

Photorepair of Ultraviolet-Induced Petite Mutational Damage in *Saccharomyces cerevisiae* Requires the Product of the *PHR1* Gene

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A wild-type (*phr*⁺) diploid yeast strain showed photorepair of petite mutational damage, whereas a photoreactivation-deficient (*phr1/phr1*) diploid strain did not, indicating that the *PHR1* gene product was required for mitochondrial photorepair.

When cells of *Saccharomyces cerevisiae* are exposed to UV light, the surviving population contains a high frequency of petite (*rho*) mutants. The sequence of events from the initial irradiation to the establishment of the mutation is poorly understood. That pyrimidine dimer formation in mitochondrial DNA induced by UV-C (200- to 290-nm) wavelengths initiates the mutational sequence is widely accepted. More than 20 years ago, Sarachek (12) and Pittman et al. (9) showed that photoreactivation would reduce the frequency of *rho* mutants in surviving populations of UV-irradiated cells. The only known function of the photoreactivation enzyme is the splitting of UV-induced pyrimidine dimers. Hence, the photoreversal of the mutational event appears to be due to the occurrence of this process in mitochondria. That this seems likely to be true follows from the demonstration that dimer content was reduced in mitochondrial DNA from yeast cells which had first been exposed to UV irradiation and then exposed to photoreactivating light (13). This observation is supported by the work of Prakash (10), who demonstrated that photoreactivation of UV-exposed cells led to reduced numbers of T4 UV-endonuclease-specific sites (presumably dimers) in yeast mitochondrial DNA.

Although these results argue strongly that dimers induced in mitochondrial DNA initiate the petite mutational sequence and that the photorepair of mitochondrial DNA by UV-A (320- to 400-nm) light is effected by the photoreactivation enzyme in mitochondria, a search for this enzyme activity in purified yeast mitochondria was unsuccessful (3). A reexamination of this latter result might be made, allowing for the possibility that the activity of the photoreactivation enzyme in mitochondria might be very low, that the enzyme might not always be present in mitochondria, or that the mitochon-

drial photoreactivation enzyme might require different assay conditions. This last point prompts the question of whether the photoreactivation enzyme which is responsible for the photorepair of UV-induced nuclear damage, leading to increased cell survival, also repairs UV-induced mitochondrial damage, resulting in fewer petite mutants. Accordingly, in this work we compared the photorepair of petite mutational damage in wild-type (*phr*⁺) *S. cerevisiae* strain X2180 and photoreactivation-deficient diploid mutant strain CP032.

The photoreactivation deficiency was induced by first treating strain CP0214 *α leu1 ilva* with ethyl methane sulfonate to yield strain CP052 *α leu1 ilva phr*. The photoreactivation-deficient mutation was considered to be in the *phr1* locus (11), as crosses of CP053-1D *α leu1 phr*, derived from CP052, with strain C457-4c *α lys1 phr1* (obtained from Allen P. James) yielded diploid progeny which did not exhibit photoreactivation. The homozygous photoreactivation-deficient diploid strain CP032 *phr1/phr1 his/HIS leu1/LEU1* was then prepared by crossing strain CP052 with strain CP011 *α his*, prepared from X2180-1A, followed by repeated backcrossing of derived *phr* haploid clones with CP011 or with strain CP012 *α leu1*, prepared from X2180-1B. Haploid strains X2180-1A and X2180-1B and diploid strain X2180 were obtained from Robert K. Mortimer.

Cells for irradiation were grown for 72 h in 2% malt extract (Difco Laboratories, Detroit, Mich.) on a rotary shaker at 30°C, harvested, washed once, and suspended in sterile distilled water at 10⁶ cells per ml. A 10-ml amount of this suspension, stirred magnetically in a 100-ml beaker, was irradiated at an incident fluence rate of 0.4 J m⁻² s⁻¹ with a General Electric germicidal lamp (G15T8-15W). Samples of 0.4 ml were removed and placed in sterile glass tubes (16 by 125 mm). Amounts of 0.1 ml from each tube were diluted in distilled water and immediately

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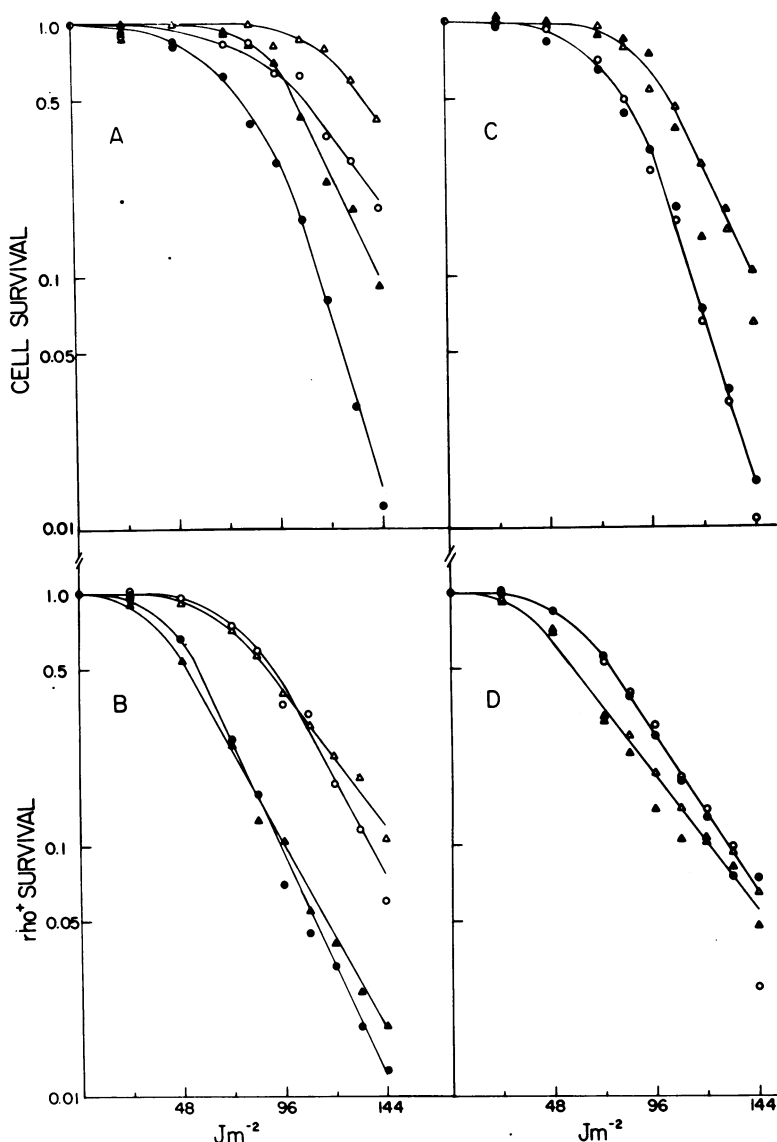


FIG. 1. Effects of black-light exposure and dark liquid holding on the survival of yeast cells and on the ρ^+ fraction among the survivors after UV irradiation. (A and B) PHR/PHR diploid strain X2180; (C and D) $phr1/phr1$ diploid strain CP032. Post-UV treatment: ●, immediate plating; ▲, 48 h of dark liquid holding; ○, 30 min of black light; △, 30 min of black light, followed by 48 h of dark liquid holding.

plated in quadruplicate on yeast extract-malt extract agar (14). The remaining fractions of irradiated samples were then subjected to dark liquid holding at 30°C or to photoreactivation and then diluted and plated. Photoreactivation of samples in horizontal glass tubes was carried out for 30 min at room temperature, with occasional shaking. The incident fluence rate of $10 \text{ J m}^{-2} \text{ s}^{-1}$ was generated by two General Electric

bulbs (F15T8-BLB). Light intensities were measured with Blak Ray UV intensity meters (models J-221 and J-225; UV Products, Inc., San Gabriel, Calif.).

After 4 days of growth, colonies were overlaid with triphenyltetrazolium chloride agar (7). White colonies were scored as ρ^0 , and both red and variegated colonies were scored as ρ^+ , as previously described (1).

To show unequivocally that UV-induced petite mutational damage can be photorepaired, it was necessary to distinguish between photorepair of lethal damage, presumably nuclear, and of mutational damage, presumably mitochondrial. The most suitable system for permitting this distinction would be one in which a relatively high incidence of mutants could be induced at UV fluences which would not kill cells. An increased frequency of respiratory-competent cells after photorepair would obviously be due to repaired mutational damage. Haploid yeast cells did not meet this requirement, as cell killing due to UV exposure occurred at much higher rates than did petite mutant induction. Diploid cells, being more UV resistant, permitted the appearance of petite mutants among the survivors at rates which were slightly higher than the rate of cell killing at fluences of less than 96 J m^{-2} (Fig. 1A and B). Thus, the plots of cell survival (Fig. 1A) and of the *rho*⁺ fraction among the survivors (Fig. 1B) after UV exposure with and without photoreactivation indicated that photorepair of petite mutational damage had occurred. The UV fluences, e.g., between 48 and 96 J m^{-2} , which induced a high incidence of petite mutants among the survivors, permitted a clear recognition of fewer mutants due to photoreactivation, but they also killed many cells. To circumvent the difficulty of attributing the increase in *rho*⁺ cells among survivors to photorepair of mutational or lethal damage, previously discussed for dark repair in yeast mitochondria (4), a period of dark liquid holding (8) was employed. Moustacchi and Enteric (6) have shown that when UV-irradiated *S. cerevisiae* suspensions of stationary-phase cells are held in the dark for a period before plating, cell survival increases, but the fraction of petite mutants among the survivors remains the same or may even increase, due to degradation of mitochondrial DNA (13). Lethal damage is repaired, but petite mutational damage is not. Our results with irradiated cells subjected to dark liquid holding (Fig. 1A and B) show this differential repair. When UV-irradiated cells were exposed to photoreactivating light before dark liquid holding, virtually no cell killing occurred at UV fluences of up to 96 J m^{-2} (Fig. 1A). The large increase in *rho*⁺ survivors (Fig. 1B) was therefore clearly due to photorepair of petite mutational damage.

The diploid strain CP032, homozygous for the *phr1* mutation, was subjected to the same regimen of irradiation and dark liquid holding (Fig. 1C and D). Virtually 100% cell survival occurred at UV-C fluences of up to 72 J m^{-2} when irra-

diation was followed by dark liquid holding with or without prior exposure to UV-A light. The incidence of petite mutants was unaltered by exposure to photoreactivating light. These results clearly suggested that the product of the nuclear gene, *PHR1*, was required for photorepair of petite mutational damage. However, strains X2180 and CP032 are probably not isogenic, exclusive of the *phr1* locus. As we have no *phr*⁺ revertant of CP032, we cannot rule out the possibility that other genetic differences have influenced our results.

Assuming that petite mutational damage occurs in the mitochondrial DNA and is photorepaired in the mitochondria, our finding implies that the same photoreactivation enzyme is responsible for photorepair of UV-induced damage to DNA in the nucleus and the mitochondria. This finding also raises the question of the manner and timing of the transport of this repair enzyme, which may consist of two subunits (2, 5), into mitochondria.

LITERATURE CITED

1. Allen, N. E., and A. M. MacQuillan. 1969. Target analysis of mitochondrial genetic units in yeast. *J. Bacteriol.* **97**:1142-1148.
2. Boatwright, D. T., J. J. Madden, J. Denson, and H. Werbin. 1975. Yeast DNA photolyase: molecular weight, subunit structure, and reconstruction of active enzyme from its subunits. *Biochemistry* **14**:5418-5421.
3. Daily, O. P., F. F. Cuttitta, Jr., and A. M. MacQuillan. 1976. The absence of DNA photoreactivation enzyme in yeast mitochondria. *Biochim. Biophys. Acta* **454**:375-381.
4. Hixon, S. C., D. Gaudin, and K. L. Yielding. 1975. Evidence for the dark repair of ultraviolet damage in *Saccharomyces cerevisiae* mitochondrial DNA (39065). *Proc. Soc. Exp. Biol. Med.* **150**:503-509.
5. Iwatsuki, N., C. O. Joe, and H. Werbin. 1980. Evidence that deoxyribonucleic acid photolyase from baker's yeast is a flavoprotein. *Biochemistry* **19**:1172-1176.
6. Moustacchi, E., and S. Enteric. 1970. Differential "liquid holding recovery" for the lethal effect and cytoplasmic "petite" induction by UV light in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **109**:69-83.
7. Ogur, M., R. St. John, and S. Nagai. 1957. Tetrazolium overlay technique for population studies of respiration deficiency in yeast. *Science* **125**:928-929.
8. Patrick, M. H., R. H. Haynes, and R. B. Uretz. 1964. Dark recovery phenomena in yeast. I. Comparative effects with various inactivating agents. *Radiat. Res.* **21**:144-163.
9. Pittman, D., B. Ranganathan, and F. Wilson. 1959. Photoreactivation studies on yeast. II. Photoreactivation of the ultraviolet damage producing respiratory deficiency in haploid and tetraploid yeasts. *Exp. Cell Res.* **17**:368-377.
10. 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.
11. Resnick, M. A. 1969. A photoreactivationless mutant of *Saccharomyces cerevisiae*. *Photochem. Photobiol.* **9**:307-312.

12. **Sarachek, A.** 1958. The induction by ultraviolet radiation and the photoreactivation of heritable respiratory deficiency in *Saccharomyces* adapted and unadapted to aerobic respiration. *Cytologia* **23**:143-158.
13. **Waters, R., and E. Moustacchi.** 1974. The fate of ultraviolet-induced pyrimidine dimers in the mitochondrial DNA of *Saccharomyces cerevisiae* following various post-irradiation cell treatments. *Biochim. Biophys. Acta* **366**:241-250.
14. **Wickerham, L. J.** 1951. Taxonomy of yeasts. U.S. Department of Agriculture technical bulletin no. 1029. U.S. Department of Agriculture, Washington, D.C.