JOURNAL OF BACTERIOLOGY, Oct. 1990, p. 6061–6065
0021-9193/90/106061-05$02.00/0
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Vol. 172, No. 10

Functional Expression of 8-Hydroxy-5-Deazaflavin-Dependent DNA Photolyase from *Anacystis nidulans* in *Streptomyces coelicolor*

FRIEDRICH MAYERL,[1] JACQUELINE PIRET,[2] ANDREAS KIENER,[1†] CHRISTOPHER T. WALSH,[1*] AND AKIRA YASUI[3]

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 25 Shattuck Street,[1] and Department of Biology, Northeastern University,[2] Boston, Massachusetts 02115, and The Research Institute for Tuberculosis and Cancer, Tohoku University, 4-1 Seiryomachi, 980 Sendai, Japan[3]

Received 26 March 1990/Accepted 10 July 1990

The gene encoding *Anacystis nidulans* 5-deazaflavin-dependent photolyase (*phr*) was inserted into the *Streptomyces* vector pIJ385 to form a transcriptional fusion with the neomycin resistance (*aph*) gene. The resulting plasmid, pANPL, was introduced into *Streptomyces coelicolor*, a host which exhibits no detectable photolyase activity and provides 5-deazaflavins. Transformants expressed functional photolyase and could be cultured at much higher cell densities than *A. nidulans*. A two-step affinity protocol was used to purify photolyase to homogeneity. High-pressure liquid chromatographic analysis established the presence of 5-deazaflavin cofactors in the enzyme, showing that this expression system allows heterologous production of 5-deazaflavin-class photolyases.

Biological photoreactivation is a light-dependent process which repairs DNA damage associated with the presence of intrastrand pyrimidine dimers. These dimers are formed by photochemical cycloaddition of adjacent pyrimidine bases, in a reaction triggered by UV irradiation. In the enzyme-mediated reversal of this process, photolyases harvest energy from the near-UV and visible portion of the spectrum to effect catalytic cleavage of the cyclobutane ring of the dimer in a transformation which reestablishes the two pyrimidine bases and restores the DNA to its intact coding form.

Photoreactivation repair has been found in many organisms (7), and several DNA photolyases have now been isolated and characterized. Two recent reviews summarize structural and functional aspects of these enzymes (22, 25). Based on their cofactor content and attendant λ_{max} for photorepair, two classes of photolyases can be distinguished, (i) a folate class ($\lambda_{max} \sim 390$ nm) whose representatives contain the cofactors 5,10-methenyltetrahydrofolate and reduced flavin adenine dinucleotide ($FADH_2$) (26), and (ii) a 5-deazaflavin class whose members incorporate an 8-hydroxy-7-desmethyl-5-deazaflavin coenzyme (F_{420}, F_0; $\lambda_{max} \sim 440$ nm) (34) and $FADH_2$. The 5-deazariboflavin F_0 is distinct from riboflavin by replacement of the N-5 nitrogen of the isoalloxazine with a carbon atom and by deletion of the 7,8-dimethyl substituents. F_{420} is a derivative of F_0 carrying a lactyl-oligoglutamyl group attached via a phosphodiester linkage to the ribityl side chain. It has been shown for *Escherichia coli* photolyase that $FADH_2$ is the physiologically significant form of the flavin cofactor in the enzyme (23, 28). The presence of FAD and FADH• species in pure enzyme results from oxidation during isolation. Photolyases from *E. coli* and *Saccharomyces cerevisiae* belong to the folate class (14), whereas the enzymes from *Methanobacterium thermoautotrophicum* (15), *Anacystis nidulans* (21), *Streptomyces griseus* (4), *Scenedesmus acutus* (5), and *Halobacterium halobium* (13) are members of the 5-deazaflavin class.

The first photolyase genes to be cloned and sequenced were for the folate-containing enzymes of *E. coli* (30) and *S. cerevisiae* (29). Subsequently, genes of the 5-deazaflavin class from *A. nidulans* (36), *S. griseus* (16), and *H. halobium* (31) were also isolated. A sequence alignment of the five photolyases (31) displayed most significant homology in the C-terminal portion, suggesting that common structural and functional domains were located in that region.

Only very small amounts of wild-type photolyase have been isolated from naturally producing organisms. For example, a concentration of 10 molecules per cell has been estimated for the *E. coli* enzyme (8). Photolyase from cultures of *M. thermoautotrophicum* (15) can be purified in yields of ca. 20 μg/liter. These results indicated that suitable overproducing systems would be required to obtain sufficient quantities of pure enzyme needed for detailed structural and mechanistic studies.

Photolyases of the folate class have been overproduced in *E. coli*, a host which provides both the requisite flavin and folate coenzymes (27, 29). However, the lack of detectable 8-hydroxy-5-deazaflavins in *E. coli* (32) renders this host unsuitable for the overexpression of a photolyase of the deazaflavin class. When the *A. nidulans* photolyase gene was expressed in *E. coli* CSR603, a strain defective in major photoreactivation activity (11), the in vivo action spectrum of the recombinant enzyme showed a maximum at 380 nm (32) as opposed to the maximum at 437 nm of the wild-type enzyme. This shift indicated that, in the absence of the deazaflavin cofactor, folate may have been incorporated by the apoenzyme. Alternatively, the new maximum may be due to the presence of the semiquinone FADH• form of the flavin cofactor.

Methanobacteria contain high levels of 5-deazaflavins (35) and would be very suitable hosts for the expression of the *A. nidulans* photolyase gene, since the apoenzyme formed could draw upon an abundant supply of the requisite cofactor for incorporation. However, with limited tools available for in vitro genetic manipulation in these archaeobacterial organisms, our attention has focused on the use of a *Strep-*

* Corresponding author.
† Present address: Department of Biotechnology, Lonza AG, 3930 Visp, Switzerland.

tomyces host in which successful expression of heterologous genes has been reported (12). In this genus, significant levels of 5-deazaflavins have been determined (3) and their roles as essential coenzymes in the biosynthesis of antibiotics of the tetracycline (19) and lincomycin classes (2) have been established. The high G+C content of the DNA of these organisms apparently does not prevent good expression levels of genes with a lower G+C content. This was an important consideration since the G+C content of the *A. nidulans* gene is 58.6% compared with 74.1% for the *phr* gene from *S. griseus* (16). Furthermore, due to their industrial significance, much accumulated knowledge about optimal growth conditions for the cultivation of *Streptomyces* species is available, facilitating high-yield preparation of cell mass for the isolation of proteins.

To obtain gene expression and eventually overproduction of the deazaflavin class of DNA repair enzymes, we undertook the heterologous expression of the *A. nidulans* photolyase gene in a host providing 5-deazaflavins. This report describes such a system in *Streptomyces coelicolor*, as well as the purification and yields of the recombinant photolyase.

MATERIALS AND METHODS

Bacterial strains. The *Streptomyces* strains used, *S. lividans* 66 (John Innes strain 1326) (17) and *S. coelicolor* J650 (20), were from the John Innes Institute (Norwich, United Kingdom) culture collection.

Plasmids and DNA manipulations. The source of the *A. nidulans* photolyase gene was pUC18 carrying a ca. 2-kilobase *Hin*cII fragment of *A. nidulans* DNA containing the *phr* gene (36). After the digestion of this plasmid with *Xba*I and *Kpn*I, the *phr* fragment was purified from an agarose gel by electroelution and phenol extraction and ligated into plasmid pIJ385 (10) digested with the same enzymes. The ligation mixture was transformed (10) into *S. lividans* 1326, in which the expected recombinant plasmid (pANPL) was obtained, and its structure was verified by restriction enzyme digestions. pANPL was also transformed into *S. coelicolor* J650.

Chemicals and media. Yeast extract, Bacto-Peptone, and Bacto-Agar were purchased from Difco Laboratories (Detroit, Mich.). Tryptic soya broth and malt extract were from Oxoid USA, Inc. (Columbia, Md.). Thiostrepton was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Phenylmethylsulfonyl fluoride was from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Neomycin sulfate, tetracycline, and heparin agarose were from Sigma Chemical Co. (St. Louis, Mo.). UV–single-stranded DNA agarose was prepared as previously described (15). TSA agar contained (per liter) 30 g of tryptic soya broth, 15 g of Bacto-Agar, and 25 mg of thiostrepton. TSB-glucose medium contained (per liter) 30 g of tryptic soya broth and 10 g of glucose. YEME was as described previously (10). R2YE liquid medium was prepared according to the published recipe (10) for R2YE agar medium except for the following modifications: 10 g of yeast extract (Difco) per liter were used and the agar, K_2SO_4, $MgCl_2$, and Casamino Acids (Difco) were eliminated. Liquid media were supplemented to a final concentration of either 5 μg of thiostrepton per ml or 1 μg of neomycin per ml when appropriate.

Buffers. Photolyase buffer (PL buffer) was composed of 50 mM Tris (pH 7.5), 1 mM EDTA, 10 mM 2-mercaptoethanol, 10% glycerol (vol/vol), and various levels of NaCl; the corresponding NaCl molarities are indicated in the text as a prefix to PL buffer, e.g., 1 M PL buffer is PL buffer containing 1 M NaCl.

Protein concentrations. Total protein concentrations in cell extracts and in fractions from the heparin agarose column were determined by the method of Bradford (1) with the reagent purchased from Bio-Rad Laboratories (Richmond, Calif.). The concentration of photolyase in fractions from the UV-DNA agarose column was measured spectrophotometrically assuming a 1:1 stoichiometry between enzyme and deazaflavin cofactor and an extinction coefficient ε_{433} = 40,000 M^{-1} cm^{-1} (15).

Photolyase assay. Photoreactivation was measured by the transformation assay (25) employing the protocol described for the isolation of *M. thermoautotrophicum* photolyase (15).

Growth of *Streptomyces* strains containing pIJ385 and pANPL. Seed cultures were inoculated with mycelium produced on TSA plates (2 days, 28°C), a medium favoring mycelium formation over sporulation. Seed cultivation was in 30 ml of TSB medium supplemented with 1 μg of neomycin per ml or 5 μg of thiostrepton per ml in 250-ml baffled flasks shaken (250 rpm) at 28°C. Growth was monitored by optical density measurements at 600 nm of sonicated (20 W, 30 s; to disrupt mycelial pellets) culture samples prepared from 500 μl of culture, 500 μl of 2.5 M HCl, and 4 ml of water (18). An entire seed culture was used to inoculate 220 ml of the same medium in a 2-liter Erlenmeyer flask; these secondary cultures were grown at 28°C with shaking at 250 rpm for 36 h. Cells from four 250-ml cultures were pooled and chilled on ice before harvest (10,000 rpm, 20 min). Yields of ca. 45 g of wet cell weight per liter were obtained.

Purification of photolyase. All steps were done at 4°C except the UV-DNA affinity column, which was run at room temperature. Cells obtained from 1 liter of culture were suspended in an equal volume of 1 M PL buffer containing 0.1 mM phenylmethylsulfonyl fluoride. The high salt concentration ensured dissociation of photolyase from DNA in the cell debris. The suspension was passed through a French press three times, and the cell debris was removed by centrifugation at 100,000 × *g* for 2 h. The supernatant was dialyzed against ca. 40 volumes of PL buffer (containing no NaCl) to bring the salt concentration to less than 0.1 M. Subsequently, the affinity protocol described previously (15) was used, with two modifications. (i) The heparin column was run with a gradient of 0.1 to 1.5 M PL buffer, with photolyase being eluted by salt concentrations from 0.6 to 0.8 M. (ii) Photolyase was eluted from the UV-DNA agarose column with a 0.6 to 2 M PL buffer gradient. To exclude light, the column was wrapped in aluminum foil during loading and the initial part of the gradient. The foil was removed when the gradient reached 1 M. At that point, the column was illuminated with light as described (15) to promote release of the photolyase from the DNA and its elution in a small volume. Active fractions were concentrated with Centricon 30 tubes (Amicon Corp., Lexington, Mass.), and the buffer was simultaneously exchanged for 0.1 M PL buffer.

HPLC analysis of cofactor content. High-pressure liquid chromatographic (HPLC) analysis was derived from a previous report on the separation of flavin coenzymes (9). A Vydac Protein and Peptide Column (C_{18}, 5 μm, 0.46 by 22 cm; The Separations Group, Hesperia, Calif.) was used and eluted with a linear gradient from 90% A (A is 50 mM ammonium acetate, pH 6.7) plus 10% B (B is methanol) to 80% A plus 20% B in 10 min at a flow rate of 1 ml/min. After completion of the gradient, elution was continued isocratically with 80% A plus 20% B. The separation was monitored at 254 nm. Retention times were 24.8 min for FAD, 16.1 min

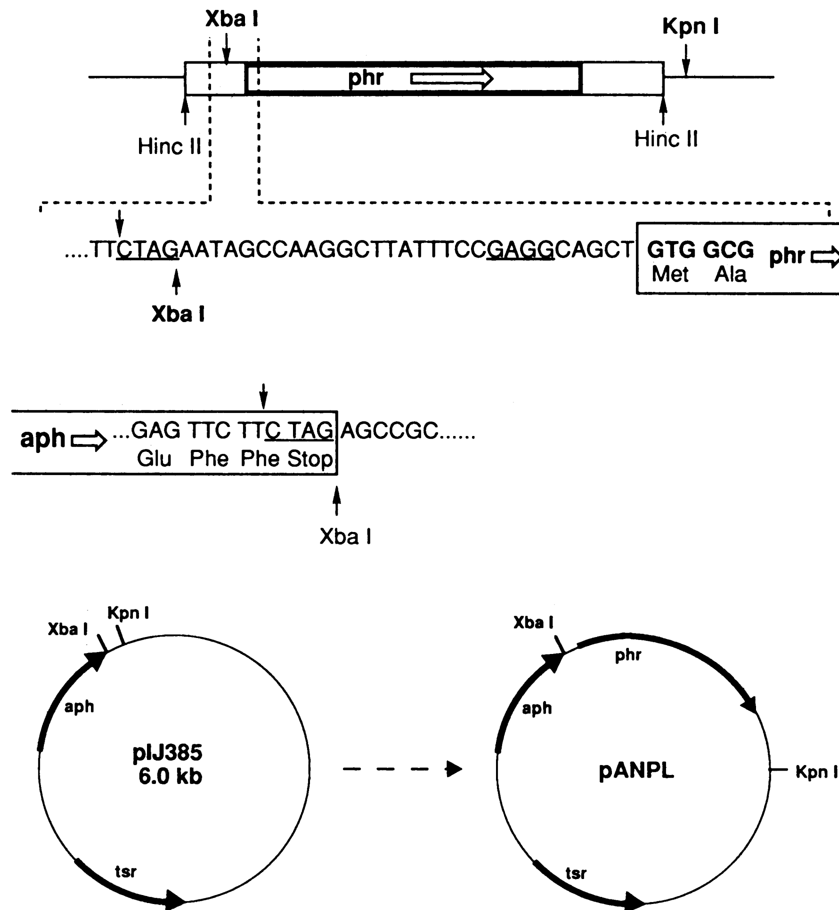

FIG. 1. Base sequence of *A. nidulans* DNA extending from the upstream *Xba*I site to the start codon of the *phr* gene. pANPL arises from replacement of the *Xba*I-*Kpn*I fragment of plasmid pIJ385 with the *phr* gene fragment. *aph* and *tsr* denote neomycin and thiostrepton antibiotic resistance markers, respectively. kb, Kilobases.

for F_0, and 8.8 min for F_{420}. To release the cofactors, we denatured the protein sample by heating it at 95°C for 5 min.

RESULTS AND DISCUSSION

The broad-host-range, multicopy *Streptomyces* plasmid vector pIJ385 was chosen for this work. It carries two antibiotic resistance determinants: a neomycin resistance gene, *aph*, and the thiostrepton resistance gene, *tsr* (10). The presence of *Xba*I and *Kpn*I sites at the downstream end of the *aph* gene allowed construction of a transcriptional fusion of the *A. nidulans* photolyase gene to this marker. Since the *Xba*I site lies in the *aph* stop codon, this placed the photolyase gene immediately downstream of the *aph* open reading frame with an intergenic space of only 29 base pairs (Fig. 1). The putative *phr* gene ribosome-binding site (GAGG) is preserved. Activation of transcription through both genes is provided by the *aph* promoter region, which has been shown to be strongly expressed and to be useful for the expression of heterologous genes (24, 33).

Two *Streptomyces* hosts, *S. lividans* 1326 and *S. coelicolor* J650, were initially selected for this study. Control strains of each organism carrying the pIJ385 plasmid without a *phr* insert were assayed in parallel for photolyase activity. None was detected in *S. coelicolor*, confirming an earlier observation that this organism exhibits no photoreactivation activity (A. P. M. Eker, personal communication). Extracts from *S. lividans* cells, however, showed *phr* activity sixfold higher than background. Consequently, we chose *S. coelicolor* as the host to avoid expressing the *A. nidulans* photolyase simultaneously with endogenous *S. lividans* enzyme. Growth of *S. coelicolor*(pANPL) was monitored in different media supplemented with thiostrepton to optimize cell yield. The yeast extract-based media YEME and R2YE proved inferior to the TSB-glucose medium, which supported maximum cell density (optical density at 600 nm, ~ 1.5) after 36 h of culture.

The TSB-glucose medium was subsequently used in cell production on a 1-liter scale, and to rule out an effect of antibiotic selection, cells were grown in cultures selecting for the presence of pANPL with either neomycin or thiostrepton. Crude cell extracts of recombinant *S. coelicolor* were subjected to affinity purification as described previously for wild-type photolyase from *M. thermoautotrophicum* (15). This method allows preparation of homogeneous enzyme from crude cell extracts in only two chromatographic steps, using heparin agarose in the first column and UV-DNA agarose in the second. When applied as described above, it initially led to an enzyme preparation contaminated with a high-molecular-weight protein (Fig. 2, lane 4) which could be separated by eluting the UV-DNA agarose column with a linear gradient as described in Materials and Methods (Fig. 2, lane 5). Purified recombinant *A. nidulans* photolyase

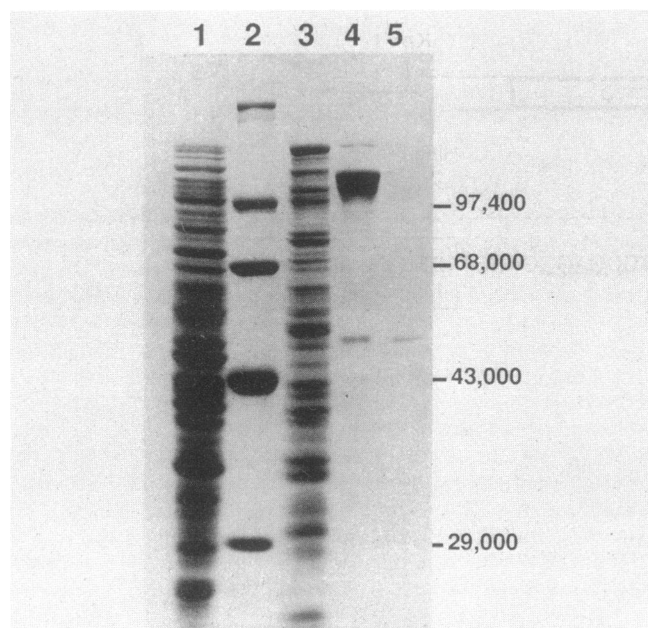

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of samples from purification of *A. nidulans* photolyase from *S. coelicolor*. Lanes 1, crude extract; 2, molecular weight standards; 3, active fractions from heparin agarose; 4, active fractions from UV-DNA agarose with a step gradient (15); 5, as in lane 4, but eluting with a linear gradient. Molecular weights are shown by the numbers on the right.

with a calculated M_r of 54,475 (36) comigrated (sodium dodecyl sulfate-13% polyacrylamide gel) with an *E. coli* photolyase reference with a calculated M_r of 53,991 (27) and was estimated to be >95% pure. The enzyme was functional in photoreactivation, as evidenced by the transformation assay used to monitor chromatographic purification. Based on the increase in the number of antibiotic-resistant colonies per microgram of protein, purification was estimated to be ca. 900-fold from neomycin-grown cells and ca. 1,600-fold from thiostrepton-grown cells.

TSB-glucose cultures (1 liter) of *S. coelicolor*(pANPL) supplemented with either thiostrepton or neomycin provided pure photolyase in quantities ranging from 50 to 100 μg. Table 1 summarizes the purification steps and protein yields obtained in the isolation of the heterologous enzyme. This final yield is three- to fourfold higher than the yield for isolation from wild-type *M. thermoautotrophicum* (15). These higher levels with *S. coelicolor* result from the much higher cell densities attainable with this host relative to other deazaflavin-class photolyase-containing organisms. When productivity is expressed in milligrams of pure enzyme per kilogram of wet cell mass, *S. coelicolor*(pANPL), wild-type *A. nidulans* (6), and wild-type *M. thermoautotrophicum* (15) all produce ca. 1 to 2 mg/kg. The more extensive cell growth of *S. coelicolor* should allow the preparation of milligram quantities of 5-deazaflavin-class photolyase by bench-scale fermentation.

TABLE 1. Isolation of *A. nidulans* photolyase from *S. coelicolor*(pANPL) grown in 1 liter of TSB-glucose medium

Step	Neomycin-grown cells		Thiostrepton-grown cells	
	Total vol	Total protein	Total vol	Total protein
Cell extract	90 ml	945 mg	107 ml	1,851 mg
Heparin agarose	80 ml	77 mg	84 ml	134 mg
UV-DNA agarose[a]	340 μl	86 μg	520 μl	64 μg

[a] The volume given is after concentration on a Centricon 30 concentrator. The amount of photolyase in the active fractions was determined before concentration.

The presence of a 5-deazaflavin cofactor was initially evident from UV measurements (λ_{max} ~ 440 nm) done to determine protein concentrations in fractions from the UV-DNA agarose column. After the homogeneity of pooled and concentrated active fractions had been ascertained (Fig. 2, lane 5), cofactors were released from the enzyme by heat denaturation and their identities were examined by reverse-phase HPLC analysis. F_{420} and F_0 were detected, together with FAD. F_0 and FAD (presumably from autooxidation of bound $FADH_2$) were present in approximately equimolar amounts, whereas F_{420} was present in ca. 10-fold excess. Predominant incorporation of F_{420} into *A. nidulans* photolyase expressed in *S. coelicolor*(pANPL) contrasts with the cofactor content of the enzyme isolated from wild-type *A. nidulans*, which was recently shown to contain F_0 and FAD in equimolar amounts (6). This difference in cofactor content may reflect the relative concentrations of F_{420} and F_0 in the host cell or may indicate relative affinities of the 5-deazaflavin-binding domain of the enzyme. The low level of FAD found in the enzyme from *S. coelicolor*(pANPL) may have resulted from preferential loss of the cofactor during column elution with salt gradients, and the absolute stoichiometry of 5-deazaflavin-$FADH_2$ bound to the DNA photolyase is yet to be determined. Full loading of enzyme may require the addition of riboflavin to the growth medium or broken cells. It has been shown for *Streptomyces lincolnensis* that F_0 can be introduced into cells of a mutant strain defective in lincomycin biosynthesis. Simple addition of the cofactor to the growth medium was sufficient to restore lincomycin production (2). In the absence of large differences in binding affinity, supplementing growing cells of recombinant *S. coelicolor* should allow directed in vivo reconstitution of the apoenzyme with different cofactors and study of concomitant variations in enzyme function.

The heterologous expression system described in this report permits the assessment of functional consequences of mutagenesis on the 5-deazaflavin class of photolyases and may also be useful for the expression of chimeric forms of folate-5-deazaflavin DNA photolyase.

ACKNOWLEDGMENTS

We thank Jorge Romero and Gilberto Hintermann for helpful discussions and experimental guidance.

This work was supported in part by Firmenich S.A., Geneva, Switzerland.

LITERATURE CITED

1. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
2. **Coats, J. H., G. P. Li, M. S. T. Kuo, and D. A. Yurek.** 1989. Discovery, production, and biological assay of an unusual flavenoid cofactor involved in lincomycin biosynthesis. J. Antibiot. **43**:472–474.
3. **Daniels, L., N. Bakhiet, and K. Harmon.** 1985. Widespread distribution of a 5-deazaflavin cofactor in *Actinomyces* and related bacteria. Syst. Appl. Microbiol. **6**:12–17.
4. **Eker, A. P. M., R. H. Dekker, and W. Berends.** 1981. Photoreactivating enzyme from *Streptomyces griseus*. IV. On the nature of the chromophoric cofactor in *Streptomyces griseus*

photoreactivating enzyme. Photochem. Photobiol. **33**:65–72.

5. **Eker, A. P. M., J. K. C. Hessels, and J. van de Velde.** 1988. Photoreactivating enzyme from the green alga *Scenedesmus acutus*. Evidence for the presence of two different flavin chromophores. Biochemistry **27**:1758–1765.
6. **Eker, A. P. M., P. Kooiman, J. K. C. Hessels, and A. Yasui.** 1990. DNA photoreactivating enzyme from the cyanobacterium *Anacystis nidulans*. J. Biol. Chem. **265**:8009–8015.
7. **Friedberg, E. C.** 1985. DNA repair, p. 85–87. W. H. Freeman and Co., New York.
8. **Harm, W., H. Harm, and C. S. Rupert.** 1968. Analysis and photoenzymatic repair of UV lesions in DNA by single light flashes. II. *In vivo* studies with *Escherichia coli* cells and bacteriophage. Mutat. Res. **6**:371–385.
9. **Hausinger, R. P., J. F. Honek, and C. T. Walsh.** 1986. Separation of flavins and flavin analogs by high performance liquid chromatography. Methods Enzymol. **122**:199–209.
10. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*. A laboratory manual. The John Innes Foundation, Norwich, United Kingdom.
11. **Husain, I., and A. Sancar.** 1987. Photoreactivation in *phr* mutants of *Escherichia coli* K-12. J. Bacteriol. **169**:2367–2372.
12. **Hütter, R., and T. Eckhard.** 1988. Genetic manipulation, p. 89–184. *In* M. Goodfellow, S. T. Williams, and M. Mordarski (ed.), Actinomycetes in biotechnology. Academic Press, Inc. (London), Ltd., London.
13. **Iwasa, T., S. Tokutomi, and F. Tokunaga.** 1988. Photoreactivation of *Halobacterium halobium*: action spectrum and role of pigmentation. Photochem. Photobiol. **47**:267–270.
14. **Johnson, J. L., S. Hamm-Alvarez, G. Payne, G. B. Sancar, K. V. Rajagopalan, and A. Sancar.** 1988. Identification of the second chromophore of *Escherichia coli* and yeast DNA photolyases as 5,10-methenyltetrahydrofolate. Proc. Natl. Acad. Sci. USA **85**:2046–2050.
15. **Kiener, A., I. Husain, A. Sancar, and C. T. Walsh.** 1989. Purification and properties of *Methanobacterium thermoautotrophicum* DNA photolyase. J. Biol. Chem. **264**:13880–13887.
16. **Kobayashi, T., M. Takao, A. Oikawa, and A. Yasui.** 1989. Molecular characterization of a gene encoding a photolyase from *Streptomyces griseus*. Nucleic Acids Res. **17**:4731–4744.
17. **Lomovskaya, N. D., N. M. Nkrtumian, N. L. Gostimskaya, and V. N. Danilenko.** 1972. Characterization of temperate bacteriophage φC31 isolated from *Streptomyces coelicolor* A3(2). J. Virol. **9**:258–262.
18. **Lubbe, C., S. Wolfe, and A. L. Demain.** 1985. Repression and inhibition of cephalosporin synthetases in *Streptomyces clavuligerus* by inorganic phosphate. Arch. Microbiol. **140**:317–320.
19. **McCormick, J. R. D., and G. O. Morton.** 1982. Identity of cosynthetic factor 1 of *Streptomyces aureofaciens* and fragment F_0 from coenzyme F_{420} of *Methanobacterium* species. J. Am. Chem. Soc. **104**:4014–4015.
20. **Merrick, M. J.** 1976. A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. J. Gen. Microbiol. **96**:299–315.
21. **Minato, S., and H. Werbin.** 1972. Excitation and fluorescence spectra of the chromophore associated with the DNA-photoreactivating enzyme from the blue-green alga *Anacystis nidulans*. Photochem. Photobiol. **15**:97–100.
22. **Myles, G. M., and A. Sancar.** 1989. DNA repair. Chem. Res. Toxicol. **2**:197–226.
23. **Payne, G., P. F. Heelis, B. R. Rohrs, and A. Sancar.** 1987. The active form of *Escherichia coli* DNA photolyase contains a fully reduced flavin and not a flavin radical, both in vivo and in vitro. Biochemistry **26**:7121–7126.
24. **Pulido, D., and A. Jiminez.** 1987. Optimization of gene expression in *Streptomyces lividans* by a transcription terminator. Nucleic Acids Res. **15**:4227–4240.
25. **Sancar, A., and G. B. Sancar.** 1988. DNA repair enzymes. Annu. Rev. Biochem. **57**:31–38.
26. **Sancar, A., and G. B. Sancar.** 1984. *Escherichia coli* DNA photolyase is a flavoprotein. J. Mol. Biol. **172**:223–227.
27. **Sancar, A., F. W. Smith, and G. B. Sancar.** 1984. Purification of *Escherichia coli* DNA photolyase. J. Biol. Chem. **259**:6028–6032.
28. **Sancar, G. B., M. S. Jorns, G. Payne, D. J. Fluke, C. S. Rupert, and A. Sancar.** 1987. Action mechanism of *Escherichia coli* DNA photolyase. III. Photolysis of the enzyme-substrate complex and the absolute action spectrum. J. Biol. Chem. **262**:492–498.
29. **Sancar, G. B., F. W. Smith, and P. F. Heelis.** 1987. Purification of the yeast PHR1 photolyase from an *Escherichia coli* overproducing strain and characterization of the intrinsic chromophores of the enzyme. J. Biol. Chem. **262**:15457–15465.
30. **Sancar, G. B., F. W. Smith, M. C. Lorence, C. S. Rupert, and A. Sancar.** 1984. Sequences of the *Escherichia coli* photolyase gene and protein. J. Biol. Chem. **259**:6033–6038.
31. **Takao, M., T. Kobayashi, A. Oikawa, and A. Yasui.** 1989. Tandem arrangement of photolyase and superoxide dismutase genes in *Halobacterium halobium*. J. Bacteriol. **171**:6323–6329.
32. **Takao, M., A. Oikawa, A. P. M. Eker, and A. Yasui.** 1989. Expression of an *Anacystis nidulans* photolyase gene in *Escherichia coli*; functional complementation and modified action spectrum of photoreactivation. Photochem. Photobiol. **50**:633–637.
33. **Thompson, C. J., and G. S. Gray.** 1983. Nucleotide sequence of a streptomycete aminoglycoside phosphotransferase gene and its relationship to phosphotransferases encoded by resistance plasmids. Proc. Natl. Acad. Sci. USA **80**:5190–5194.
34. **Walsh, C. T.** 1986. Naturally occurring 5-deazaflavin coenzymes: biological redox roles. Acc. Chem. Res. **19**:216–221.
35. **Wolfe, R. S.** 1985. Unusual coenzymes of methanogens. Trends Biochem. Sci. **10**:396–399.
36. **Yasui, A., M. Takao, A. Oikawa, A. Kiener, C. T. Walsh, and A. P. M. Eker.** 1988. Cloning and characterization of a photolyase gene from the cyanobacterium *Anacystis nidulans*. Nucleic Acids Res. **16**:4447–4463.