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Expression of a *Saccharomyces cerevisiae* Photolyase Gene in *Escherichia coli*

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A 3.3-kilobase *Pvu*II fragment carrying the *PHR1* gene of *Saccharomyces cerevisiae* has been cloned into an *Escherichia coli* expression vector and introduced into *E. coli* strains deficient in DNA photolyase. Complementation of the *E. coli phr-1* mutation was observed, strongly suggesting that the yeast *PHR1* gene encodes a DNA photolyase.

Cis-syn cyclobutane dipyrimidines (pyrimidine dimers) are induced in DNA by 254-nm irradiation and are one of the primary lesions responsible for UV-induced carcinogenesis, mutation, and cell lethality. Enzymatic photoreactivation, mediated by DNA photolyases, is one pathway by which these lesions are repaired; photolyase binds to pyrimidine dimers in DNA and upon exposure to 300- to 600-nm light splits the cyclobutane ring and restores the pyrimidines to the monomeric form (for a review, see reference 20). In *Saccharomyces cerevisiae* two loosely linked genes, *PHR1* (9) and *PHR2* (6), have been implicated in photoreactivation; however, it is not known whether these are structural or regulatory genes, nor has the relationship of these genes to the two photolyases (I and II) from *S. cerevisiae* been established. I have attempted to answer these questions for the *PHR1* gene by introducing the cloned gene into *Escherichia coli* strains which lack *E. coli* photolyase by virtue of either point mutation or deletion but which produce the chromophore of the *E. coli* enzyme (14).

Plasmid YEp13-*PHR1* (generously provided by David Schild) contains an ~6-kilobase (kb) insert of *S. cerevisiae* DNA carrying the *PHR1* gene. Subcloning experiments indicated that the *PHR1* gene is contained on a 3.3-kb *Pvu*II fragment from this insert (16). For the purpose of expressing the *PHR1* gene in *E. coli*, I subcloned this fragment into the expression vector pUNCO9 (15), a pBR328 derivative which carries the *tac* promoter and the *lacZ* ribosome-binding site immediately 5′ to a unique *Pvu*II site (12). The construction of the resulting plasmids pGBS100 and pGBS101, which contain the 3.3-kb *Pvu*II fragment in opposing orientations relative to *tac*, is shown in Fig. 1. These plasmids were isolated and propagated in *E. coli* K12 strain CSR603 F′ *lacI*^{q} (*recA1 uvrA6 phr-1* Pro$^+$; 10–12).

To determine whether the *S. cerevisiae PHR1* gene complements the *phr-1* mutation in strain CSR603, plasmid-containing strains were grown in Luria broth plus 20 μg of tetracycline per ml to an absorbance value at 600 nm of 0.4, at which time isopropylthio-β-D-galactoside (IPTG) was added to 5 mM. (The *tac* promoter is under the control of the *lac* repressor and thus is induced by IPTG.) After 2 h of further growth, the cells were washed and resuspended in phosphate-buffered saline to an absorbance value at 600 nm of 0.3 and irradiated with a General Electric germicidal lamp. Samples were either held in the dark for 1 h or exposed to photoreactivating light for 1 h and then were diluted and plated on Luria broth agar; survivors were counted after 24 h of incubation at 37°C. All operations during and subsequent to the 254-nm of irradiation were performed under yellow light (15). The results of this experiment are shown in Fig. 2; as can be seen, strains carrying either pGBS100 or pGBS101 display significant photoreactivation. That this effect is plasmid mediated is indicated by the fact that when purified plasmid DNA was used to transform CSR603 F′ *lacI*^{q}, 100% (10 of 10) of the colonies obtained with either plasmid were Phr$^+$ (data not shown). Furthermore, photoreactivation is not the result of suppression of the *phr-1* mutation, as the presence of either plasmid in strain MCL21 F′ *lacI*^{q} (*recA* Δ*uvrB* Δ*phr*, kindly provided by M. C. Lorence and C. S. Rupert) also conferred a Phr$^+$ phenotype.

Strains carrying plasmid pGBS101 consistently displayed slightly greater photoreactivation than did strains containing pGBS100. To determine whether this resulted from transcription from the *tac* promoter, I used the flash photolysis technique of Harm et al. (3) to count the number of enzyme-substrate complexes present 10 min after exposure to 254-nm irradiation. Only the strain carrying pGBS101 displayed IPTG-dependent enhancement of photoreactivation, indicating that in this plasmid the photolyase gene is in the same orientation as *tac* (Fig. 3A and B). This result establishes the direction of transcription of the photolyase gene in pGBS101 as counterclockwise relative to the plasmid map. The fact that some photoreactivation is observed in the absence of induction in strains carrying either plasmid (Fig. 3A) suggests that a promoter and ribosome-binding site recognized by the *E. coli* transcription and translation systems lie upstream of *PHR1* and within the 3.3-kb *Pvu*II fragment; a similar observation has been made for the yeast *HIS3* gene (18, 19). Induction after the addition of IPTG in plasmid pGBS101 presumably results from increased transcription initiating from the *tac* promoter and extending through *PHR1*. The flash photolysis technique can be used to determine the mean number of active photolyase molecules per cell, provided that at some point the number of dimers repaired reaches a constant value which is not dependent on the UV dose (i.e., the number reaches constant dose decrement as defined by Harm et al. [3]); under such conditions the substrate (dimers) is saturating. From the data shown in Fig. 3B, the number of dimers pre- and post-photoreactivation were calculated according to the formula $S/S_0 = e^{-D}$ where S/S_0 is the ratio of the survival of the UV-irradiated culture (+ or − photoreactivation) to the nonirradiated culture and D is the mean number of dimers per genome. Thus, it was found that the mean number of dimers repaired

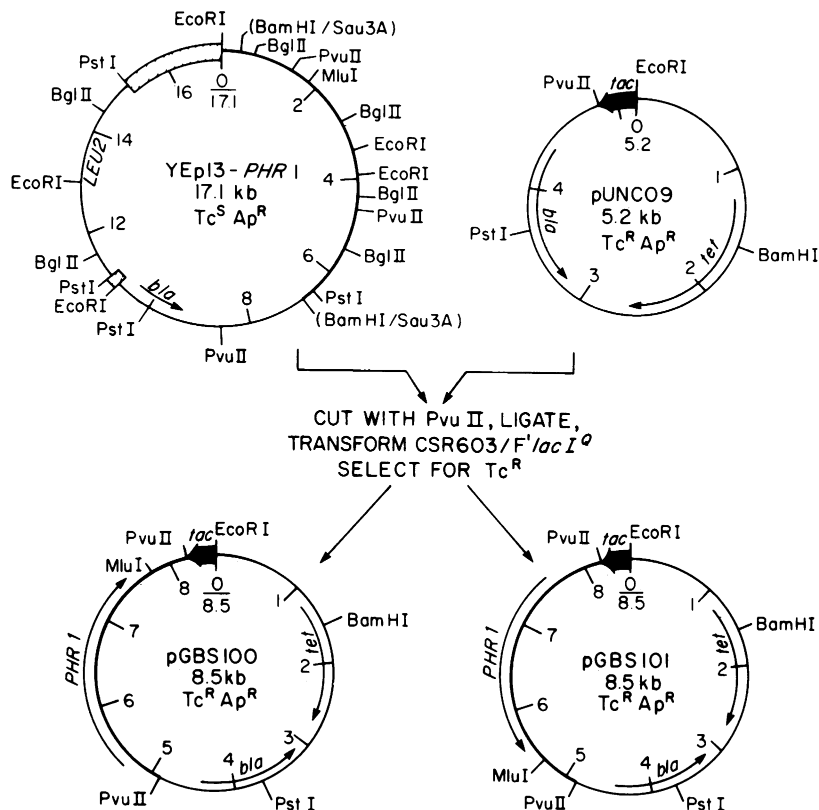

FIG. 1. Construction of plasmids pGBS100 and pGBS101. The restriction map of YEp13-*PHR1* is from reference 16, except that the 3.3-kb *Pvu*II fragment carrying *PHR1* is inverted compared with the map in reference 16, based upon restriction mapping performed in this laboratory; this orientation has been confirmed by David Schild (personal communication). Tetracycline-resistant transformants were analyzed by restriction endonuclease mapping. Symbols: solid box with arrow, position and direction of transcription from the *tac* promoter; heavy line, chromosomal insert from *S. cerevisiae*; hatched region, region from the yeast endogenous 2-μm DNA plasmid. In plasmids pGBS100 and pGBS101 the arrow for *PHR1* indicates the direction of transcription but not the extent of the gene.

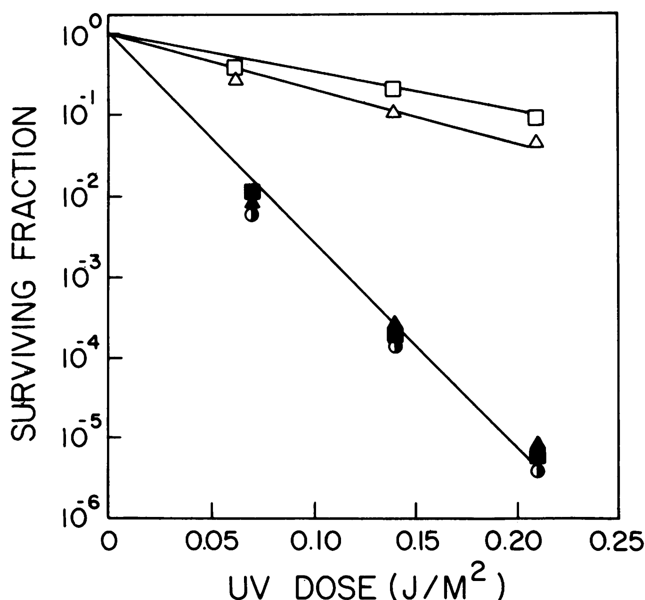

FIG. 2. UV survival of CSR603 F' *lacI*^{q} containing various plasmids. Cells were grown, induced, and irradiated with stirring as described in the text; the incident dose rate of 254 nm of light was 10^{-2} J/cm^2 per s and that of photoreactivating light (supplied by two Sylvania Black Light bulbs) was 2 J/cm^2 per s. The Morowitz correction (8) was used to determine the actual dose received by the cells. Symbols: ○, pUNCO9; △, pGBS100; □, pGBS101. Closed symbols, cultures that were irradiated with 254 nm of light and then held in the dark for 1 h; open symbols, cells that were irradiated with 254 nm of light and then exposed to photoreactivating light for 1 h.

at saturating substrate and thus the number of photolyase molecules per cell was 2.5 in the case of the strain carrying pGBS100, whereas the minimum number for the strain carrying pGBS101 was 7.3 (constant dose decrement was not reached for this strain).

The results reported here strongly suggest that the *PHR1* gene of *S. cerevisiae* encodes a DNA photolyase, although at present the existence of a photolyase adjacent to the *PHR1* gene on the 3.3-kb *Pvu*II fragment cannot be ruled out. Yasui and Chevallier (21) were unable to detect complementation by the *PHR1* gene of the *phrA* mutation in *E. coli*. The discrepancy between their results and those presented here probably resides in the fact that strains carrying the so-called *phrA* mutation retain full photoreactivating activity (11, 17) and that the relatively low level of photoreactivating activity seen in the absence of induction is difficult to detect except in a *recA uvr*$^-$ background. Two photolyases, designated I and II, have been isolated from *S. cerevisiae* (2, 7); the former is a single polypeptide with an M_r of 53,000 (4, 7), whereas the latter is a dimer composed of polypeptides with

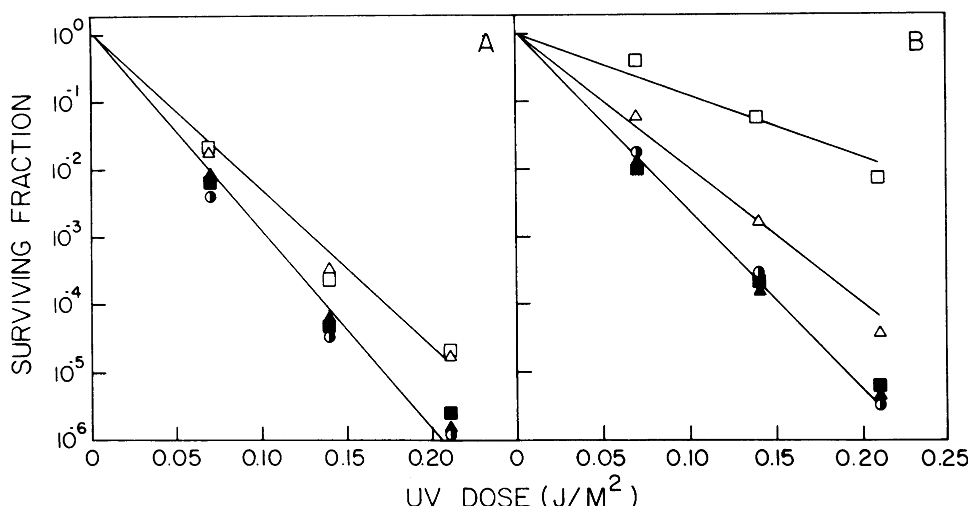

FIG. 3. UV survival after flash photolysis of CSR603 F′ *lacI*q containing various plasmids. (A) Cells were irradiated without prior induction with IPTG; 10 min after 254 nm of irradiation, the cells were exposed to two simultaneous flashes from Yashika Pro50 flash units. (B) Cells were irradiated and flashed as in (A), except that 2 h before irradiation the cells were induced with IPTG. Symbols are the same as those described in the legend to Fig. 2.

M_rs of 54,000 and 82,000, neither of which has activity alone (2, 20). Since at least 3.8 kb of DNA, excluding nontranslated regions, would be required to code for the two subunits of photolyase II, it is likely that the *PHR1* gene encodes photolyase I. The chromophore of photolyase I is a flavin (4); in this respect the enzyme is similar to *E. coli* photolyase, which contains a neutral flavin free radical (5, 13). Thus, it is probable that when the *PHR1* gene is expressed in *E. coli*, the chromophore of the enzyme is provided by the host.

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