mutually exclusive. For instance, it has been found that certain genes which affect radiosensitivity, such as *rad14*, *rad50*, r_1^s and *cdc8*, appear to belong simultaneously to two or three of the different epistatic groups (12). Moreover, *rad6* and *rad18* are not epistatic with regard to X-ray sensitivity (11), whereas they are both members of the same UV-epistatic group.

In spite of these difficulties, at present it is generally accepted that there is no major contradiction in the definition of the three predominant epistatic groups of repair genes as defined by the double-mutant phenotypes and the biochemical and phenotypical properties of the mutants. This is particularly true for the *rad3*-type mutations assigned to the same epistatic group on the basis of the survival of double mutants (9, 28). All of those mutants which have been individually examined for excision of UV radiation-induced pyrimidine dimers (25, 28, 30–32, 35, 36) turned out to be defective in the removal of such lesions. The nature of the enzymatic defect(s) in these strains remains, however, unknown. Another common phenotype limited to this class of mutants is the exhibition of enhanced UV radiation-induced mutations (1, 7, 17, 22, 30, 37).

Keeping in mind both the usefulness and the limitations of the analysis of the survival of double mutants compared to single mutants, the interactions observed can be formally described as follows.

8-MOP photoaddition. For 8-MOP photoaddition, *pso1-1* and *rad3-12* or *rad2-1* demonstrat-

ing a nonepistatic interaction must have been involved in two independent pathways. The quantitative examination of Fig. 2A, especially of the first part of the curves, due to the most sensitive fraction of the population (cells in the G1 phase of the cell cycle), indicates that there was a synergistic interaction.

On the other hand, *pso1-1* was epistatic to *pso2-1* (Fig. 1), *rad6-1* (Fig. 2B), and *rad52-1* (Fig. 2C). These last two mutations demonstrated a nonepistatic interaction with each other (Fig. 4B and C), whereas *rad3-12* and *rad52-1* are epistatic (R. Chanet, personal communication). Consequently, the simplest interpretation is that the *PSO1-1* gene product may govern a step that produces a substrate which is then common to these three independent pathways (*PSO2*, *RAD6*, *RAD52*). It has already been established that double mutations blocked in the excision-resynthesis (*rad2*) and the error-prone (*rad9*) pathways exhibit an additive interaction for 8-MOP photoaddition (2). In other words, such mutants are associated with two independent pathways. We show here that *rad6-1* and *rad52-1* are synergistic for 8-MOP photoaddition: these two independent pathways may possibly act on a common substrate.

From Fig. 4A it is clear that *pso2-1* was epistatic to *rad3-12* (and *rad2-1*, data not shown): they were, consequently, associated with the same pathway. The genes which govern the excision repair probably defined an earlier step than *PSO2-1*. Indeed, it has recently been shown that mutants defective in incision of UV-induced pyrimidine dimers are also unable to remove psoralen photoinduced cross-links (W. J. Jachymzsick and R. C. Von Borstel, personal communication; N. Magana-Schwencke et al., submitted for publication). However, after 8-MOP photoaddition the *pso2-1* mutation performs this incision but is blocked in the restitution of high-molecular-weight DNA in sucrose gradient analysis (Magana-Schwencke et al., submitted for publication). It should be noted that *pso2-1* exhibits a wild-type response to 254-nm UV radiation treatment (14). This implies that the incision of UV radiation-induced pyrimidine dimers by *RAD3*-type gene products leads to a substrate which does not require the *PSO2* function for the achievement of the following repair steps.

Figure 6 schematically summarizes this interpretation of the data. 8-MOP photoaddition induced both interstrand cross-links and monoadducts in DNA. The actual data for the interaction between *pso* and *rad* genes do not allow a distinction between these two types of lesions. The analysis of the survival of the sets of tetrads examined in this work after treatment by a monofunctional agent such as 3-carbethoxypsor-

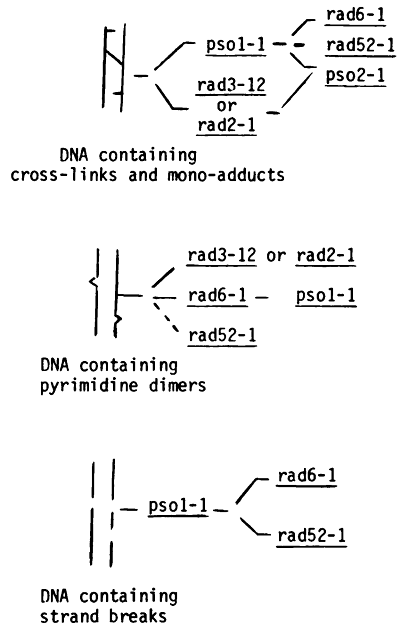

FIG. 6. *Illustration of the repair pathway situations which would account for the observations after treatment with 8-MOP plus 365-nm light (top), 254-nm UV radiation (middle), and gamma rays (bottom). An undetermined number of metabolic steps may take place along each pathway. The broken line indicates that rad52 is associated with a minor pathway for repair of UV radiation-induced lesions (6).*

alen (3, 20) will tell us, by comparison with the response to the bifunctional agent used here (8-MOP), (i) whether the two types of lesions are initially recognized as unique or distinct substrates and (ii) whether or not the gene interactions in subsequent steps remain the same as those observed here.

254-nm UV treatment. For 254-nm UV treatment, the *pso1-1* mutation demonstrated a synergistic interaction with *rad3-12* (or *rad2-1*) and *rad52-1*. Consequently, the *PSO1* gene was apparently associated with a pathway that was independent from the excision and the double-strand break repair pathways. An epistatic interaction was observed between the *pso1-1* and *rad6-1* mutations, provided that the comparison between single and double mutations was performed in conditions in which the *rad6-1* mutation was unsuppressed (i.e., by treating cells in the exponential phase of growth). In other words, the *PSO1* and *RAD6* genes apparently were associated with the same repair pathway, and this was true for UV and 8-MOP photoinduced lesions. It should be recalled that in both mutants the frequency of reversion and forward mutation induced by the two agents is very much reduced (5). This suggests that UV radia-

tion-induced lesions, as well as 8-MOP photoinduced cross-links and monoadducts, are subject to repair in the wild type by the same error-prone pathway. Why the suppressivity of *rad6-1* is dependent upon the growth phase and why this suppressivity is specific for the repair of UV radiation-induced lesions remain open questions.

Ionizing radiation damage. For ionizing radiation damage, several results are consistent with the existence of two main types of repair in *S. cerevisiae* (4, 11, 21). One type is defined by the *rad52* epistasis group and the other by the *rad6* epistasis group. The *pso1-1* gene was found to be epistatic to both the *rad6* and *rad52* genes. Unless the possible leakiness of the alleles used or their suppressivity masked a synergistic interaction, it is possible that, as in the case of 8-MOP photoaddition, the *PSO1-1* gene product acted at first on gamma ray-induced lesions, leaving a substrate(s) which was in turn repaired by the two independent pathways controlled by *rad6* and *rad52*.

The next question worth asking is what metabolic roles the *PSO* pathways perform. We can be reasonably confident that *pso2-1* governs a process of restitution of high-molecular-weight DNA after a cross-link incision of treated parental DNA (Magana-Schwencke et al., submitted for publication). We have earlier shown that the *pso2-1* mutation in G2 haploid cells and in homozygous diploid cells loses resistance to 8-MOP photoaddition (14). A similar defect, but in daughter DNA strands after 254-nm radiation treatment, has been observed for *rad18* (L. Di Caprio and B. S. Cox, personal communication). In the case of both *pso2-1* (5) and *rad18* (17) this is accompanied by a reduction in UV radiation-induced mutants. On the other hand, both *pso2-1* and *rad6-1* are mutationally defective after 8-MOP photoaddition (5). In other words, this process of restitution of high-molecular-weight DNA would be error-prone in wild-type cells.

The function of *pso1-1* is less easy to define. From Fig. 6 it appears that *pso1-1* may play a role as an alternative to the excision-resynthesis pathway. However, a primary difference in properties between the *pso1-1*- and the *rad3*-type mutations is that the former are mutationally defective (5), whereas the latter show enhanced mutability after 254-nm UV radiation or 8-MOP photoaddition. These uncertainties are likely to be resolved in the near future through the biochemical analysis of the fate of DNA-induced lesions in *pso1-1*.

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LITERATURE CITED

1. **Averbeck, D., W. Laskowski, F. Eckardt, and E. Lehmann-Brauns.** 1970. Four radiation sensitive mutants of *Saccharomyces*. Survival after UV- and X-ray-irradiation as well as UV-induced reversion rates from isoleucine-valine dependence to independence. Mol. Gen. Genet. **107**:117-127.
2. **Averbeck, D., and E. Moustacchi.** 1975. 8-Methoxypsoralen plus 365 nm light effects and repair in yeast. Biochim. Biophys. Acta **395**:393-404.
3. **Averbeck, D., E. Moustacchi, and E. Bisagni.** 1978. Biological effects and repair of damage photoinduced by a derivative of psoralen substituted at the 3,4 reaction site. Photoreactivity of this compound and lethal effect in yeast. Biochim. Biophys. Acta **518**:464-481.
4. **Brendel, M., and R. H. Haynes.** 1973. Interactions among genes controlling sensitivity to radiation and alkylation in yeast. Mol. Gen. Genet. **125**:197-216.
5. **Cassier, C., R. Chanet, J. A. P. Henriques, and E. Moustacchi.** 1980. The effects of three *PSO* genes on induced mutagenesis: a novel class of mutationally defective yeast. Genetics **96**:841-857.
6. **Cox, B. S., and J. C. Game.** 1974. Repair systems in *Saccharomyces*. Mutat. Res. **26**:257-264.
7. **Eckardt, F., and R. H. Haynes.** 1977. Induction of pure and sectored mutant clones in excision-proficient and deficient strains of yeast. Mutat. Res. **43**:327-338.
8. **Game, J. C., and B. S. Cox.** 1971. Allelism tests of mutants affecting sensitivity to radiation in yeast and a proposed nomenclature. Mutat. Res. **12**:328-331.
9. **Game, J. C., and B. S. Cox.** 1972. Epistatic interactions between four *rad* loci in yeast. Mutat. Res. **16**:353-362.
10. **Game, J. C., and B. S. Cox.** 1973. Synergistic interactions between *rad* mutations in yeast. Mutat. Res. **20**:35-44.
11. **Game, J. C., and R. K. Mortimer.** 1974. A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24**: 281-292.
12. **Haynes, R. H., L. Prakash, M. A. Resnick, B. S. Cox, E. Moustacchi, and J. B. Boyd.** 1978. DNA repair in lower eucaryotes: workshop summary, p. 405-411. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
13. **Henriques, J. A. P., and E. Moustacchi.** 1980. Sensitivity to photoaddition of mono and bifunctional furocoumarins of X-ray sensitive mutants of *Saccharomyces cerevisiae*. Photochem. Photobiol. **31**:557-563.
14. **Henriques, J. A. P., and E. Moustacchi.** 1980. Isolation and characterization of *pso* mutants sensitive to photoaddition of psoralen derivatives in *Saccharomyces cerevisiae*. Genetics **95**:273-288.
15. **Khan, N. A., M. Brendel, and R. H. Haynes.** 1970. Supersensitive double mutants in yeast. Mol. Gen. Genet. **107**:376-378.
16. **Lawrence, C. W., J. W. Stewart, F. Sherman, and R. Christensen.** 1974. Specificity and frequency of ultraviolet-induced reversion of an Iso-1-cytochrome *c* ochre mutant in radiation-sensitive strains of yeast. J. Mol. Biol. **85**:137-162.
17. **Lawrence, C. W., and R. Christensen.** 1976. UV mutagenesis in radiation-sensitive strains of yeast. Genetics **82**:207-232.
18. **Lawrence, C. W., and R. Christensen.** 1979. Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae rad 6* mutants.

J. Bacteriol. 139:866–876.

19. **Lemontt, J. F.** 1971. Pathways of ultraviolet mutability in *Saccharomyces cerevisiae*. I. Some properties of double mutants involving *uvs9* and *rev*. Mutat. Res. 13: 311–317.
20. **Magana-Schwencke, N., D. Averbeck, J. A. P. Henriques, and E. Moustacchi.** 1980. Absence de pontage inter-chaines dans l'ADN traité par le 3-carbéthoxypsoralène et une irradiation à 365 nm. C.R. Acad. Sci. (Paris) 291:207–210.
21. **McKee, R. H., and C. W. Lawrence.** 1980. Genetic analysis of γ-ray mutagenesis in yeast. III. Double-mutant strains. Mutat. Res. 70:37–48.
22. **Moustacchi, E.** 1969. Cytoplasmic and nuclear genetic events induced by UV light in strains of *Saccharomyces cerevisiae* with different UV sensitivities. Mutat. Res. 7:171–185.
23. **Moustacchi, E., R. Chanet, and M. Heude.** 1977. Ionizing and ultraviolet radiations: genetic effects and repair in yeast, p. 197–206. *In* A. Castellani (ed.), Research in photobiology. Plenum Publishing Corp., New York.
24. **Nakai, S., and S. Matsumoto.** 1967. Two types of radiation sensitive mutants in yeast. Mutat. Res. 4:129–136.
25. **Prakash, L.** 1975. Repair of pyrimidine dimers in nuclear and mitochondrial DNA of yeast irradiated with low doses of ultraviolet light. J. Mol. Biol. 98:781–795.
26. **Prakash, L.** 1977. Repair of pyrimidine dimers in radiation-sensitive mutants *rad3*, *rad4*, *rad6* and *rad9* of *Saccharomyces cerevisiae*. Mutat. Res. 45:13–20.
27. **Prakash, L.** 1977. Defective thymine dimer excision in radiation-sensitive mutants *rad10* and *rad16* of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 152:125–128.
28. **Prakash, L., and S. Prakash.** 1979. Three additional genes involved in pyrimidine dimer removal in *Saccharomyces cerevisiae*: *RAD7*, *RAD14* and *MMS19*. Mol. Gen. Genet. 176:351–359.
29. **Prakash, S., L. Prakash, W. Burke, and B. A. Montelone.** 1980. Effects of the *rad52* gene on recombination in *Saccharomyces cerevisiae*. Genetics 94:31–50.
30. **Resnick, M.** 1969. Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics 62:519–531.
31. **Resnick, M., and K. Seltow.** 1972. Repair of pyrimidine dimer damage induced in yeast by ultraviolet light. J. Bacteriol. 109:979–986.
32. **Reynolds, R. J.** 1978. Removal of pyrimidine dimers from *Saccharomyces cerevisiae* nuclear DNA under non-growth conditions as detected by a sensitive enzymatic assay. Mutat. Res. 50:43–56.
33. **Song, P. S., and K. J. Tapley.** 1979. Photochemistry and photobiology of psoralens. Photochem. Photobiol. 29: 1177–1197.
34. **Tuite, M. F., and B. S. Cox.** 1980. Ultraviolet mutagenesis studies of [*psi*], a cytoplasmic determinant of *Saccharomyces cerevisiae*. Genetics 95:611–630.
35. **Unrau, R., R. Wheatcroft, and B. S. Cox.** 1971. The excision of pyrimidine dimers from DNA of ultraviolet irradiated yeast. Mol. Gen. Genet. 113:359–362.
36. **Waters, R., and E. Moustacchi.** 1974. The disappearance of ultraviolet-induced pyrimidine dimers from the nuclear DNA of exponential and stationary phase cells of *Saccharomyces cerevisiae* following various post-irradiation treatments. Biochim. Biophys. Acta 353:407–419.
37. **Zakharov, I. A., T. N. Kozina, and I. V. Fedorova.** 1970. Effets de mutations vers la sensibilité au rayonnement ultraviolet chez la levure. Mutat. Res. 9:31–39.