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Further phenotypic characterization of *pso* mutants of *Saccharomyces cerevisiae* with respect to DNA repair and response to oxidative stress

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ABSTRACT. The sensitivity responses of seven *pso* mutants of *Saccharomyces cerevisiae* towards the mutagens N-nitrosodiethylamine (NDEA), 1,2:7,8-diepoxyoctane (DEO), and 8-hydroxyquinoline (8HQ) further substantiated their allocation into two distinct groups: genes *PSO1* (allelic to *REV3*), *PSO2* (*SNM1*), *PSO4* (*PRP19*), and *PSO5* (*RAD16*) constitute one group in that they are involved in repair of damaged DNA or in RNA processing whereas genes *PSO6* (*ERG3*) and *PSO7* (*COX11*) are related to metabolic steps protecting from oxidative stress and thus form a second group, not responsible for DNA repair. *PSO3* has not yet been molecularly characterized but its pleiotropic phenotype would allow its integration into either group. The first three *PSO* genes of the DNA repair group and *PSO3*, apart from being sensitive to photo-activated psoralens, have another common phenotype: they are also involved in error-prone DNA repair. While all mutants of the DNA repair group and *pso3* were sensitive to DEO and NDEA the *pso6* mutant revealed WT or near WT resistance to these mutagens. As expected, the repair-proficient *pso7-1* and *cox11-Δ* mutant alleles conferred high sensitivity to NDEA, a chemical known to be metabolized via redox cycling that yields hydroxylamine radicals and reactive oxygen species. All *pso* mutants exhibited some sensitivity to 8HQ and again *pso7-1* and *cox11-Δ* conferred the highest sensitivity to this drug. Double mutant *snm1-Δ*

cox11-Δ exhibited additivity of 8HQ and NDEA sensitivities of the single mutants, indicating that two different repair/recovery systems are involved in survival. DEO sensitivity of the double mutant was equal or less than that of the single *snm1-Δ* mutant. In order to determine if there was oxidative damage to nucleotide bases by these drugs we employed an established bacterial test with and without metabolic activation. After S9-mix biotransformation, NDEA and to a lesser extent 8HQ, lead to significantly higher mutagenesis in an *Escherichia coli* tester strain WP2-IC203 as compared to WP2, whereas DEO-induced mutagenicity remained unchanged.

Key words: DNA damage, Drug metabolism, Oxidative stress, DNA repair, Yeast, *Escherichia coli* mutagenesis

INTRODUCTION

The *pso* mutants of *Saccharomyces cerevisiae* were isolated due to their sensitivity to photoactivated psoralens (Henriques and Moustacchi, 1980; Henriques et al., 1989; Querol et al., 1994). With increasing phenotypic characterization a division of the hitherto known seven *PSO* genes into two groups, based on their involvement with DNA repair, was suggested (Henriques et al., 1997). While *PSO1* (allelic to *REV3*), *PSO2* (allelic to *SNM1*), *PSO4* (allelic to *PRP19*), and *PSO5* (allelic to *RAD16*) encode proteins with known or apparent functions in either error-prone or error-free DNA repair, the *PSO6* (allelic to *ERG3*) and *PSO7* genes (allelic to *COX11*) encode proteins apparently not involved in a DNA repair mechanism. Mutant alleles of the latter genes most probably confer sensitivity to certain mutagens as a result of loss from protection to reactive oxygen species (ROS) by low ergosterol in *pso6/erg3* (Schmidt et al., 1999) or due to altered mutagen metabolism in *pso7/cox11* (induced by an electron flow impairment; Pungartnik et al., 1999). *PSO3* has not yet been molecularly characterized and, though it exhibits defects in error-prone repair (Cassier et al., 1980) and, therefore, should belong to the first group of repair-deficient *pso* mutants, it might as well be associated with *PSO6* and *PSO7*, since the *pso3-1* mutation, as all mutant alleles from the second group of repair-proficient *pso* mutants, confers sensitivity to oxidative stress-inducing treatments (Querol et al., 1994; Brendel et al., 1998; Schmidt et al., 1999; Pungartnik et al., 1999), as do all mutant alleles from the second group of repair-proficient *pso* mutants.

In the present study, we show some new phenotypes for the *pso* mutants in which we have determined their sensitivities to the three selected mutagens: N-nitrosodiethylamine (NDEA, which after hydroxyl radical formation decomposes to acetaldehyde and an alkylating agent; Bartsch et al., 1989); 1,2:7,8-diepoxyoctane (DEO, a cross-linking and deletogenic agent; Yunes et al., 1996; Picada et al., 1999), and 8-hydroxyquinoline (8HQ, which is only mutagenic after biotransformation/metabolic activation; Nagao et al., 1977). Furthermore, we have applied these chemicals to yeast double mutants containing one *pso* mutant allele from each group in order to assess whether an epistasis/synergism analysis (Brendel and Haynes, 1973; Brendel and Ruhland, 1984) might indicate possible interactions of the respective *PSO* gene-encoded proteins in protection from, or repair of, DNA damage induced by these agents in yeast. Finally we have indirectly verified the production of ROS in the course of NDEA and probably also 8HQ meta-

bolic activation by showing their elevated mutagenicity in *Escherichia coli* tester strain WP2-IC203 that specifically indicates ROS-induced DNA lesions (Blanco et al., 1998).

MATERIAL AND METHODS

Strains and media

The yeast strains used are given in Table 1. Strains were usually grown and stored on YPD media. To ascertain their respiratory competence and for elimination of spontaneously accumulated petites all strains (except *cox11* deletion mutants) were pre-grown on YP glycerol media (glucose replaced by 3% glycerol) before being grown in YPD. All media, general and genetical procedures were as described before (Ruhland et al., 1981a). *E. coli* strains WP2 *uvrA*/pKM101 (WP2) and WP2 *uvrA oxyR*/pKM101 (IC203) were obtained from M. Blanco, Instituto de Investigaciones Citológicas, Spain.

Table 1. Yeast strains used.

Name	Genotype	Source
N123	*MAT***a** *PSO his1-1*	Henriques and Moustacchi, 1980
MB1615-2B	*MAT***a** *PSO his5-2 arg4-17 ade2-101*	Brendel, M., unpublished results
pso1-1	*MAT***a** *pso1-1 his1-1*	Henriques and Moustacchi, 1980
pso2-1	*MAT***a** *pso2-1 his1-1*	Henriques and Moustacchi, 1980
snm1-Δ	*MAT***a** *snm1-Δ::URA3 his5-2*	Wolter et al., 1996
MB1615-4A	*MATα pso3-1 ura3-52 ade2-101 lys2-801*	Brendel et al., 1998
MG5002-3B	*MAT***a** *pso4-1 ura3-53 ade2-1 his3 trp1 leu2-3,112*	Grey et al., 1996
PT2314-11	*MATα pso5-1 his1-1 ura3-52 leu2-Δ1*	Querol et al., 1994
CL1503-15A	*MATα pso6-1 lys2-1 ura3-52 leu2-3,112*	Schmidt et al., 1999
CH1804-2C	*MATα pso7-1 lys2-1 his3-Δ200 trp1-Δ901 ura3-52 leu2-3,112 ade2-1 can1-100*	Pungartnik et al., 1999
BY4742	*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 urs3Δ0 cox11::kanMX4*	Euroscarf

Treatment with chemicals

Yeast strains were grown in YPD under shaking at 30°C for 2 days (stationary phase). These cells were screened for their sensitivity in the center well test (Ruhland et al., 1981a). Having established the appropriate range of concentration for each mutagen, the sensitivities of WT, *pso* single and double mutants were determined as follows: a) NDEA was added to YPD agar plates to a final concentration of 40 and 80 mM and 5 µl drops of a 1:10 dilution series from undiluted culture (approximately 2 x 10^8 cells/ml) to dilution 10^{-5} (approximately 2 x 10^3 cells/ml) of up to 5 different strains were applied to the same plate. Incubation was for 2 to 3 days at 30°C. Strains from the tetrad analysis were treated with lower NDEA concentrations; b) Sensitivity to DEO and to 8HQ was routinely determined in liquid YPD media. Exposure concentrations in 1 ml samples were 10 and 20 mM (for DEO) and 20 and 40 mM (for 8HQ), and exposure time was 1 h at 30°C. Treated cells were diluted in distilled water as in (a) and 5 µl drops from each dilution were spotted onto YPD agar plates. In some instances DEO was applied to washed cells suspended in either phosphate buffer, pH 7.0, or distilled water for testing of a possible influence of medium components and pH on DEO reactivity.

Mutagenicity testing

E. coli strains WP2 and IC203 were exposed to the three chemicals that had been/had not been treated with S9-mix (Molecular Toxicology Inc.; Moltox™, prepared from livers of Sprague-Dawley rats pretreated with polychlorinated biphenyls (Aroclor 1254)). Testing for mutagenicity was as described by Blanco et al. (1998).

RESULTS AND DISCUSSION

The survival of haploid WT and *pso* mutant cells of yeast after exposure to the three chemicals is shown for some strains in Figure 1 and summarized for all *pso* mutants in Table 2. Exposure concentrations of the mutagens were such that the WT cells showed no killing at the highest exposure dose, whereas mutant cells normally exhibited a varying degree of sensitivity in survival. Two WT strains were used because the tested *pso* mutants were derived from either one of them (Table 2).

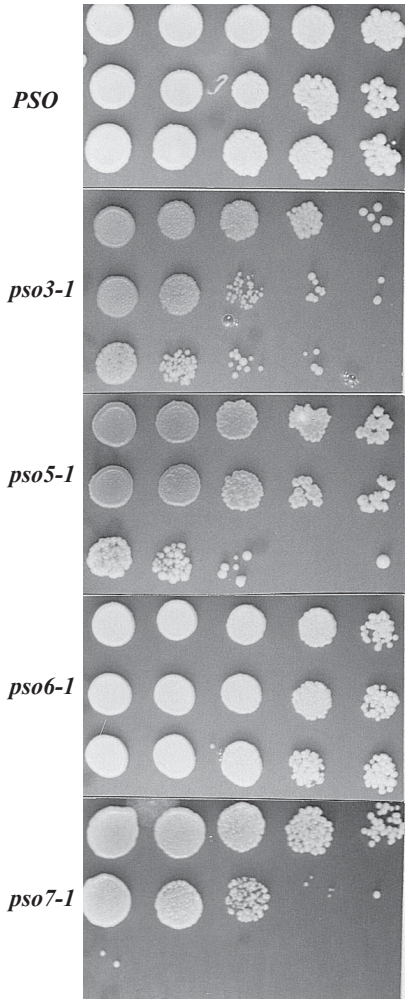

Figure 1. Sensitivity to 8HQ of haploid yeast strains containing different *PSO* mutant alleles. From top to bottom: *PSO* (WT); *pso3-1*; *pso5-1*; *pso6-1*; *pso7-1*. Three exposure concentrations (from top to bottom: 0, 20 and 40 mM, 1-h exposure) and five 1:10 dilutions (left to right) are given for each strain.

Table 2. Sensitivities to 3 mutagens of haploid yeast containing different *pso* mutant alleles.

Strain	8HQ 20 mM	8HQ 40 mM	NDEA 40 mM	NDEA 80 mM	DEO 8 mM	DEO 16 mM
N123	R	R	R	R	R	R
pso1-1	S	SS	SS	SSSS	SSSS	SSSSS
pso2-1	S	SS	S	SS	SS	SSSSS
snm1-Δ	S	SS	S	SS	SSS	SSSSS
pso6-1	R	R	R	SS	R	S
pso7-1	SS	SSSSS	S	SSSSS	R	SS
cox11-Δ (a)	Nd	Nd	S	SSSS	S	SS
cox11-Δ (b)	Nd	Nd	S	SSSS	R	SS
MB1615	R	R	R	R	R	R
pso3-1	S	SS	SSS	SSSSS	SS	SSSSS
pso4-1	SS	SS	SS	SSSS	S	SS
pso5-1	R	SSS	SSSS	SSSSS	R	S

R, WT resistance; S to SSSSS, each S stands for one decade´s killing (sensitivity of survival) when compared to the control without mutagen exposure. Two WT strains used as the *pso* mutant strains were derived from either of them; (a) treated in distilled water, (b) treated in phosphate buffer, pH 7.0; Nd, not determined.

Sensitivity to 8HQ

The chemical 8HQ is not genotoxic unless metabolically activated, e.g., by rodent liver or by S9-mix (Hollstein et al., 1978; Willems et al., 1992); mutagenic activity of 8HQ was correlated to the formation of water-soluble quinoline intermediate metabolites, which constitute epoxide derivatives that could form DNA adducts (Tada et al., 1980).

After 1-h exposure to 20 and 40 mM 8HQ the seven *pso* mutants display a wide spectrum of sensitivities to this pro-mutagen, whereas the two WT strains were completely resistant (Table 2). Interestingly, the *pso7/cox11* mutation confers the highest 8HQ-sensitivity amongst all yeast strains tested, whereas all mutants from the DNA repair-deficient group, while also 8HQ-sensitive, were clearly much more resistant than the DNA repair-proficient *pso7/cox11* mutant (Table 2). Mutant alleles *pso2-1/snm1-Δ* confer low 8HQ-sensitivity which is not surprising as this mutant is known to be specifically sensitive to interstrand cross-link (ICL) producing mutagens (Ruhland et al., 1981b) and the *pso6/erg3* mutant allele does not confer any sensitivity at all and survives like a WT. The highly reactive diol epoxides that may emerge at some point of 8HQ biotransformation (Willems et al., 1992) and that can form DNA adducts may explain the sensitivity of group I *pso* mutants. On the other hand, the extreme sensitivity of DNA repair-proficient mutant *pso7/cox11* suggests that 8HQ, very much like 4NQO, might be metabolized via a redox cycle induced by altered electron flow that would generate nitroquinoline radicals and consequently highly genotoxic ROS (Pungartnik et al., 1999).

Sensitivity to NDEA

NDEA is a potent carcinogen that turns mutagen in *Salmonella typhimurium* (TA98 and TA100) when activated by alcohol dehydrogenase (ADH) from yeast or horse liver. Nitrosamine compounds can easily be activated by redox cycling, producing nitrosamine-derivative radicals and ROS (Boveris, 1998); the latter may induce lipid peroxidation (LP) (Ahotupa et al., 1987; Kappus and Sies, 1981). All *pso* mutants, being proficient in at least 5 different ADHs

(Wehner et al., 1993) showed higher-than-WT sensitivity to NDEA (Figure 1 and Table 2). Highest sensitivities were displayed by the group of repair-deficient mutants *pso1*, *pso4*, *pso5*, and *pso3*, whereas ICL-specific repair mutant *pso2/snm1* (Ruhland et al., 1981b) had only intermediate NDEA-sensitivity. These responses clearly show that activated NDEA caused significant numbers of DNA lesions (few or none of the ICL type) throughout the yeast genome, the *pso5/rad16* mutant's (Paesi-Toresan et al., 1995) high sensitivity pointing to the importance of a functional Rad16p/Pso5p for excision repair in silent regions of the genome or in non-transcribed strands of DNA (Verhage et al., 1994).

As would be expected, the DNA repair-proficient *pso6* and especially *pso7* mutants (*pso7-1* and *cox11-Δ*) were also NDEA-sensitive (Table 2). For the *pso6/erg3* mutant this may be explained by its lower ergosterol content (Schmidt et al., 1999) that would offer less protection from NDEA-induced LP (which was shown to rapidly occur in rat liver after NDEA had been metabolized into a pro-oxidant state; Ahotupa et al., 1987). This is coupled with increased production of ROS, ultimately leading to a larger number of oxidative DNA lesions. NDEA metabolism may also be accelerated in *pso7* by enhanced redox cycling due to altered electron flow (Pungartnik et al., 1999) leading to high levels of nitrosamine derivatives and ROS. Finally, the *pso3* mutant is known to be sensitive to ROS (Brendel et al., 1998) and also has an impaired error-prone DNA repair (Henriques et al., 1997). Its high NDEA-sensitivity may, therefore, indicate that the Pso3p has a special role in repair of oxidative DNA damage, and that nonrepair of such lesions would result in lower-than-WT mutagenesis and higher-than-WT sensitivity.

Sensitivity to DEO

The carcinogenicity of epoxide compounds has been attributed to their covalent binding to DNA. While monoepoxides form only monoadducts, diepoxides can also form ICL. Depending on their chain length diepoxides may form DNA-ICL and show higher carcinogenicity and mutagenicity than their monoepoxide analogues (for a review, see Yunes et al., 1996; Vock et al., 1999). Sensitivity to the ICL forming and deletogenic (Picada et al., 1999) agent DEO of all 7 *pso* mutants is shown in Table 2. As with NDEA all mutants of the repair-deficient group (*pso1*, *pso2*, *pso4*, with exception of *pso5*) were highly sensitive to DEO, again demonstrating the importance of error-free and error-prone repair in removal of DNA lesions induced by the mono- and bifunctional action of DEO. The low DEO-sensitivity of *pso5/rad16* indicates that there is either very low DNA damage (in number or in stability of DNA lesions) in silent and non-transcribed regions of the yeast genome (Verhage et al., 1994) or that the still existing residual excision repair in a *rad16* mutant (Reed et al., 1998) suffices to remove most of DEO-induced DNA lesions. Indeed a *rad16-1* mutant had no repair of 8-methoxypsoralen (8-MOP + UVA)-induced ICL in the silent mating type locus HMLa whereas incision near ICL in the transcriptionally active *MATα* locus was normal (Meniel et al., 1995). Alternatively, ICL repair in transcribed regions of the genome might be more severely inhibited or lead to lethal DNA processing in the other repair-deficient *pso* mutants and hence enhance their sensitivity over that of *rad16/pso5*.

The increased sensitivity of *pso7/cox11* mutants to DEO, however, is surprising since these mutants have WT-like capacity for DNA repair (Henriques et al., 1997) and since DEO has not yet been shown to be involved in, or capable of, inducing ROS (see below). We might,

therefore, speculate that DEO metabolism in the *pso7/cox11* mutant could lead to altered derivatives with significantly higher DNA-damaging potential while the WT-like resistance of the *pso6* mutant indicates that lack of ergosterol does not lead to a larger number of DNA lesions. Finally, the sensitivity of *pso3-1* to DEO was expected, as this mutant is known to be sensitive to 8-MOP + UVA treatment that leads to DNA-ICL (Henriques and Moustacchi, 1980).

Sensitivities to DEO, NDEA and 8HQ of *snm1-Δ cox11-Δ* double mutants

A synergistic or epistatic interaction of two mutant alleles which each alone confer a similar phenotype, in our case sensitivity to a given mutagen, could give a first indication whether the two mutant alleles affect interrelated metabolic steps (epistasis), competitive (synergism) or unrelated (additivity) metabolic pathways of damage prevention/repair (Brendel and Haynes, 1973). Sensitivity to the three mutagens of haploid strains containing all possible allele combinations of *PSO2* and *PSO7* (WT, single and double mutants represented by the *snm-Δ* and *cox11-Δ* mutant alleles) is shown in Table 3. In addition to DEO three typical DNA cross-linking agents or treatments, nitrogen mustard (HN_2), triaziquone (Ruhland et al., 1981b) and

Table 3. Sensitivity of *snm1-Δ cox11-Δ* double mutants.

Relevant	8HQ		NDEA		DEO		HN_2	Triaziquone	8-MOP + UVA
genotype	20 mM	40 mM	40 mM	80 mM	7 mM	14 mM	10 μM	100 nM	1 kJ/m²
snm1-Δ cox11-Δ	SSS	SSS	SSS	SSSSS	SS	SSSSS	SSSS	SSSSS	SSS
SNM1 cox11-Δ	S	SS	S	SSS	S	SS	S	SS	R
snm1-Δ COX11	S	S	S	SS	SSSS	SSSSS	SSSS	SSSSS	SSS
SNM1 COX11	R	S	R	R	R	R	R	R	R

Four ascosporal clones (tetratype tetrad) tested for sensitivity to 8HQ, NDEA, and to four DNA interstrand cross-linking agents DEO, HN2, triaziquone, and 8-MOP + UVA. R, S as in Table 2

photoactivated 8-MOP + UVA (Bankmann and Brendel, 1989) were used to verify the *pso2/snm1* mutant's sensitivity to DNA-ICL (Table 3). The four haploid yeast strains tested are from one tetratype tetrad derived from one ascus of the sporulated diploid MG5503 that was generated by the cross of the haploid *cox11* and *snm1* mutants (Table 1).

Analyses of the 8HQ-sensitivities of the four allele combinations revealed a non-epistatic (additive or synergistic) interaction of the two mutant alleles at 20 mM but not at 40 mM exposure concentration (first two columns in Table 3). This points to unrelated metabolic activities of the two related proteins in protection from the toxic/genotoxic effects of biotransformed 8HQ in yeast.

The sensitivity to NDEA of both *pso2-1/snm1-Δ* and *pso7-1/cox11-Δ* mutants, the former representing impaired DNA repair and the latter with a defect in mitochondrial function, enabled us to test our hypothesis that these two *PSO* genes do not contribute to the same repair/protection mechanism against NDEA-induced DNA damage but that the two mutants' sensitivities are caused by totally unrelated metabolic deficiencies. We would thus expect, given a conjunction of both NDEA sensitivity-conferring mutant alleles in one haploid strain, an additive sensitivity to NDEA in the double mutant. By constructing haploid *pso2pso7* double mutants and testing their NDEA-sensitivity we could clearly show that the two mutant alleles yielded strains more sensitive than either parental mutant alone (Table 3), i.e., they displayed additivity of sensitivities. The absence of super-sensitivity to NDEA of the double mutant (no syner-

gistic interaction of the two mutant alleles), shows the noncompetitivity of the two metabolic "repair and protection capacities" encoded in the two respective *PSO* genes (Brendel and Haynes, 1973).

The analyses of all four cross-linking treatments revealed epistatic interaction of the two mutant alleles for these mutagens. In the case of 7 mM DEO even a slight protection could be detected in the double mutant and could be repeated with this and other double mutants derived from other asci from the MG5503 diploid (data not shown). This hints at a slightly different DEO metabolism in the presence of *cox11-Δ* that perhaps would lower the number of DNA-ICL lesions produced by this mutagen, thus allowing for a better survival in the absence of ICL repair. Epistatic interaction was also found after treatment with the other three ICL-forming mutagens. Sensitivity to HN_2, triaziquone and to 8-MOP + UVA was identical in *snm1-Δ* single and *snm1-Δ cox11-Δ* double mutants, clearly showing that the reactions of these mutagens were not affected by the quality of the *COX11* allele (Table 3, last three columns).

NDEA and 8HQ, but not DEO increase oxidative DNA damage in *Escherichia coli* tester strain WP2-IC203

Finally, we tested the hypothesis that the biotransformation of NDEA and 8HQ produces ROS that ultimately might damage DNA in pro- and eukaryotes by exposing cells of *E. coli* mutant WP2-IC203, known to be a reliable indicator for oxidative stress-causing chemicals (Martinez et al., 2000), to the three mutagens used in our yeast study. *E. coli* WP2-IC203 has a proven detection ability for mutagens that generate ROS and cause oxidative DNA damage (Blanco et al., 1998; Ohta et al., 1998) and has been successfully used in detecting such mutagens (Martinez et al., 2000).

S9-mix activated NDEA and 8HQ, but not DEO, clearly had higher mutagenicity in IC203 than in WP2 (Figure 2), which indicates a significant contribution of ROS-induced oxidative DNA lesions in mutagenesis (Martinez et al., 2000). By comparing mutagenicity with

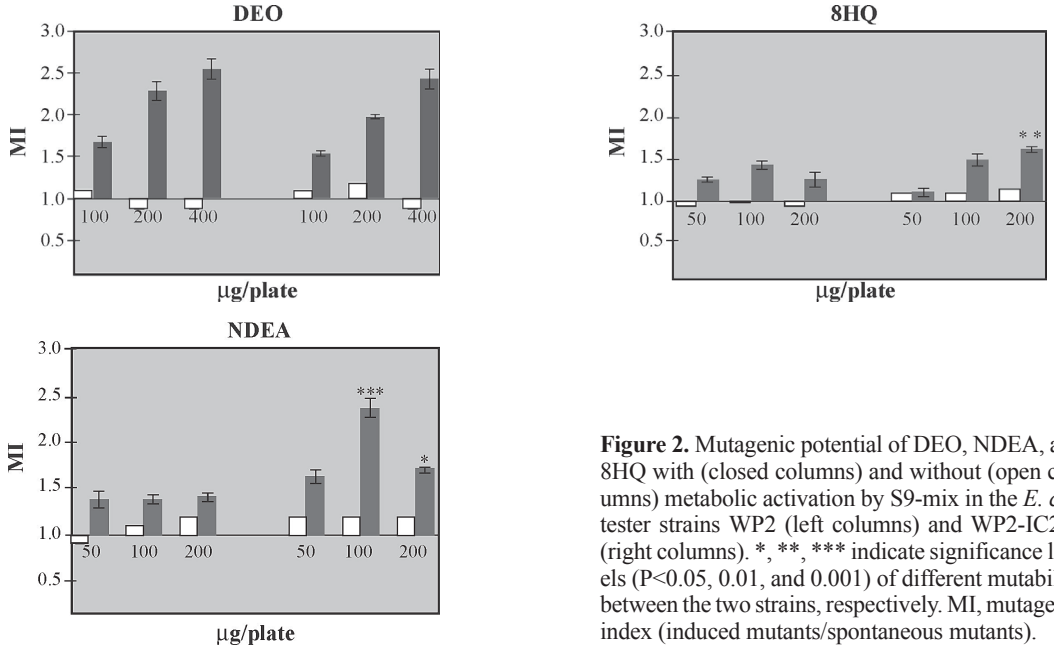

Figure 2. Mutagenic potential of DEO, NDEA, and 8HQ with (closed columns) and without (open columns) metabolic activation by S9-mix in the *E. coli* tester strains WP2 (left columns) and WP2-IC203 (right columns). *, **, *** indicate significance levels ($P<0.05$, 0.01, and 0.001) of different mutability between the two strains, respectively. MI, mutagenic index (induced mutants/spontaneous mutants).

and without metabolic activation it is clear that all three chemicals had greater mutagenic potential after biotransformation in both strains (Figure 2). NDEA is known for its high ROS production (Ahotupa et al., 1987; Boiter et al., 1995), while the mutagenicity of biotransformed 8HQ has been shown in several studies (Nagao et al., 1977; Willems et al., 1992). We might take these and our data as an indirect proof that yeast biotransformation of NDEA and 8HQ might also lead to elevated level of nitro radical intermediates and of ROS and thus to more DNA damage. In the case of the *pso* mutants, especially in *pso3*, *pso6*, and *pso7*, predicted to be susceptible to ROS-generating treatments (Querol et al., 1994; Henriques et al., 1997), this would result in a high sensitivity phenotype as indeed was found (Table 2).

CONCLUDING REMARKS

The available information on the *pso* mutants suggests the following grouping of these genes: four *PSO* genes, *PSO1*, *PSO2*, *PSO4*, and *PSO5* encode proteins known to be involved in DNA repair which, except for *PSO5*, control steps in a specific error-prone recombinational repair pathway for 8-MOP photo-induced stable and highly toxic ICL. *PSO3* has not yet been molecularly characterized but may encode a repair protein specific for oxidative DNA damage (Brendel et al., 1998). Two *PSO* genes, *PSO6* and *PSO7* encode proteins not involved in DNA repair but in the biosynthesis of ergosterol and in correct assembly of the mitochondrial cytochrome c oxidase (and hence allowing oxidative phosphorylation), respectively. Althoug rather detached from each other, these two metabolic activities may, each in a different way, influence the dynamics and levels of ROS that ultimately might interact with DNA. Lack of ROS-quenching effects by low ergosterol-containing membranes might be the culprit of the NDEA-sensitivity of the *pso6/erg3* mutant (Schmidt et al., 1999). If this were true, a yeast cell's sensitivity to 8HQ and to DEO would not depend on correct ergosterol content of biomembranes, since *pso6/erg3* mutant has more or less WT resistance to these chemicals (Table 2). Our new data found in a sensitivity screening against NDEA, 8HQ, and DEO do not contradict but rather support our initial grouping of the *PSO* genes into the above-mentioned two main categories.

In summary it can be said that the *PSO* genes belonging to the DNA repair group have been rather important to elucidate genotoxicity (survival, repair, mutagenesis, and recombination; Henriques et al., 1997) of photo-induced psoralen DNA damage (Bankmann and Brendel, 1989), thereby controlling events that, when not properly coordinated, might eventually lead to cell death. In higher eukaryotes non-correct DNA damage processing might also lead to carcinogenesis. However, our data also point to the importance of the second group of *PSO* genes related to oxidative stress responses, where altered lipid composition or drug metabolism may enhance genotoxic potential (e.g., for NDEA, and to a lesser degree 8HQ, this report) and for 4NQO (Pungartnik et al., 1999). Perhaps some of the *pso* mutant alleles might be useful for developing yeast tester strains for selective detection of ROS-producing agents. By eliminating proteins neutralizing or destroying ROS and by partially blocking mitochondrial activity in some DNA repair mutants, yeast tester strains with a wide sensitivity range against ROS-producing chemicals could be provided.

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