Klee, C. B., & Singer, M. F. (1967) *Biochem. Biophys. Res. Commun. 29*, 356.
Lee, C. H., & Tinoco, I., Jr. (1980) *Biophys. Chem. 11*, 283.
McFarland, G. D., & Borer, P. N. (1979) *Nucleic Acids Res. 7*, 1067.
Prestegard, J. H., & Chan, S. I. (1969) *J. Am. Chem. Soc. 91*, 2843.
Quigley, G. C., & Rich, A. (1976) *Science (Washington, D.C.) 194*, 796.
Reid, B. R., & Hurd, R. E. (1977) *Acc. Chem. Res. 10*, 396.
Rich, A., Quigley, G. C., & Wang, A. H. J. (1979) in *Stereodynamics of Molecular Systems* (Sarma, R. H., Ed.) p 315, Pergamon Press, New York.
Stout, C. D., Mizuno, H., Rao, S. T., Swaminathan, P., Rubin, J., Brennan, T., & Sundaralingam, M. (1978) *Acta Crystallogr. B34*, 1529.
Sundaralingam, M. (1974) *Struct. Conform. Nucleic Acids Protein-Nucleic Acid Interact., Proc. Annu. Harry Steenbock Symp., 4th*, 487.
Ts'o, P. O. P. (1974) in *Basic Principles in Nucleic Acid Chemistry* (Ts'o, P. O. P., Ed.) Vol. I, p 453, Academic Press, New York.
Uhlenbeck, O. C., Borer, P. N., Dengler, B., & Tinoco, I., Jr. (1973) *J. Mol. Biol. 73*, 483.
Van Geet, A. L. (1968) *Anal. Chem. 40*, 2227.
Van Geet, A. L. (1970) *Anal. Chem. 42*, 679.

Poly(adenosine diphosphoribose) Synthesis in Ultraviolet-Irradiated Xeroderma Pigmentosum Cells Reconstituted with *Micrococcus luteus* UV Endonuclease†

Nathan A. Berger* and Georgina W. Sikorski

ABSTRACT: Synthesis of DNA and poly(adenosine diphosphoribose) [poly(ADPR)] was examined in permeabilized xeroderma pigmentosum lymphoblasts (XP3BE) before and after UV irradiation and in the presence and absence of *Micrococcus luteus* UV endonuclease. *M. luteus* UV endonuclease had no effect on the level of DNA or poly(ADPR) synthesis in control, unirradiated cells. UV irradiation caused a decrease in replicative DNA synthesis without any significant change in poly(ADPR) synthesis. In UV-irradiated cells treated with *M. luteus* UV endonuclease, DNA synthesis was restored to a level slightly greater than in the unirradiated control cells, and poly(ADPR) synthesis increased by 2- to 4-fold. Time–course studies showed that the UV endonuclease dependent poly(ADPR) synthesis preceded the endonuclease-dependent DNA synthesis. Inhibition of endonuclease-dependent poly(ADPR) synthesis with 3-aminobenzamide, 5-methylnicotinamide, or theophylline produced a partial inhibition of the endonuclease-dependent DNA synthesis. Conversely, inhibition of the endonuclease-dependent DNA synthesis with dideoxythymidine triphosphate, phosphonoacetic acid, or aphidicolin had no effect on the endonuclease-dependent poly(ADPR) synthesis. These studies show that stimulation of poly(ADPR) synthesis in UV-irradiated cells occurs subsequent to the DNA strand breaks created by the specific action of the UV endonuclease on UV-irradiated DNA. The effect of the inhibitors of poly(ADPR) synthesis in UV-irradiated cells indicates that the endonuclease-stimulated DNA synthesis is dependent in part on the prior synthesis of poly(ADPR).

Poly(adenosine diphosphoribose) is synthesized from NAD^+ by poly(ADPR)[1] polymerase, which is a tightly bound chromosomal enzyme activated when cells are treated with various agents that damage DNA (Miller, 1975; Hayaishi & Ueda, 1977; Davies et al., 1977; Berger et al., 1979a–c). The rapid synthesis and degradation of ADP-ribose polymers in the nucleus have the potential for causing drastic but reversible alterations in chromatin conformation. Since poly(ADPR) synthesis increases in response to DNA damage, it was proposed that poly(ADPR) might be involved in the DNA repair process, altering chromatin structure so as to make regions of DNA damage more readily accessible to the enzymes of DNA repair (Miller, 1975; Davies et al., 1977; Cleaver, 1978; Berger et al., 1979c). This proposal has been partially confirmed by the demonstration that inhibitors of poly(ADPR) synthesis interfere with the ability of cells to recover and proliferate following DNA damage (Durkacz et al., 1980). In addition, cells made NAD^+ deficient by nicotinamide starvation are unable to carry out unscheduled DNA synthesis after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG); they are also unable to reseal DNA strand breaks after treatment with dimethyl sulfate (Durkacz et al., 1980; Jacobson et al., 1980).

We have shown that cells from normal human donors develop an increase in poly(ADPR) synthesis after treatment with various DNA damaging agents including MNNG, (*N*-acetoxyacetylamino)fluorene, bleomycin, and UV irradiation

† From the Department of Medicine, Washington University School of Medicine, Division of Hematology/Oncology, The Jewish Hospital of St. Louis, St. Louis, Missouri 63110. *Received July 30, 1980.* This work was supported by National Institutes of Health Grants GM26463 and CA23986 and American Cancer Society Grant CH-134. Cell culture medium was prepared in a Cancer Center Facility funded by the National Cancer Institute. N.A.B. is a Leukemia Society of America Scholar.

[1] Abbreviations used: ADPR, adenosine diphosphoribose; AP, apyrimidinic; aCTP, cytosine arabinoside triphosphate; d_2TTP, dideoxythymidine triphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NAD, nicotinamide adenine dinucleotide; UV, ultraviolet; XP, xeroderma pigmentosum; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/81/0420-3610$01.25/0

(Berger et al., 1979c). In cells from patients with xeroderma pigmentosum, poly(ADPR) synthesis increases in response to DNA damage induced by MNNG but not in response to damage from UV irradiation (Berger et al., 1980). We proposed that the failure of XP cells to increase their poly(ADPR) synthesis in response to UV irradiation was due to their defective ability to perform the initial incision at the sites of UV-induced DMA damage (Fornace et al., 1976; Berger et al., 1980). Tanaka et al. (1975) showed that the defective ability of XP cells to repair UV-induced DNA damage could be corrected by supplying cells with T_4 endonuclease V, an endonuclease which specifically acts at the site of thymidine dimers. The UV endonuclease from *M. luteus* should also serve this purpose since it also cleaves the phosphodiester backbone of DNA at the site of thymidine dimers (Riazuddin & Grossman, 1977; Haseltine et al., 1980). In the present study, we permeabilized control and UV-irradiated XP cells and then supplied them with *M. luteus* UV endonuclease to determine how this would affect the synthesis of poly(ADPR) and to investigate the relation of poly(ADPR) synthesis to the DNA repair process.

Experimental Procedures

The long-term lymphocyte cell line XP3BE derived from a patient with XP, group C (Andrews et al., 1974), was obtained from Mutant Cell Repository, Camden, NJ. *M. luteus* UV endonuclease, purified through the Sephadex G-75 stage (Riazuddin & Grossman, 1977), was a gift from Dr. Lawrence Grossman. Aphidicolin was obtained from Imperial Chemical Industries, Ltd., dideoxythymidine triphosphate (d_2TTP) and cytosine arabinoside triphosphate (aCTP) were from P-L Biochemicals, 5-methylnicotinamide was from the Lilly Research Laboratories, phosphonoacetic acid was from Abbott Laboratories, 3-aminobenzamide was from Pfaltz & Bauer, Inc., theophylline was from Sigma Chemical Co., and nicotinamide adenine dinucleotide ([U-^{14}C]adenosine) and deoxythymidine 5′-triphosphate (methyl-^{3}H-labeled) were from New England Nuclear.

Cells were maintained in suspension culture at 37 °C in α-modified Eagle's medium, buffered with 25 mM Hepes, pH 7.2, and supplemented with 20% fetal calf serum, 4 mM fresh glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. All experiments were performed when cells were in logarithmic growth between 2×10^5 and 5×10^5 cells/mL.

For UV irradiation, cells were suspended at 2×10^6 cells/mL in phosphate-buffered saline, spread in a thin layer in plastic petri dishes, and irradiated with a General Electric 15-W germicidal lamp at an incident dose of 1 J/(m^2 s) as previously described (Berger et al., 1979c, 1980).

Measurements of DNA and poly(ADPR) synthesis were performed in cells that were first made permeable to exogenously supplied nucleotides and enzymes by a technique that has previously been described in detail (Berger, 1978; Berger et al., 1978a,b). Briefly, cells were collected by centrifugation at 3000*g* for 10 min at 4 °C, then resuspended at 2×10^6 cells/mL in a hypotonic buffer composed of 0.01 M Tris-HCl, pH 7.8, 1 mM EDTA, 4 mM $MgCl_2$, and 30 mM 2-mercaptoethanol, and incubated in an ice–water bath for 15 min. The cells were collected again by centrifugation and resuspended at 2×10^7 cells/mL in the same buffer. The proof that these cells are permeable to nucleotides and proteins and the characterization of the products as DNA and poly(ADP-ribose) have already been detailed (Berger, 1978; Berger et al., 1978a,b).

To measure synthesis of DNA and poly(ADPR), 50-μL portions of cell suspension were added to tubes containing reaction mix, *M. luteus* UV endonuclease, and the appropriate inhibitors as indicated to a final volume of 100 μL. The UV endonuclease was dissolved in 0.1 M potassium phosphate, pH 7.6, 5 mM 2-mercaptoethanol, 5 mM EDTA, and 10% glycerol. When the UV endonuclease was added to reaction tubes, the same amount of this buffer was added to control tubes. Inhibitors were all dissolved in 10 mM Tris-HCl adjusted to pH 7.8. The final concentrations of the components in the reaction were 1×10^6 permeabilized cells, 5 mM Tris-HCl, pH 7.8, 22 mM Hepes (pH 7.8), 15 mM 2-mercaptoethanol, 9.5 mM $MgCl_2$, 0.5 mM EDTA, 33 mM NaCl, 3.3 mM ATP, 60 μM dATP, 60 μM dCTP, 60 μM dGTP, 0.17 μM dTTP (methyl-^{3}H-labeled) (specific activity 96×10^3 dpm/pmol), 0.1 mM NAD^+ ([U-^{14}C]adenosine) (specific activity 27.6 dpm/pmol), and 0.05% Triton X-100 in a final volume of 100 μL. *M. luteus* UV endonuclease was included in the indicated reactions at a final concentration of 10 units/100 μL of system. In preliminary experiments, we found that the degree of restoration of DNA synthesis in UV-irradiated cells was dependent on the amount of added UV endonuclease until at saturating levels of endonuclease there was no further increase in the level of DNA synthesis. The concentration of 10 units/100 μL of reaction system was selected for all further experiments because it provided a saturating level of UV endonuclease that completely reversed the effects of UV irradiation on DNA synthesis in permeable cells.

Components were combined in an ice–water bath, and reactions were started by shifting tubes to a 37 °C shaking water bath. Incubations were for 30 min except where indicated otherwise. Reactions were stopped by precipitation with an excess of cold 20% trichloroacetic acid and 2% sodium pyrophosphate. Samples were collected on Whatman GF/C filter disks and prepared for scintillation counting as previously described (Berger et al., 1978a). Radioactivity was analyzed and automatically corrected for cross contribution by using a dual label program of a Mark III Liquid Scintillation System, Tracor Analytic, Inc., Elk Grove Village, IL. Results are presented as the means of triplicate assays which agreed within 10%. All experiments were performed at least 3 times.

Results

When normal cells were treated with UV irradiation, there was a dose-dependent decrease in replicative DNA synthesis and dose-dependent increases in DNA repair and poly(ADPR) synthesis (Berger et al., 1979a–c). UV irradiation of XP cells also produces a decrease in replicative DNA synthesis; however, there is essentially no increase in the level of poly(ADPR) synthesis or DNA repair (Berger et al., 1980). Table I shows the effects of UV irradiation and the *M. luteus* UV endonuclease on DNA and poly(ADPR) synthesis in XP3BE cells. The levels of DNA and poly(ADPR) synthesis in the control unirradiated cells are typical values for cells in mid log phase growth. UV irradiation with 10, 50, or 100 J/m^2 resulted in a dose-dependent decrease in DNA synthesis; however, there was essentially no change in the level of poly(ADPR) synthesis. It should be noted that these cells were permeabilized and DNA syntheses measured immediately after UV irradiation. The UV-induced suppression of DNA synthesis becomes greater at progressive time intervals after UV irradiation. For example, immediately after UV irradiation with 50 J/m^2, the level of DNA synthesis is suppressed to 58% of control, within 1.5 h it is suppressed to 40%, and by 3 h it is 33% of the level of DNA synthesis in control cells. When the UV-irradiated cells were treated with the UV endonuclease, DNA synthesis increased to levels slightly greater than those present in the

Table I: Effect of *M. luteus* UV Endonuclease on Synthesis of DNA and Poly(ADPR) in XP3BE Lymphoblasts[a]

treatment	DNA synthesis (dpm/ 10^6 cells)	poly(ADPR) synthesis (dpm/ 10^6 cells)
control	98 300	2000
control + UV endonuclease	97 800	2000
UV (10 J/m^2)	72 800	2100
UV (50 J/m^2)	56 900	2300
UV (100 J/m^2)	36 200	2200
UV (10 J/m^2) + UV endonuclease	106 200	5400
UV (50 J/m^2) + UV endonuclease	102 000	6600
UV (100 J/m^2) + UV endonuclease	103 200	7900

[a] XP3BE lymphoblasts in mid log phase growth were UV irradiated as described under Experimental Procedures. Immediately after irradiation, cells were permeabilized, and 1 × 10^6 permeable cells were incubated with the reaction mixture containing [^{3}H]dTTP and [^{14}C]NAD for 30 min at 37 °C. The indicated reactions contained 10 units of *M. luteus* UV endonuclease.

unirradiated control cells. Concomitantly, the levels of poly(ADPR) synthesis increased to between 2 and 4 times those of the control cells. The demonstration that the UV endonuclease caused DNA and poly(ADPR) synthesis to increase in UV-irradiated cells but had no effect on control cells indicates that such increases are dependent on the enzyme carrying out its specific function, which is to induce DNA strand breaks at the sites of pyrimidine dimers.

As noted above, the level of DNA synthesis in the unirradiated cells is typical for cells in mid log phase growth and represents the replicative mode of DNA synthesis (Berger, 1978). UV irradiation caused a dose-dependent suppression of this replicative DNA synthesis. Regardless of the degree of suppression, treatment of the cells with UV endonuclease under the conditions described above restored DNA synthesis to slightly greater than normal levels. The increment in DNA synthesis that occurred when UV-irradiated cells were treated with this dose of UV endonuclease was too great to be accounted for by DNA repair alone. It is probable that this increment contained a small contribution from the repair mode of DNA synthesis and a large contribution from the replicative mode of DNA synthesis, the latter being restored to preirradiation levels immediately after the UV damage was excised. For the purpose of this study, we describe this increment in DNA synthesis by the operational term of UV endonuclease dependent DNA synthesis. We emphasize again that this term is composed of contributions from both the replicative and repair modes of DNA synthesis.

One approach to determining the relation of poly(ADPR) synthesis to the process of DNA repair and the restoration of replicative DNA synthesis is to examine the time course of their synthesis in UV-irradiated cells in the presence and absence of UV endonuclease. The first panel in Figure 1 shows that in permeable mid log phase cells the synthesis of DNA and poly(ADPR) continued for 30 min and then leveled off. The second panel shows that UV irradiation resulted in a marked depression of DNA synthesis. However, even at the reduced rate, both DNA and poly(ADPR) continued to be synthesized during the first 30 min before leveling off. The third panel shows that when the UV endonuclease was added to the UV-irradiated cells, there was a rapid increase in poly(ADPR) synthesis which appeared to precede the increase in DNA synthesis.

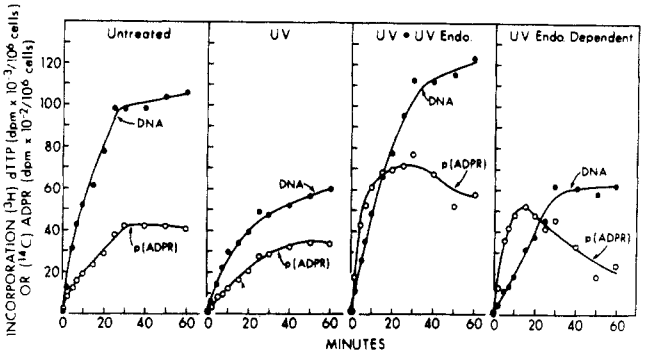

FIGURE 1: Time course of DNA (●) and poly(ADPR) (○) synthesis in XP3BE lymphoblasts. Cells were permeabilized and the reactions performed as described under Experimental Procedures. (First panel on left) Cells in mid log phase growth. (Second panel) Cells irradiated with 50 J/m^2 and then incubated without UV endonuclease. (Third panel) Cells irradiated with 50 J/m^2 and then incubated with 10 units of UV endonuclease in each reaction. (Fourth panel) UV endonuclease dependent reaction obtained by subtracting results depicted in second panel from those in the third.

The amount of endonuclease-dependent DNA synthesis is determined by subtracting the DNA synthesis values obtained in cells treated with UV irradiation alone from the values obtained in cells treated with UV irradiation and UV endonuclease. Similarly, the amount of endonuclease-dependent poly(ADPR) polymerase activity is determined by subtracting the values for UV-irradiated cells from those for UV-irradiated cells treated with UV endonuclease. The values derived from these calculations are plotted in the fourth panel of Figure 1. Clearly, the addition of the UV endonuclease to UV-irradiated XP cells resulted in an abrupt increase in poly(ADPR) synthesis which preceded the increment in DNA synthesis. The rapid increase in poly(ADPR) synthesis leveled off after 15 min; then the amount of radioactive poly(ADPR) in the system decreased, suggesting that it was undergoing degradation. The amount of DNA synthesized leveled off after 30-min incubation and remained constant thereafter.

To investigate further the relation of poly(ADPR) synthesis to DNA synthesis in the UV endonuclease dependent reaction, we examined the effects of inhibitors which were selected for their abilities to inhibit selectively the synthesis of either DNA or poly(ADPR). aCTP, d_2TTP, phosphonoacetic acid, and aphidicolin are all DNA polymerase inhibitors (Edenberg et al., 1978; Berger et al., 1979a). 5-Methylnicotinamide, 3-aminobenzamide, and theophylline were selected for their ability to inhibit poly(ADPR) polymerase (Berger et al., 1978a; Purnell & Whish, 1980). As shown in Table II, 3-aminobenzamide, 5-methylnicotinamide, and theophylline all inhibited the UV endonuclease dependent stimulation of poly(ADPR) synthesis. In addition, all three agents caused a partial inhibition of the endonuclease-dependent DNA synthesis. When aCTP was added to inhibit DNA synthesis, there was also a partial inhibition of poly(ADPR) synthesis. This inhibition of poly(ADPR) synthesis may be explained by partial deamination of the cytosine moiety to uracil since uracil acts as a direct inhibitor of poly(ADPR) polymerase (Berger et al., 1978a). d_2TTP, phosphonoacetic acid, and aphidicolin all inhibited the endonuclease-dependent DNA synthesis but had no effect on poly(ADPR) synthesis. These studies demonstrate that inhibition of poly(ADPR) synthesis interferes with the DNA repair process that occurs after UV-irradiated XP cells are treated with *M. luteus* UV endonuclease. In contrast, several agents which inhibited the UV endonuclease dependent DNA synthesis had no effect on poly(ADPR) synthesis. Thus, the UV endonuclease dependent DNA syn-

Table II: Effect of Inhibitors on UV Endonuclease Dependent Synthesis of DNA and Poly(ADPR) in UV-Irradiated XP3BE Lymphoblasts[a]

inhibitor	DNA synthesis (% of control)	poly(ADPR) synthesis (% of control)
control	100	100
5 mM 3-aminobenzamide	43	5
5 mM 5-methylnicotinamide	66	32
5 mM theophylline	65	24
1 mM aCTP	26	67
1 mM d_2TTP	7	101
1 mM phosphonoacetic acid	19	107
1 μg/mL aphidicolin	14	103

[a] Cells were UV irradiated with 50 J/m². Cell permeabilization and reaction conditions are indicated under Experimental Procedures. The effect of each agent on synthesis of DNA and poly(ADPR) was determined in the presence and absence of 10 units of UV endonuclease. The UV endonuclease dependent synthesis of DNA and poly(ADPR) was determined as in Figure 1. The specific effect of each agent on the UV endonuclease dependent reaction was determined by subtracting the values obtained in the absence of endonuclease from those obtained in the presence of endonuclease. Percentages were then calculated relative to the amount of UV endonuclease dependent synthesis in cells incubated without any inhibitors. The control values were 44 400 dpm for endonuclease-dependent DNA synthesis and 4200 dpm for endonuclease-dependent poly(ADPR) synthesis.

thesis appears to depend, in part, on the prior synthesis of poly(ADP-ribose) but not vice versa.

Discussion

In the present study, UV irradiation of XP3BE cells caused a dose-dependent decrease in replicative DNA synthesis. Treatment of the UV-irradiated cells with *M. luteus* UV endonuclease restored DNA synthesis to a level slightly greater than the controls. The decrease in replicative DNA synthesis that occurs when cells are UV irradiated has been attributed to the induction of pyrimidine dimers which interfere with DNA replication by blocking the progression of DNA polymerase along the DNA template (Moore & Strauss, 1979). These lesions can be removed and the integrity of the DNA strand restored by the excision repair process (Grossman et al., 1975; Hanawalt et al., 1979), following which DNA polymerase can progress along the template and replicative DNA synthesis can be resumed. The excision repair process can be initiated by endonucleases that specifically cleave DNA at the site of pyrimidine dimers. Several of these enzymes including the UV endonuclease from *M. luteus* function in a compound or multistep process (Haseltine et al., 1980). First, they act as a glycosylase, cleaving the bond between the 5′-pyrimidine of the thymidine dimer and its sugar. Next, they act as an apyrimidinic (AP) endonuclease to incise the DNA backbone at the AP site left by the glycosylase action. The incision step is presumably followed by the action of other enzymes which excise the dimer-containing DNA. This in turn is followed by synthesis of a patch of DNA to replace the excised nucleotides. The patch is subsequently ligated to the remaining portion of the original DNA (Grossman et al., 1975; Cleaver, 1978; Hanawalt et al., 1979).

Patients with XP have a genetic defect in their ability to repair UV-induced DNA damage (Cleaver et al., 1969, 1978; Setlow et al., 1969). Tanaka et al. (1975) showed that the UV-repair defect could be corrected by supplying XP cells with the T_4 UV endonuclease, indicating that after the initial incision is made by the endonuclease at the site of UV damage, XP cells contain all the components required to complete the

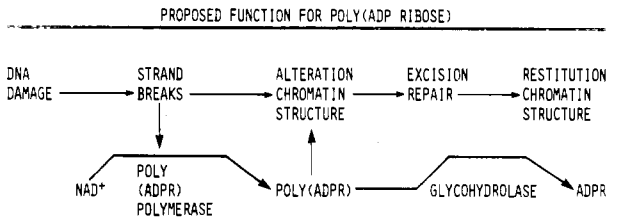

FIGURE 2: Proposed function for poly(ADP-ribose).

DNA repair process. In the present study, we permeabilized UV-irradiated XP cells and then supplied them with *M. luteus* UV endonuclease. The high levels of DNA synthesis that occurred in the UV-irradiated endonuclease treated cells probably reflect the combination of the endonuclease-initiated DNA repair synthesis and the restoration of replicative DNA synthesis.

We have also conducted similar experiments with lymphoblasts derived form normal donors. Normal cells excise UV-induced DNA damage and show an associated increase in poly(ADPR) synthesis over a period of several hours following UV irradiation (Berger et al., 1979c, 1980). Since all the UV-induced pyrimidine dimers are not excised immediately, addition of saturating amounts of UV endonuclease results in incisions at the sites of residual dimers, and an associated increase in synthesis of DNA and poly(ADPR) occurred even in normal cells. The studies described in this paper were specifically conducted in XP cells to take advantage of the fact that they do not excise UV-induced pyrimidine dimers and therefore show no spontaneous increase in poly(ADPR) synthesis following UV irradiation. Thus we can reconstitute these cells with *M. luteus* UV endonuclease to examine the relation between poly(ADPR) synthesis and DNA damage and repair. While UV irradiation of the XP cells produced a dose-dependent decrease in replicative DNA synthesis, there was essentially no change in the level of poly(ADPR) synthesis. When UV-irradiated cells were treated with the UV endonuclease, there was a 2–4-fold increase in poly(ADPR) synthesis. These studies confirm our proposal that poly(ADPR) polymerase activity is not directly stimulated by UV-induced DNA damage but rather by the breaks in the DNA backbone created by the specific action of the UV endonuclease on the UV-irradiated DNA. Stimulation of poly(ADPR) synthesis by the strand break phase of DNA repair is consistent with previous studies in which DNase and other treatments which induce DNA strand breaks have been shown to cause maximal stimulation of poly(ADPR) synthesis (Miller, 1975; Halldorsson et al., 1978; Berger et al., 1978a, 1979c). In the present studies, the rapid increase in poly(ADPR) synthesis that occurred in UV-irradiated cells treated with UV endonuclease preceded the increase in DNA synthesis. Inhibition of this poly(ADPR) synthesis partially prevented the endonuclease-dependent increase in DNA synthesis. These findings indicate that stimulation of poly(ADPR) synthesis occurs after the incision step of DNA repair but before the resumption of normal DNA synthesis.

The results of our experiments are consistent with the pathway for repair of UV-induced DNA damage outlined in Figure 2. According to this proposal, UV irradiation induces DNA damage which is recognized by an endonuclease system whose function includes nicking the DNA phosphodiester backbone at or near the site of the damage. Poly(ADPR) polymerase, which is tightly bound and widely distributed in reverse quantities in the chromatin (Berger et al., 1978b), is stimulated by DNA strand breaks to synthesize poly(ADPR). This in turn alters the chromatin structure so as to permit the enzymes of DNA repair to gain access to and repair the regions

of DNA damage. After the damage is repaired and the DNA strand breaks are ligated, the stimulus for poly(ADPR) synthesis is removed. Another chromatin enzyme, poly(ADPR) glycohydrolase, is suppressed by single-stranded DNA but is fully active in the presence of double-stranded DNA (Burzio et al., 1976). When the DNA strand breaks are repaired, the glycohydrolase is free to degrade te poly(ADPR), and the chromatin can return to its predamaged conformation.

Acknowledgments

We thank Dr. L. Grossman for the *M. luteus* UV endonuclease, Dr. B. Hesp for the aphidicolin, Abbott Laboratories for the phosphonoacetic acid, and Lilly Research Laboratories for the 5-methylnicotinamide.

References

Andrews, A. D., Robbins, J. H., Kraemer, K. H., & Buell, D. N. (1974) *J. Natl. Cancer Inst. (U.S.) 53*, 691–693.

Berger, N. A. (1978) *Methods Cell Biol. 20*, 325–339.

Berger, N. A., Weber, G., & Kaichi, A. S. (1978a) *Biochim. Biophys. Acta 519*, 87–104.

Berger, N. A., Kaichi, A. S., Steward, P. G., Klevecz, R. R., Forrest, G. L., & Gross, S. D. (1978b) *Exp. Cell Res. 117*, 127–135.

Berger, N. A., Kurohara, K. K., Petzold, S. J., & Sikorski, G. W. (1979a) *Biochem. Biophys. Res. Commun. 89*, 218–225.

Berger, N. A., Petzold, S. J., & Berger, S. J. (1979b) *Biochim. Biophys. Acta 564*, 90–104.

Berger, N. A., Sikorski, G. W., Petzold, S. J., & Kurohara, K. K. (1979c) *J. Clin. Invest. 63*, 1164–1171.

Berger, N. A., Sikorski, G. W., Petzold, S. J., & Kurohara, K. K. (1980) *Biochemistry 19*, 289–293.

Burzio, L. O., Riquelme, P. T., Ohtsuka, E., & Koide, S. S. (1976) *Arch. Biochem. Biophys. 173*, 306–319.

Cleaver, J. E. (1969) *Proc. Natl. Acad. Sci. U.S.A. 63*, 428–435.

Cleaver, J. E. (1978) in *The Metabolic Basis of Inherited Disease* (Stanbury, J. N., Wyngaarden, J. B., & Fredrickson, D. S. Eds.) pp 1072–1095, McGraw-Hill, New York.

Davies, M. I., Shall, S., & Skidomore, C. J. (1977) *Biochem. Soc. Trans. 5*, 949–950.

Durkacz, B. W., Omidiji, O., Gray, D. A., & Shall, S. (1980) *Nature (London) 283*, 593–596.

Edenberg, H. J., Anderson, S., & DePamphilis, M. L. (1978) *J. Biol. Chem. 253*, 3273–3280.

Fornace, A. J., Jr., Kohn, K. W., & Kann, H. E., Jr. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 39–43.

Grossman, L. Braun, A., Feldberg, R., & Mahler, I. (1975) *Annu. Rev. Biochem. 44*, 19–43.

Halldorsson, H., Gray, D. A., & Shall, S. (1978) *FEBS Lett. 85*, 349–352.

Hanawalt, P. C., Cooper, P. K., Ganesan, A. K., & Smith, C. A. (1979) *Annu. Rev. Biochem. 48*, 783–836.

Haseltine, W. A., Gordon, L. K., Lindan, C. P., Grafstrom, R. H., Shaper, N. L., & Grossman, L. (1980) *Nature (London) 285*, 634–641.

Hayaishi, O., & Ueda, K. (1977) *Annu. Rev. Biochem. 46*, 95–116.

Jacobson, E. L., Juarez, D., & Sims, J. L. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 1739.

Miller, E. G. (1975) *Biochim. Biophys. Acta 395*, 191–200.

Moore, P., & Strauss, B. S. (1979) *Nature (London) 278*, 664–666.

Purnell, M. R., & Whish, W. J. D. (1980) *Biochem. J. 185*, 775–777.

Riazuddin, S., & Grossman, L. (1977) *J. Biol. Chem. 252*, 6280–6286.

Setlow, R. B., Regan, J. D., German, J., & Carrier, W. L. (1969) *Proc. Natl. Acad. Sci. U.S.A. 64*, 1035–1041.

Tanaka, K., Sekiguchi, M., & Okada, Y. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4071–4075.