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Photoreactivation and Gene Dosage in Yeast

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The amount of photoreactivating enzyme in tetraploid cells of *Saccharomyces cerevisiae* and the ability of the cells to be photoreactivated after ultraviolet irradiation are directly proportional to the number of genes per cell involved in the synthesis of photoreactivating enzyme.

Pyrimidine dimers have been shown to be induced in the deoxyribonucleic acid (DNA) of *Saccharomyces cerevisiae* by ultraviolet (UV) radiation (11). This damage can be repaired by a dark-repair mechanism that alters or removes the dimers or by a light-repair system (photoreactivation) that monomerizes them. The splitting of the dimers is mediated by photoreactivating enzyme (PR; for review see reference 12), an enzyme that can be removed in yeast by a mutation in the *PHR1* gene (9). In the present study, we have examined the relationship between the number of functional *PHR1* genes and the PR enzyme activity in tetraploid cells of *S. cerevisiae* by measuring the ability of irradiated cells to be photoreactivated and by determining the specific activity of PR enzyme in crude cell extracts. Previously it was shown that the amount of enzyme varies with the phase of growth of the culture (1).

The methods of growth (9), measurement of survival (10), and preparation of crude extracts (1) have been reported. Cells were grown to stationary phase (2 to 4 days). Protein concentrations in crude extracts were adjusted to 1.5 mg/ml. The PR enzyme activity was measured by the method of Muhammed (6), by determining the ability of the enzyme to photoreactivate UV-irradiated transforming DNA. To 0.1 ml of extract was added 0.1 ml of *Haemophilus influenzae* DNA which had been exposed to 3,000 ergs/ml[2] at 254 nm (13), and the mixture was exposed to photoreactivating light (11). Samples were withdrawn after various times and assayed for ability to transform cells to streptomycin resistance. The relative activity was determined as the ratio of the number of transformants after 10 min of exposure to the number after no exposure. Measurements of in vivo PR enzyme were made by exposing UV-irradiated cells (10^7/ml) to short periods of photoreactivating light (0 to 8 min, reference 11) and determining survival. The dose reduction factor (4) after 1 min of photoreactivation of cells previously exposed to 20 sec of UV (5 ergs per mm^2 per sec at 254 nm) was used as a measure of the relative amount of PR enzyme in the cells.

Tetraploid stocks with zero to four *PHR1* genes were formed by forced mating of auxotrophic diploids on nutritionally defined medium that allowed only the growth of the corresponding tetraploids (8). No colonies were observed when only one or the other diploid was plated to the defined medium. That the strains were tetraploid was further verified by tetrad analysis of spores from the tetraploids (except for the *PHR1/PHR1/phr1/phr1* strain, which exhibited poor sporulation). The diploids were originally formed from haploid strains of opposite mating type that were *rad2-17* (formerly *uvs9-3*; references 2, 10) and were either *PHR1* (wild types) or *phr1* (nonphotoreactivable, reference 9). These haploid strains therefore lacked the ability to excise UV-induced pyrimidine dimers (11) and were either capable or incapable of photoreactivating these dimers.

The ability of the crude extracts obtained from strains having zero to four *PHR1* genes to photoreactivate the transforming ability of UV-irradiated transforming DNA is seen in Fig. 1. The strain with four *PHR1* genes clearly has the greatest amount of PR enzyme, and there is progressively less enzyme with a decrease in the number of *PHR1* genes.

The ability to survive UV exposure was not very different among the five tetraploid strains examined, the greatest difference being 1.5 in terms of dose required to kill 99% of the cells. An example of survival of one of the tetraploids (four *PHR1* genes) is seen in Fig. 2; in-

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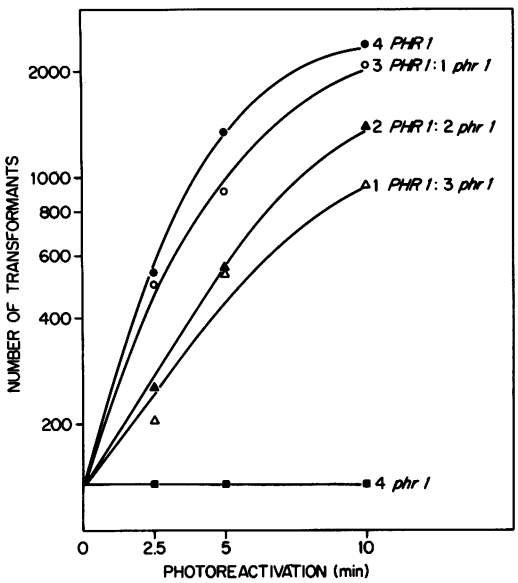

FIG. 1. *Photoreactivation of UV-irradiated transforming DNA by crude extracts of tetraploid yeast having from zero to four PHR1 genes.*

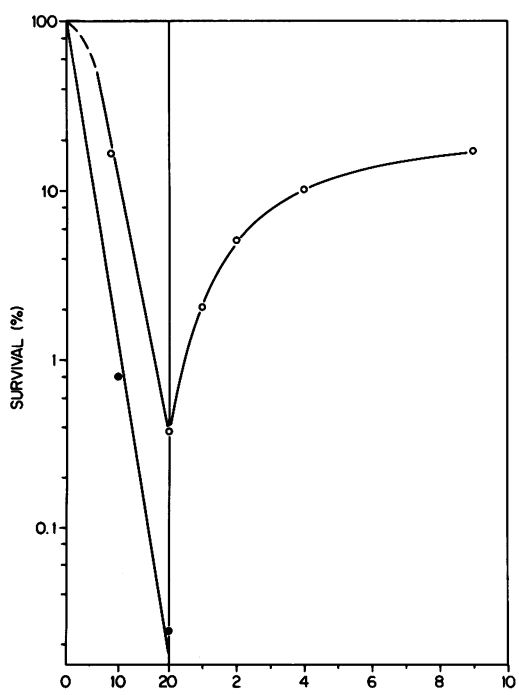

FIG. 2. *Survival after UV irradiation of a haploid strain (●) or UV irradiation and photoreactivation of a tetraploid strain (PHR1/PHR1/PHR1/PHR1; ○).*

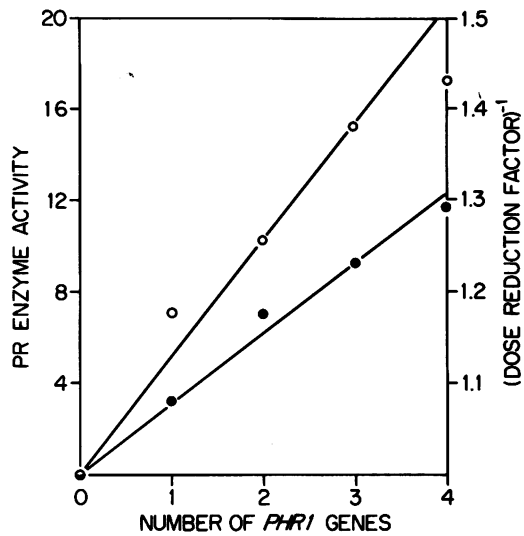

FIG. 3. *Photoreactivating enzyme activity as determined in vitro (○ summarized from Fig. 1) or in vivo (●) by (dose reduction factor)$^{-1}$ in survival (100 ergs/mm^2) after 1 min of photoreactivation.*

cluded for comparison is a *rad2-17* haploid strain. Photoreactivation after 20 sec of exposure to UV leads to a very large increase in survival; after only 8 min, it increases 50-fold. As shown in Fig. 3, the ability to increase survival by photoreactivation is related to the number of functional *PHR1* genes in the cell. From the in vitro measurements of PR enzyme and the in vivo action of the enzyme, we conclude that the amount of enzyme synthesized in a cell and the amount of enzyme available for in vivo photoreactivation of survival are proportional to the number of functional *PHR1* genes present. Similar gene-dosage effects as measured in diploids and tetraploids have been found for galactokinase (7) and methionine permease (3), and for enzymes involved in pyrimidine biosynthesis (5).

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