

MUTATIONAL RESPONSE OF A DIPLOID *ps04-1* YEAST STRAIN DEFECTIVE IN ERROR-PRONE RECOMBINATIONAL DNA REPAIR

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ABSTRACT

The mutagenic response of diploid *Saccharomyces cerevisiae* cells homozygous for the *ps04-1* mutation was compared to that of the corresponding wild-type strain. The *ps04-1* diploid presented a marked reduction in the reverse mutation frequencies induced by 254 nm-UV radiation or by mono- and bifunctional furocoumarin photoaddition. For these different agents, the frequency of revertants was reduced within a range of high doses. It has been previously shown that, in addition to affecting the mutagenesis response, the *ps04-1* mutant is completely defective in both reciprocal (crossing-over) and non-reciprocal (gene conversion) mitotic recombination. These data indicate that the *PSO4* gene simultaneously controls the mutagenesis and recombination processes by acting on an error-prone recombinational repair pathway comparable to the SOS repair present in *Escherichia coli*.

INTRODUCTION

Even though the yeast *Saccharomyces cerevisiae* is quite suitable for the study of genetic aspects of DNA repair processes in eukaryotes, some points concerning the mode of action and the interaction of the different repair mechanisms present in this organism still need to be clarified. When compared to bacteria, the error-prone repair processes identified in yeast are not well understood but there are strong indications that they may be dissociated from recombinational events (for reviews, see Sied and Eckardt, 1984; Moustacchi, 1987).

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Starting with the pioneering work by Nakai and Matsumoto (1967), three major classes of radiation-sensitive mutants have been identified in yeasts, corresponding to three distinctive modes of DNA damage repair: a) excision-resynthesis repair-deficient mutants which are extremely sensitive to inactivation and to mutagenesis induced by 254 nm-UV radiation or by so-called UV-mimetic chemical agents (Haynes and Kunz, 1981; Wilcox and Prakash, 1981); b) recombination-deficient mutants, which are essentially involved in an error-free repair process that utilizes recombinational events, and are particularly sensitive to X-rays and to agents inducing double DNA breaks; and c) mutants having more than one error-prone repair pathway, which show a lower mutation frequency than the wild type at the same mutagen doses (for reviews, see Siede and Eckardt, 1984; Moustacchi, 1987; Friedberg, 1988).

Excision-resynthesis-deficient mutants (*RAD3* epistasis group), as well as mutants blocked in error-prone repair (*RAD6* epistasis group) present normal mitotic and meiotic recombination frequencies, except for *rad6-1* (Montelone *et al.*, 1981). In contrast, recombination-deficient mutants (*RAD52* epistasis group) present a normal mutagenic response (Game, 1983). These findings led to the suggestion that a dissociation exists between mutational ability and recombination proficiency in yeast, in contrast to what is observed for the *Rec A* mutants of *Escherichia coli* which are simultaneously blocked in mutagenesis and recombinogenesis.

The *ps04-1* mutant was first isolated by Benathen and Beam (1977) because of its sensitivity to X-rays. Later studies demonstrated that this mutant is extremely sensitive to the lethal effect of the photoaddition of the bi-functional furocoumarin 8-methoxypsoralen (8-MOP), especially when haploid cells are treated in the exponential growth phase. The haploid *ps04-1* mutant is also sensitive to mono- or bi-functional nitrogen mustards, it is slightly sensitive to UV radiation and shows nearly normal sensitivity to mono-functional furocoumarin 3-carbethoxypsoralen (3-CPs) photoaddition (Henriques *et al.*, 1989).

On the other hand, in the homozygous diploid condition, the *ps04-1* mutant is more sensitive than the wild-type strain to the photoaddition of mono- and bi-functional furocoumarins and to treatment with 254 nm-UV radiation and with mono- and bi-functional nitrogen mustards. This phenotypic response of the *ps04-1* diploid is much more marked when the mutant cells are treated during the exponential growth phase (Andrade *et al.*, 1989). In addition, the *ps04-1* mutant in the diploid homozygous state is completely blocked in both reciprocal (crossing-over) and non-reciprocal (gene conversion) recombinogenic events, induced by any agent tested (Andrade *et al.*, 1989).

All of these observations suggest that the repair process responsible for diploid recovery and for the resistance of exponentially growing cells is directly correlated with the occurrence of mitotic recombinational events which depend on the expression of the wild-type *PSO4* allele. In addition to playing a role in mitotic recom-

ination, the *PSO4* gene in the haploid state causes a generalized blockage of reverse mutation induction, as well as deficient induction of forward mutation after treatment with different genotoxic agents (Henriques *et al.*, 1989). Thus, it may be inferred that, in addition to having a recombinational effect, the product of the *PSO4* gene may also be involved in error-prone repair activity. However, blockage of error-prone repair activity mediated by the *PSO4* gene was observed only when these mutant cells were treated in the haploid state.

In view of these considerations, it became of interest to study the role of the *PSO4* gene in mutagenic events when treated in the diploid state. Thus, the induction of reverse mutation at the *his1-1* locus by 3-CPs and 8-MOP photoaddition and by 254 nm-UV radiation treatment was compared for the wild-type (*PSO4*) and mutant (*pso4-1*) diploid strains.

MATERIAL AND METHODS

Strains

The diploid wild-type strain *XS2316* previously described by Machida and Nakai (1980) was used, and its genotype is as follows:

+	<i>leu1-1</i>	<i>trp5-48</i>	+	+	+	<i>a</i>	<i>PSO4</i>	<i>his1-1</i>
o								
<hr style="width: 100%; border: 0.5px solid black;"/>	<i>ade6</i>	<i>leu1-12</i>	+	<i>cyh2</i>	<i>met13</i>	<i>lys5-1</i>	<hr style="width: 100%; border: 0.5px solid black;"/>	<i>α PSO4 his1-1</i>

and the diploid strain *HAH 1-1* was constructed for the present study and its genetic constitution is as follows:

+	<i>leu1-1</i>	<i>trp5-48</i>	+	+	+	<i>a</i>	<i>pso4-1</i>	<i>his1-1</i>
o								
<hr style="width: 100%; border: 0.5px solid black;"/>	<i>adeb</i>	<i>leu1-12</i>	+	<i>cyh2</i>	<i>met13</i>	<i>lys5-1</i>	<hr style="width: 100%; border: 0.5px solid black;"/>	<i>α pso4-1 his1-1</i>

Culture media

The following media were used: YEPD, a liquid medium consisting of 0.5% Difco yeast extracts, 2% Difco bacto-peptone and 2% glucose; minimal medium (MM) contained 0.67% Difco yeast nitrogen base without amino acids, 2% glucose and 3% Difco bacto agar; complete synthetic medium (SC), a medium of the same composition as MM and supplemented with 2 mg adenine, 5 mg lysine, 1 mg histidine, 3 mg leucine, 2 mg methionine and 2 mg tryptophan per 100 ml; omission medium (SC-histidine), SC medium in which the amino acid histidine was omitted.

Mutagens

Furocoumarins: Chromatographically purified 3-carbethoxypsoralen (3-CPs; molecular weight 258) was kindly provided by Dr. E. Bisagni. 8-Methoxypsoralen (8-MOP; molecular weight 216) (Sigma, St. Louis, MO, USA) was also used. After treatment with 365 nm-UV radiation (UVA), the bi-functional furocoumarin 8-MOP induces mono and bi-adducts (crosslinks) (for reviews, see Song and Tapley, 1979; Parsons, 1980; Averbeck, 1982). In contrast, the monofunctional furocoumarin (3-CPs) only forms monoadducts with pyrimidine bases (Averbeck *et al.*, 1978; Magana-Schwencke *et al.*, 1980).

Stock 3-CPs and 8-MOP solutions were prepared using 5.2 mg of the furocoumarins in 40% absolute ethanol in twice-distilled water, as described by Averbeck and Moustacchi (1975).

Growth conditions

Cells in the stationary growth phase were obtained from precultures maintained in liquid YEPD medium. Aliquots of approximately 2 to 3×10^6 cells/ml were inoculated in 10 ml YEPD medium and incubated for 48 hours at 28°C with aeration by shaking. The cultures thus maintained reached a concentration of 2 to 4×10^6 cells/ml, corresponding to the stationary growth phase. The cells were harvested, washed three times with saline (0.9% NaCl) at 4°C , and then sonicated for 15 seconds in a 100 w ultrasonic disintegrator (INPEC, São Paulo, Brazil). The cell concentration and the percentage of budding cells in each culture were checked with a microscope.

Treatment with furocoumarins

A suspension of cells (2×10^7 cells/ml) was incubated for 20 minutes at 4°C , with equimolar 5×10^{-5} M concentrations of mono- (3-CPs) and bi-functional (8-MOP) furocoumarins. After incubation, the cells were irradiated with an UVA radiation source as described by Averbeck *et al.* (1978) and Henriques and Moustacchi (1980b).

Treatment with 254-nm UV radiation

A suspension of cells (2×10^7 cells/ml) was irradiated with different UV doses at 254 nm, as described by Henriques and Moustacchi (1980b).

Measurement of survival and mutation induction after each treatment

The cells (2×10^7 cells/ml) treated with different mutagenic agents were concentrated by centrifugation to a density of 2×10^8 cells/ml and plated onto six dishes containing SC-his medium for the detection of HIS^+ revertants. To determine cell viability, part of the irradiated suspension was appropriately diluted with saline and plated onto SC medium.

The number of revertants and of viable colonies (survivors) was determined after incubation at 28°C for eight days. The experiments were repeated at least three times.

RESULTS

The mutagenic response of diploid *ps04-1* cells at the *his1-1* locus was compared with that of wild-type *PSO4* cells after treatment with mono- and bifunctional furocoumarins plus UVA or after exposure to 254-nm UV radiation.

Figures 1, 2 and 3 show the survival curves (upper panels) as well as the revertant frequencies (lower panels) as a function of dose (lower right panels) or survival (lower left panels) after treatment with the different genotoxic agents tested. As previously reported by Andrade *et al.* (1989), the *ps04-1* mutant in the homozygous diploid state presents marked sensitivity to the lethal effect of the three mutagens used.

Revertant induction at the *HIS 1-1* locus

As illustrated in Figures 1 through 3, furocoumarin photoaddition or exposure to 254-nm UV radiation induces mutations leading to the HIS^+ phenotype in the homozygous diploid *ps04-1/ps04-1* strain. However, the mutagenic response of this strain appears to be dose dependent. In the low-dose range, the frequency of induced revertants follows about the same kinetics as the wild-type diploid *PSO4* strain, whereas at higher levels, the average mutation frequencies remain constant and are significantly lower than in the wild-type *PSO4* strain (left panels in Figures 1 through 3).

On the other hand, when these frequencies are expressed as a function of survival, a strong reduction in the number of revertants induced in the *ps04-1* diploid strain is observed at all survival levels after treatment with these three mutagens (right panels in Figures 1 through 3). It should be noticed that in the haploid state the *ps04-1* strain shows a complete blockage of the induction of HIS^+ revertants after furocoumarin photoaddition or after exposure to 254 nm-UV radiation or to nitrogen mustards. These results are observed when the reverse mutation frequencies are expressed as a function of dose or of survival (Henriques *et al.*, 1989).

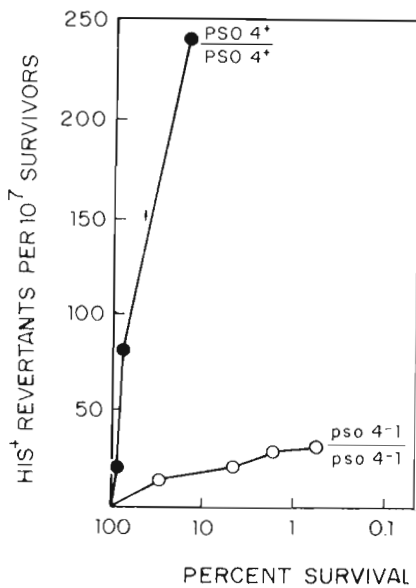
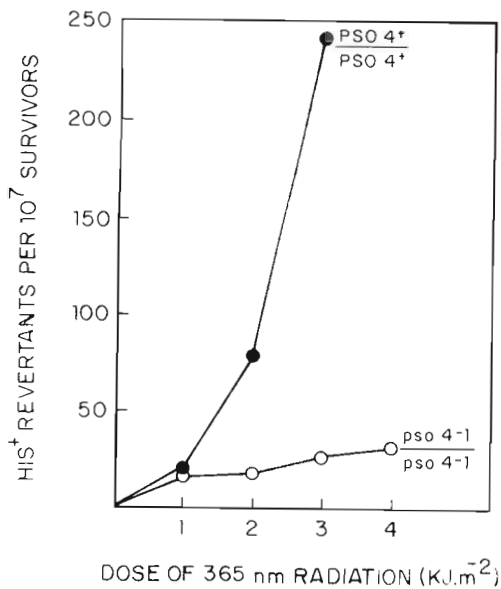
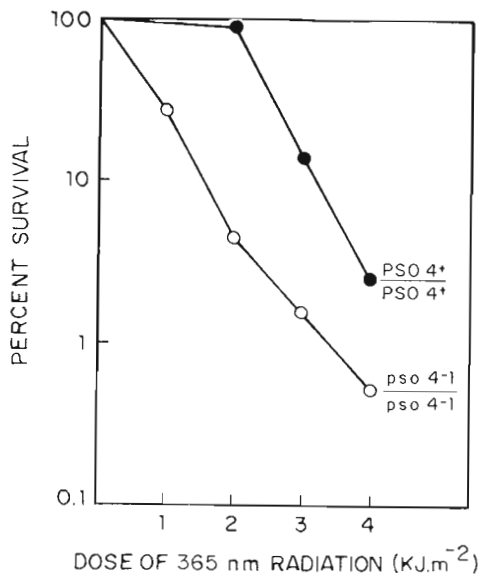


Figure 1 - Survival (upper panel) and HIS^+ revertant frequency as a function of dose (lower left panel) and of survival (lower right panel) of the *PSO4* wild-type (o) and *pso4-1* diploid cells (o) after 8-MOP photoaddition.

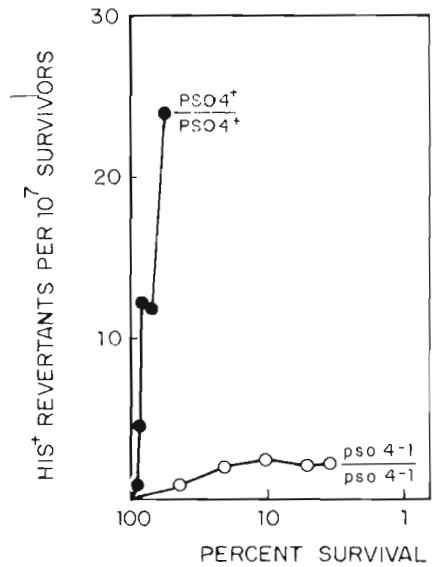
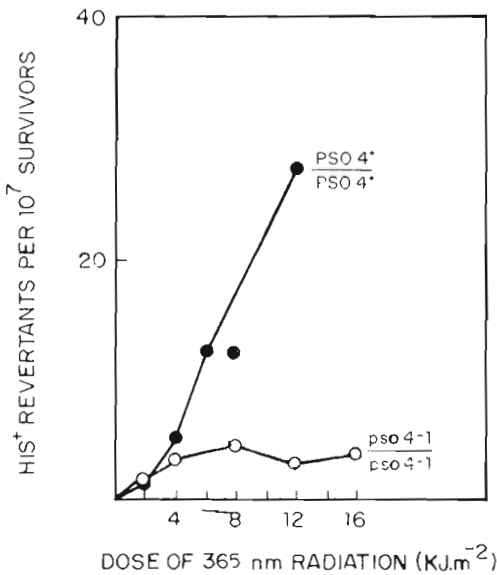
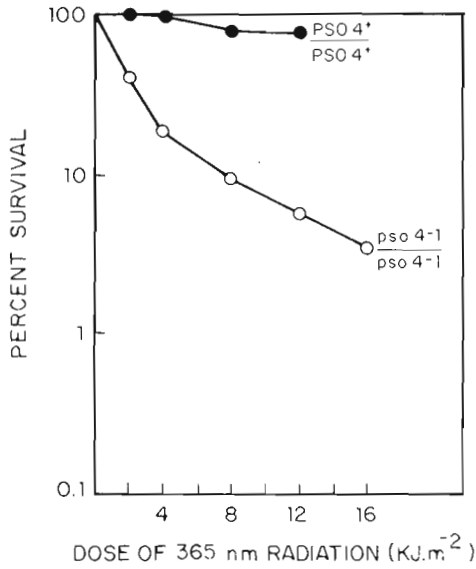


Figure 2 - Survival (upper panel) and HIS⁺ revertant frequency as a function of dose (lower left panel) and of survival (lower right panel) of *PSO4* wild-type (o) and *pso4-1* diploid cells (o) after 3-CPs photoaddition.

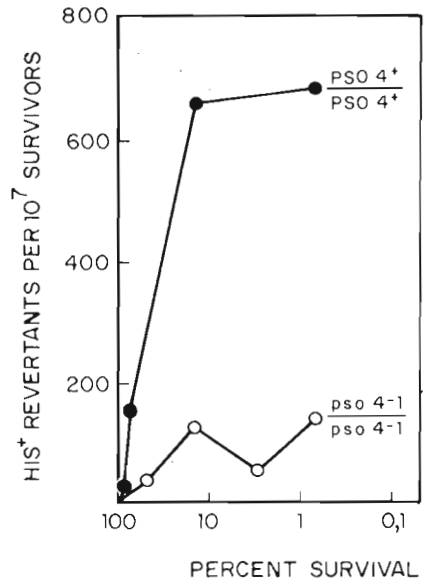
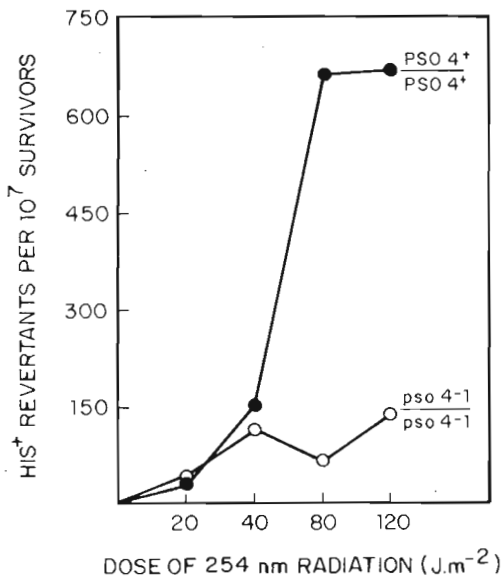
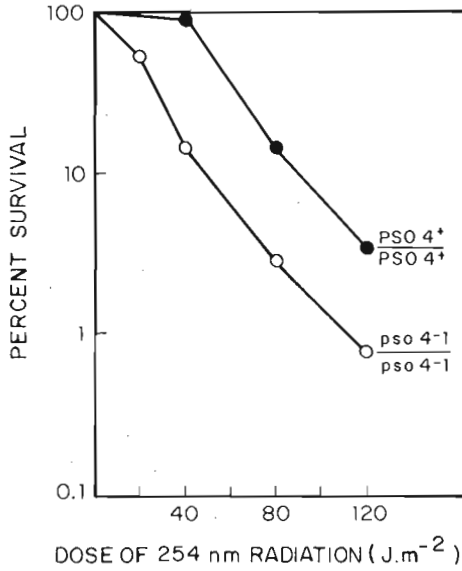


Figure 3 - Survival (upper panel) and HIS^+ revertant frequency as a function of dose (lower left panel) and of survival (lower right panel) of the $PSO4^+$ wild-type (o) and $pso4-1$ diploid cells (o) after treatment with 254 nm-UV radiation.

When the data related to revertant frequency are represented graphically on a log-log plot as shown in Figure 4, it can be seen that the mutations induced in the wild-type diploid *PSO4* strain follow two-hit kinetics for the treatment with 8-MOP plus UVA, as previously described by Grant *et al.* (1979) and Cassier *et al.* (1980). However, after mono-functional furocoumarin 3-CPs photoaddition, the phenomenon observed is one-hit kinetics (Figure 5, Cassier *et al.*, 1980). In contrast, the mutant *ps04-1* diploid strain responds with one-hit kinetics to 8-MOP photoaddition and maintains the same one-hit kinetic response as the wild-type diploid strain after treatment with 3-CPs plus UVA (Figures 4 and 5).

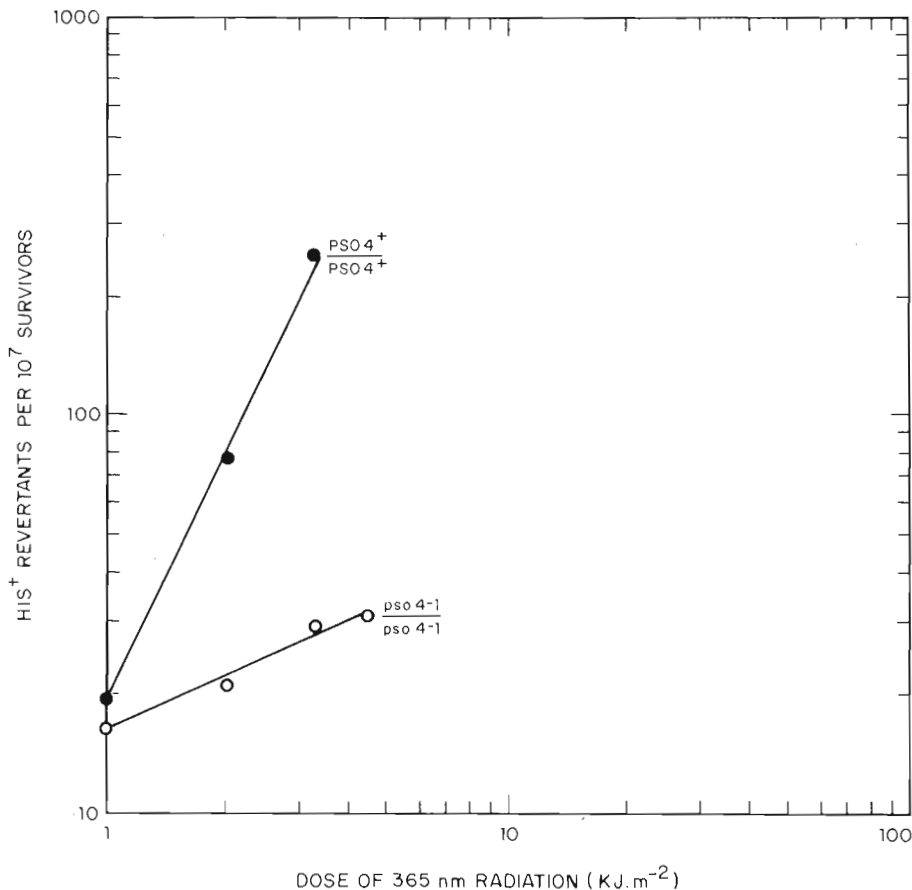


Figure 4 - Log-log plot of the His⁺ revertant frequencies in the wild-type (o) and *ps04-1* diploid strains (o).

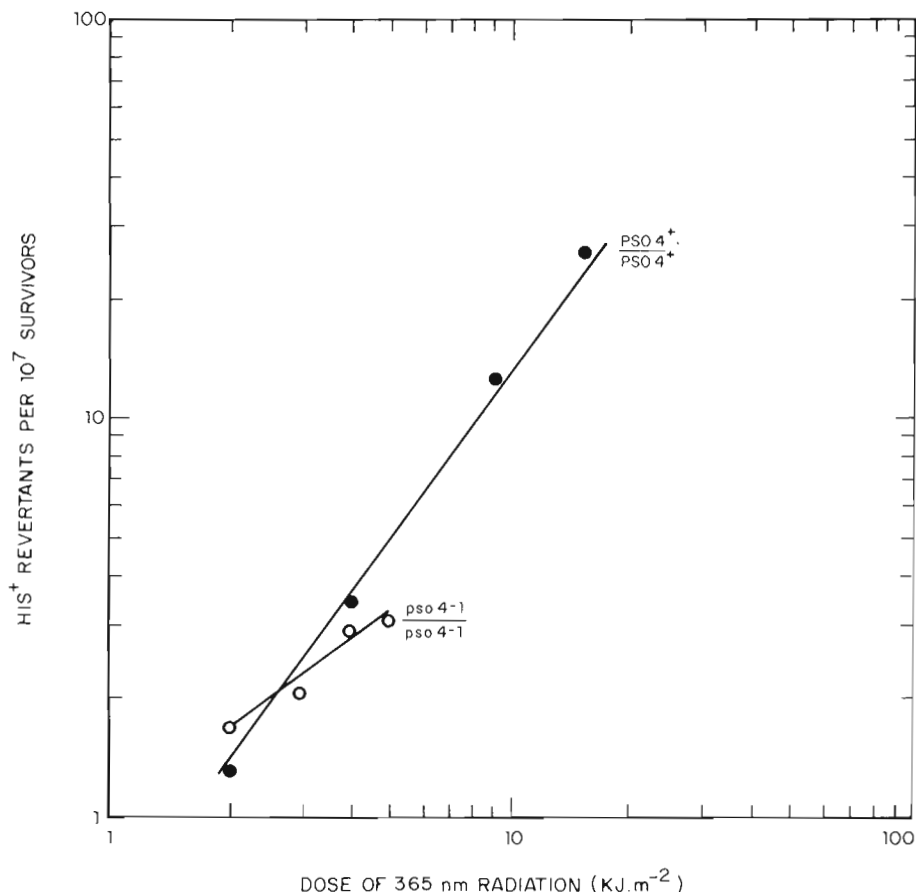


Figure 5 - Log-log plot of the HIS^+ revertant frequencies in the wild-type (o) and $pso4-1$ diploid strains (o).

DISCUSSION

Table I summarizes the main phenotypic characteristics of the $pso4-1$ mutant related to survival (Figures 1-3, Henriques *et al.*, 1989; Andrade *et al.*, 1989), recombination (Andrade *et al.*, 1989) and mutagenesis (Figures 1-3, Henriques *et al.*, 1989) when compared with the corresponding wild-type strain.

The diploid $pso4-1$ strain presented a marked reduction in the frequency of HIS^+ revertants induced after treatment with 254 nm-UV radiation or with mono- and bi-functional furocoumarins plus UVA (Figures 1-3 and Table I). The frequency of induced revertants is comparable to those of the wild-type $PSO4$ strain in the low dose range, whereas it remains at a low level for high doses (left panels in Figures

Table I - Main phenotypic characteristics of the *ps04-1* mutant as compared to the wild-type strain.

Treatment	Stationary phase haploid		Exponential phase haploid		Stationary phase diploid		Exponential phase diploid	
	Lethal effect	Mutagenesis Lys ⁺ CAN ^R	Lethal effect	G ₂ cell resistance	Lethal effect	Mutagenesis HIS ⁺	Recombination Crossing-over	Lethal effect
	<i>ps04-1</i>	<i>ps04-1</i>	<i>ps04-1</i>	<i>ps04-1</i>	<i>ps04-1/ps04-1</i>	<i>ps04-1/ps04-1</i>	<i>ps04-1 / ps04-1</i>	<i>ps04-1/ps04-1</i>
3-CPs + UVA	R	---	R	+++	SSS	--	---	S
8-MOP + UVA	SSS	---	SSSS	-	SSS	--	---	SSS
UV-254 nm	R	--	SS	-	SSS	--	---	SSS
X-rays	± S*	---*	SS*	+	SSS	ND	ND	SSS*
HN1	S	---	SSS	-	SSS	ND	--	SSSS
HN2	± S	---	SS	+	SSS	ND	--	SSSSS

Data referring to diploid cell recombination are from Andrade *et al.* (1989) and data referring to haploid cells are from Henriques *et al.* (1989). R, Same sensitivity as the wild-type strain; S, SS, SSS, SSSS and SSSSS, increased mutant cell sensitivity to the lethal effect of different treatments as compared to the wild-type strain; N refers to a mutagenic frequency similar to that of the wild-type strain; ---, and -- refer to a mutagenesis or recombination frequency blocked or extremely reduced compared to the wild-type strain; + + +, + +, +, and + refer to the presence of a resistant fraction in an exponential phase population with a decreasing sensitivity to the different treatments; - refers to the absence of a resistant fraction; ND, not determined; * data from Benathen and Beam (1977).

1-3). On the other hand, when these frequencies are expressed as a function of survival, a great reduction in induced mutagenesis is observed in comparison with the wild type at all survival levels analyzed (right panels in Figures 1-3). These differences reflect the correlation between cell death and mutagenic effect (Munson and Goodhead, 1977; Averbeck and Moustacchi, 1979). Additionally, in the haploid state the *ps04-1* mutation completely blocks the induced mutagenesis at different loci for all agents utilized (Henriques *et al.*, 1989).

The locus-specific reversion of his 1-1 follows two-hit kinetics for the wild-type PSO4 strain after bi-functional furocoumarin (8-MOP) photoaddition, whereas after mono-functional furocoumarin (3-CPs) one-hit kinetics is observed (Figures 4 and 5). These results confirm that in repair-proficient wild-type yeast the interstrand DNA crosslink and monoadducts are mutagenic (Grant *et al.*, 1979; Henriques *et al.*, 1989). On the other hand, the *ps04-1* mutant shows one-hit kinetics after 8-MOP as well as 3-CPs photoaddition. This is a response similar to that observed for an excision-defective strain (*RAD3* type) treated with mono- and bi-functional psoralen derivatives plus UVA (Grant *et al.*, 1979; Henriques *et al.*, 1989). In addition, Cassier *et al.* (1980) demonstrated that in the homozygous diploid state the *ps02-1* mutant, which is blocked in an error-prone recombinational repair pathway (Saeki *et al.*, 1983), shows one-hit kinetics after 8-MOP photoaddition. Subsequent studies by Henriques and Moustacchi (1981) and Henriques *et al.* (1985) confirmed the participation of this mutant in the excision-resynthesis repair pathway. Recently, Benfato and Henriques (personal communication), in a study of the mode of interaction of the *ps04-1* mutant with *rad* mutant which are blocked in the excision-resynthesis repair pathway (*RAD3* type) after furocoumarin derivative photoaddition and 254 nm-UV irradiation, demonstrated that *ps04-1* belongs to the excision repair pathway. It is thus possible to suggest that the monoadducts induced by 3-CPs are mutagenic in *ps02-1*, *ps04-1* and in the excision-defective strains, although to a much lesser extent than in the wild type. On the other hand, the croolinkss photoinduced by 8-MOP in repair-defective strains may be considered not to be mutagenic but to be essentially lesions leading to cell lethality.

In addition, the survival rate of the diploid *ps04-1* mutant after treatment with the three mutagens confirms, as previously reported (Andrade *et al.*, 1989), that these mutant cells are more sensitive than those of the corresponding wild-type strain to the lethal effect induced by the different genotoxic agents tested. This sensitivity is significantly more marked in the diploid state (Figures 1-3, upper panels, Andrade *et al.*, 1989) than in the haploid state (Henriques *et al.*, 1989), demonstrating the disappearance of the ploidy effect characteristic of wild-type diploid strains (Table I).

These observations, taken together with the fact that the *ps04-1* mutation caused a generalized blockade of both gene conversion and crossing-over mitotic recombination (Table I, Andrade *et al.*, 1989), lead us to suggest that the repair processes responsible for recovery of the diploid depend on the occurrence of recom-

binational events governed by the product of the wild-type *PSO4* allele. Similar results were obtained for the *ps02.1* mutant, which, however, is specifically sensitive to agents inducing crosslinks in DNA (Henriques and Moustacchi, 1980b) showing deficiency in both mutation and mitotic recombination induced by bifunctional furocoumarins (Cassier *et al.*, 1980; Cassier and Moustacchi, 1981; Saeki *et al.*, 1983). On the other hand, as determined by Henriques *et al.* (1989), these two genes are not alleles.

Indeed, the phenotypic response of *ps04-1* in terms of blockade of mitotic recombination and mutagenesis is close to that of mutants of the *RAD52* pathway, which are blocked in an error-free recombination process (Lawrence and Christensen, 1976; McKee and Lawrence, 1979; Game *et al.*, 1980; Malone and Esposito, 1980; Prakash and Taillon-Miller, 1981; Lawrence, 1982; Saeki *et al.*, 1983), and to that of mutants of the *RAD6* pathway, which are blocked in an error-prone repair process dissociated from recombination (for reviews, see Lawrence, 1982; Moustacchi, 1987). However, no allelism relationships were detected between *ps04-1* and different mutant alleles belonging to the *RAD52* and *RAD6* epistasis groups (Henriques *et al.*, 1989).

Studies of gene interaction carried out by Benfato and Henriques (personal communication) have demonstrated that for lesions photoinduced by 3-CPs and 8-MOP, the *PSO4* and *RAD6* genes act on different repair pathways. However, for these same lesions an epistatic interaction was observed between the *ps04-1* mutant and the *ps01-1* (= *rev3-1*, Cassier-Chauvat and Moustacchi, 1988) mutant which belongs to the error-prone repair pathway (*RAD6* type) (Benfato and Henriques, personal communication).

Thus far, none of the *S. cerevisiae* mutants analyzed seems to be comparable with the *rec A* and *lex A* mutants of *E. coli*, since they dissociate mutational ability from recombination proficiency (Moustacchi, 1987). In fact, only the *PSO4* gene seems to be the first yeast gene in which mutagenic deficiency is associated with generalized blockade of recombination. The observation that the *PSO4* function controls an error-prone recombination process regardless of the type of DNA damage suggests a strong analogy between the *ps04.1* mutation and the *rec A* and *lex A* mutations of *E. coli*.

RESUMO

Foi examinada a resposta mutagênica de células diplóides de *S. cerevisiae*, homozigotas para a mutação *ps04-1* em comparação com a cepa selvagem correspondente. Observou-se que o diplóide *ps04-1* apresenta uma acentuada redução nas frequências de mutação reversa induzidas após tratamento com radiação UV, ou furocumarinas mono e bifuncionais mais UVA. Esta severa inibição na mutagênese induzida ocorre principalmente em doses mais elevadas dos diferentes agentes genotóxicos utilizados. Em adição ao seu efeito sobre o processo de mutagênese o mutante *ps04-1* exibe um bloqueio generalizado na recombinação mitótica-recíproca (recombinação) ou não recíproca - espontânea e in-

duzida. Estas observações indicam que o gene *PSO4* controla simultaneamente os processos de mutagênese e de recombinação, atuando sobre um mecanismo de reparo recombinacional sujeito a erro, comparável ao reparo SOS presente em *E. coli*.

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(Received May 29, 1989)