Veeger, C., DerVartanian, D. V., & Zeylemaker, W. P. (1969) *Methods Enzymol. 13*, 81-90.
Venable, J. H., & Coggeshall, R. (1965) *J. Cell Biol. 25*, 407-408.
Watson, M. L. (1958) *J. Biophys. Biochem. Cytol. 4*, 475-478.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406-4412.
Weibel, E. R. (1969) *Int. Rev. Cytol. 26*, 235-299.
Widnell, C. C., & Unkless, J. (1969) *Proc. Natl. Acad. Sci. U.S.A. 61*, 1050-1057.

Defective Poly(adenosine diphosphoribose) Synthesis in Xeroderma Pigmentosum†

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ABSTRACT: The response of poly(adenosine diphosphoribose) [poly(ADPR)] polymerase to treatment of cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or ultraviolet (UV) irradiation was evaluated in long-term lymphocyte lines derived from three normal donors and from five patients with xeroderma pigmentosum representing complementation groups A-E. Measurements of poly(ADPR) synthesis were conducted in cells that were made permeable to exogenously supplied nucleotides and then incubated with [^{3}H]NAD$^+$ and optimal concentrations of other components required for poly(ADPR) synthesis. All of the lymphocyte cell lines obtained from normal donors showed an increase in poly(ADPR) synthesis in response to treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or UV irradiation. While the xeroderma pigmentosum cell lines showed increased poly(ADPR) synthesis in response to treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, they were all defective in their poly(ADPR) synthesis response to UV irradiation. In cells from xeroderma pigmentosum complementation group A, the increase in poly(ADPR) synthesis was similar to that seen in normal cells but it occurred at a prolonged interval after UV irradiation. Cells from xeroderma pigmentosum complementation groups B and C showed no increase, and cells from complementation groups D and E showed only slight increases in poly(ADPR) synthesis in response to UV irradiation. When xeroderma pigmentosum cells were UV irradiated and then permeabilized and treated with deoxyribonuclease I, they showed a normal increase in poly(ADPR) synthesis, demonstrating that their ability to synthesize poly(ADPR) in response to deoxyribonucleic acid (DNA) damage was not destroyed by treatment with UV irradiation. The failure of xeroderma pigmentosum cells to demonstrate an increase in poly(ADPR) synthesis in response to UV irradiation is consistent with a role for poly(ADPR) in the DNA repair process.

Poly(ADPR)[1] polymerase is a chromosomal enzyme which uses the ADPR moiety of NAD$^+$ to synthesize the nucleic acid homopolymer poly(adenosine diphosphoribose) (Hayaishi & Ueda, 1977). The activity of this enzyme increases when cells are subjected to a variety of treatments which cause DNA damage (Berger et al., 1979b; Davies et al., 1977; Jacobson & Jacobson, 1978; Miller, 1975). For example, normal human lymphocytes treated with UV irradiation, *N*-acetoxy-*N*-acetyl-2-aminofluorene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), or bleomycin all showed an abrupt increase in poly(ADPR) synthesis along with an increase in DNA repair synthesis (Berger et al., 1979b). While these studies showed that poly(ADPR) synthesis was stimulated by DNA damage and also that the increase in activity occurred in temporal association with DNA repair (Berger et al., 1979b), they did not demonstrate whether one process was dependent on the other.

To further investigate the relation between poly(ADPR) synthesis and DNA repair, we examined poly(ADPR) synthesis in cells obtained from patients with xeroderma pigmentosum (XP). Such cells are defective in their ability to repair UV-induced DNA damage (Cleaver, 1969; Setlow et al., 1969). They do repair some types of DNA damage such as that caused by MNNG (Cleaver, 1973). We reasoned that if poly(ADPR) synthesis is a component of the DNA repair process, then cells from patients with xeroderma pigmentosum might show a defective poly(ADPR) synthesis response to UV irradiation but a normal response to treatment with MNNG. These studies were conducted with long-term lymphocyte cell lines derived from three normal donors and from five patients with xeroderma pigmentosum representing each of the complementation groups from A to E. Cells from each of the different complementation groups are presumably defective in a different aspect of the repair of UV-induced DNA damage since the defect in DNA repair is corrected in heterocaryons obtained by fusing cells from any two of the different complementation groups (Kraemer et al., 1975). The long-term lymphocyte cell lines were used in order to provide sufficient cells for the studies described. These cell lines from patients with xeroderma pigmentosum have previously been shown to be abnormal in their response to UV irradiation (Andrews et

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[1] Abbreviations used: ADPR, adenosine diphospohoribose; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NAD, nicotinamide adenine dinucleotide; UV, ultraviolet; XP, xeroderma pigmentosum.

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al., 1974; Cheng et al., 1978).

Methods

The long-term lymphocyte lines derived from patients with xeroderma pigmentosum were GM2250 (patient XP12BE, complementation group A), GM2252 (patient XP11BE, complementation group B), XP3BE (complementation group C), GM2253 (patient XP17BE, complementation group D), and GM2450 (patient XP3RO, complementation group E). The long-term lymphocyte cell lines derived from normal donors were GM0892, GM0558, and GM0605. The XP3BE cell line was obtained directly from Dr. Allen Andrews at the National Institutes of Health. All of the other cell lines were purchased from The Mutant Cell Repository, Camden, NJ. The age, sex, and other characteristics of each of the donors have been published (Cheng et al., 1978) and are also listed in the catalog of The Mutant Cell Repository.

Fetal calf serum was purchased from K C Biological, Inc., Lenexa, KS, and heated to 56 °C for 30 min before use. Cells were maintained in unstirred suspension cultures 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. Cultures were incubated at 37 °C and diluted with fresh medium every 24–48 h to maintain growth between 2×10^5 and 11×10^5 cells/mL. All experiments were performed when cells were in the logarithmic phase of growth between 2×10^5 and 5×10^5 cells/mL.

For most experiments, 10^9 cells were used. All procedures during the induction of DNA damage and subsequent manipulation of cells for measurement of poly(ADPR) synthesis were performed in a darkened room. MNNG treatment was performed by dissolving and diluting the drug in Me_2SO and then immediately adding appropriate aliquots to cell suspensions. Control cells were adjusted to contain the same final concentrations of 1% Me_2SO as the MNNG-treated cultures.

For UV irradiation, cells were collected from suspension culture by centrifugation at 800*g* for 5 min at room temperature. The growth medium was reserved. The cells were resuspended at 2×10^6 cells/mL in phosphate-buffered saline (10 g of NaCl, 250 mg of KCl, 3.6 g of $Na_2HPO_4 \cdot 7H_2O$, and 250 mg of KH_2PO_4 per L, pH 7.5). Ten-milliliter samples of the cell suspension were spread in 150-mm diameter plastic petri dishes and irradiated with a General Electric 15-W germicidal lamp, G15T8 (principle irradiation 2537 Å), at an incident dose of 1 J/(m^2·s) calibrated with a J225 Black Ray UV meter (Ultra Violet Products, Inc., San Gabriel, CA). Following irradiation, cells were washed from the plates, medium was added to the suspension, and cells were collected again by centrifugation and then resuspended in their original growth medium at concentrations between 2×10^5 and 4×10^5 cells/mL and incubated at 37 °C. Control, mock irradiated, cells were subjected to the same procedures of centrifugation, resuspension in phosphate-buffered saline, spreading in petri dishes, collection by centrifugation, and resuspension in original growth medium. The time of drug addition or UV irradiation was taken as the 0 time for all experiments.

At intervals after drug treatment or UV irradiation, samples were removed from control and treated cultures and used to measure the rates of poly(ADPR) synthesis. These measurements were conducted in cells that were first made permeable to exogenously supplied nucleotides by a technique that has previously been described in detail (Berger, 1978; Berger et al., 1978a,b). Briefly, after cells were removed from suspension culture, they were collected by centrifugation at 3000*g* for 10 min at 4 °C and 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, that their nucleotide pools are effectively depleted, and that they use exogenously supplied NAD^+ for the synthesis of poly(ADPR) has already been detailed (Berger, 1978; Berger et al., 1978a,b).

To measure poly(ADPR) synthesis, 50-μL portions of a given cell suspension were added to a reaction mix tube in which the final concentration of components was 33 mM Tris-HCl, pH 7.8, 20 mM mercaptoethanol, 0.6 mM EDTA, 42.5 mM $MgCl_2$, 0.33 mM [*adenine*-2,8-$^3H_2$]NAD^+([^{3}H]-NAD^+) [specific activity (17–24) $\times 10^3$ dpm/nmol], and 1×10^6 permeabilized cells in a final volume of 75 μL. Each component of this reaction system is present at concentrations that were previously determined to be optimum for this system (Berger et al., 1978a,b). To measure poly(ADPR) synthesis under conditions of maximum DNA damage, the reaction system was adjusted to contain 0.05% Triton X-100 and 30 μg of DNase I for a final concentration of 300 μg/mL. This concentration of DNase has been previously demonstrated to produce maximal stimulation of poly(ADPR) synthesis (Berger et al., 1978a,b).

Reaction components were combined in tubes in an ice–water bath, and reactions were started by shifting tubes to a 30 °C water bath. After 30-min incubations at 30 °C, reactions were terminated by precipitation with an excess of cold 20% trichloroacetic acid. Samples were sonicated, collected on Whatman GF/C filter disks, and washed 5 times with 20% trichloroacetic acid and twice with ethanol; radioactivity was then determined in a toluene-based scintillation fluid as previously described (Berger et al., 1978a). Each experiment presented in the results was performed at least 3 times, and each point within each experiment was performed in triplicate. Results are presented as the means of triplicate assays which agreed within 10%. The response pattern presented for each cell type was typical of those obtained in the repeats of each experiment. That these reactions measured the synthesis of poly(ADPR) has been shown by several criteria including the demonstration that the reaction is inhibited by well characterized inhibitors of poly(ADPR) polymerase such as 5-methylnicotinamide and also by the demonstration that the product of this reaction has the characteristic nuclease susceptibilities of poly(ADPR) in that it is degraded by venom phosphodiesterase but not by DNase or RNase (Berger et al., 1978a,b).

Results

We measured the stimulation of poly(ADPR) synthesis that occurred when long-term lymphocytes prepared from normal human donors and patients with xeroderma pigmentosum were treated with MNNG or UV irradiation. The levels of poly(ADPR) synthesis in the MNNG-treated or UV-irradiated cells are expressed relative to the rates of poly(ADPR) synthesis measured at the same time in the appropriately treated control cells. Figure 1 shows that MNNG stimulated increases in poly(ADPR) synthesis in each of the three different normal cell lines. Maximal responses to MNNG in the normal cells were in the range of 250–400%. Similarly, UV irradiation induced increases in poly(ADPR) synthesis in each of the three normal cell types with maximal responses in the range of 150–300%. While the poly(ADPR) synthesis response to MNNG was usually greater and more rapid than the response

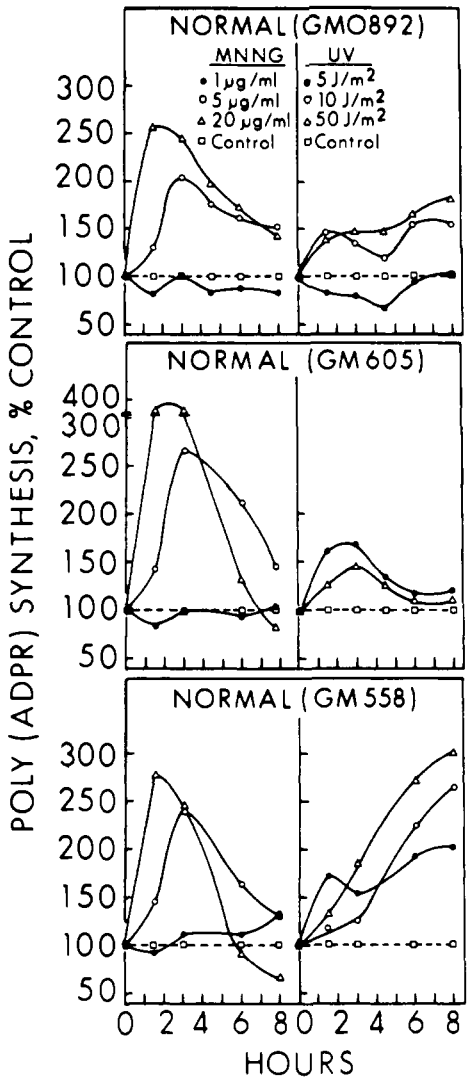

FIGURE 1: Effect of treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or UV irradiation on the synthesis of poly(ADPR) in long-term lymphocyte cell lines derived from normal donors. The number of each cell line is included across the top of each set of figures. Treatment with DNA-damaging agent was at 0 h. At each time point, poly(ADPR) synthesis is expressed as a percentage of the control levels measured simultaneously in cells that were mock treated as described under Methods. Left-side panels represent cells treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine which was present at final concentrations of 1 μg/mL (●), 5 μg/mL (○), 20 μg/mL (△), or no drug in the case of control cultures (□). Right-side panels represent cultures UV irradiated to final doses of 5 J/m² (●), 10 J/m² (○), 50 J/m² (△), or mock-irradiated in the case of the control cells (□). The ordinates are linear scales; however, note the break in the scale between 300 and 400% in the middle panel. The initial levels of poly(ADPR) synthesis (dpm/10⁶ cells) in control cells for the MNNG experiments were the following: GM0892, 17 800; GM605, 7 600; GM558, 10 919. The levels in control cells for the UV experiments were the following: GM0892, 5 700; GM605, 7 600; GM558, 4 600.

to UV irradiation, all the normal cell types increased their poly(ADPR) synthesis in response to both types of DNA damage.

Figure 2 shows the results of similar experiments performed in cells obtained from patients in five different complementation groups of xeroderma pigmentosum. All of the cell types responded to the MNNG treatment with an abrupt increase in poly(ADPR) synthesis. In the XP-E cell line the maximal response to MNNG treatment was 170% while the maximal responses in the other XP cell lines were between 500 and 1500%. Thus, the responses to MNNG treatment were quite exaggerated in most of the XP cell lines when compared to

Table I: Response of Poly(ADPR) Synthesis to UV Irradiation and DNase Treatment in Long-Term Lymphocyte Cell Lines from Normal Donors and Patients with Xeroderma Pigmentosum[a]

cell line (complementation group)	conditions	poly(ADPR) synthesis, incorp of [³H]ADPR (dpm/10⁶ cells) −DNase	+DNase
GM0558 (normal)	control	4 600	34 000
	UV irradiated	6 200	45 800
GM0605 (normal)	control	7 600	88 200
	UV irradiated	9 800	101 700
GM0892 (normal)	control	5 700	65 000
	UV irradiated	8 110	61 700
GM2250 (XP-A)	control	13 200	92 900
	UV irradiated	13 000	90 200
GM2252 (XP-B)	control	5 100	49 600
	UV irradiated	3 600	52 700
XP3BE (XP-C)	control	14 000	99 300
	UV irradiated	12 700	92 600
GM2253 (XP-D)	control	14 500	88 800
	UV irradiated	12 700	92 700
GM2450 (XP-E)	control	13 500	54 400
	UV irradiated	14 300	59 100

[a] Cells from each of the normal and XP cell lines were treated with 50 J/m² of UV irradiation or mock irradiated in the case of the control cells as outlined under Methods. Cells were then incubated in complete growth medium for 90 min, and then control and irradiated cells were permeabilized and incubated with [³H]NAD⁺ for 30 min at 30 °C to measure poly(ADPR) synthesis as described under Methods. The cells incubated in the absence of DNase were used to measure alterations in the level of poly(ADPR) synthesis due to UV irradiation. Both the control and UV-irradiated cells were treated with DNase as described under Methods to determine whether UV irradiation caused any alteration in the ability of their poly(ADPR) polymerase to respond to other types of DNA damage.

the responses in the normal cells. In contrast to the ability of cells derived from normal donors to respond to UV irradiation, the XP cells were all defective in their ability to synthesize poly(ADPR) in response to UV irradiation.

Cells from a patient in XP group A showed an increase in poly(ADPR) synthesis which reached 160% of the control level by 8 h after UV irradiation. An increase of this magnitude was characteristic of some of the normal cells. However, the response from the XP group A cells was delayed in comparison to that observed in the normal cells.

Cells from XP groups B and C showed essentially no increase in poly(ADPR) synthesis in response to UV irradiation. There was also no increase in poly(ADPR) synthesis in all the repeats of these experiments. Cells from XP groups D and E showed very slight increases in poly(ADPR) synthesis during the 8 h following UV irradiation. The maximum response to UV irradiation in group D cells was 115% of control, and the maximum in group E cells was 120% of control. No further increase in poly(ADPR) synthesis occurred when these cells were examined at 10 or 24 h after UV irradiation.

Experiments were then conducted to evaluate the possibility that in XP cells UV irradiation might in some way inactivate the ability of poly(ADPR) polymerase to respond to DNA damage. To examine this possibility, we made use of an earlier observation that cells permeabilized in the presence of 0.05% Triton X-100 could be treated with DNase to directly damage DNA, thereby inducing marked increases in the level of poly(ADPR) synthesis (Berger et al., 1978a,b). In this study we compared the levels of poly(ADPR) synthesis in irradiated and mock-irradiated control cells in the absence and presence of added DNase. Table I shows that in the absence of DNase, each of the three normal cell lines increased their level of poly(ADPR) synthesis in response to irradiation with 50 J/m².

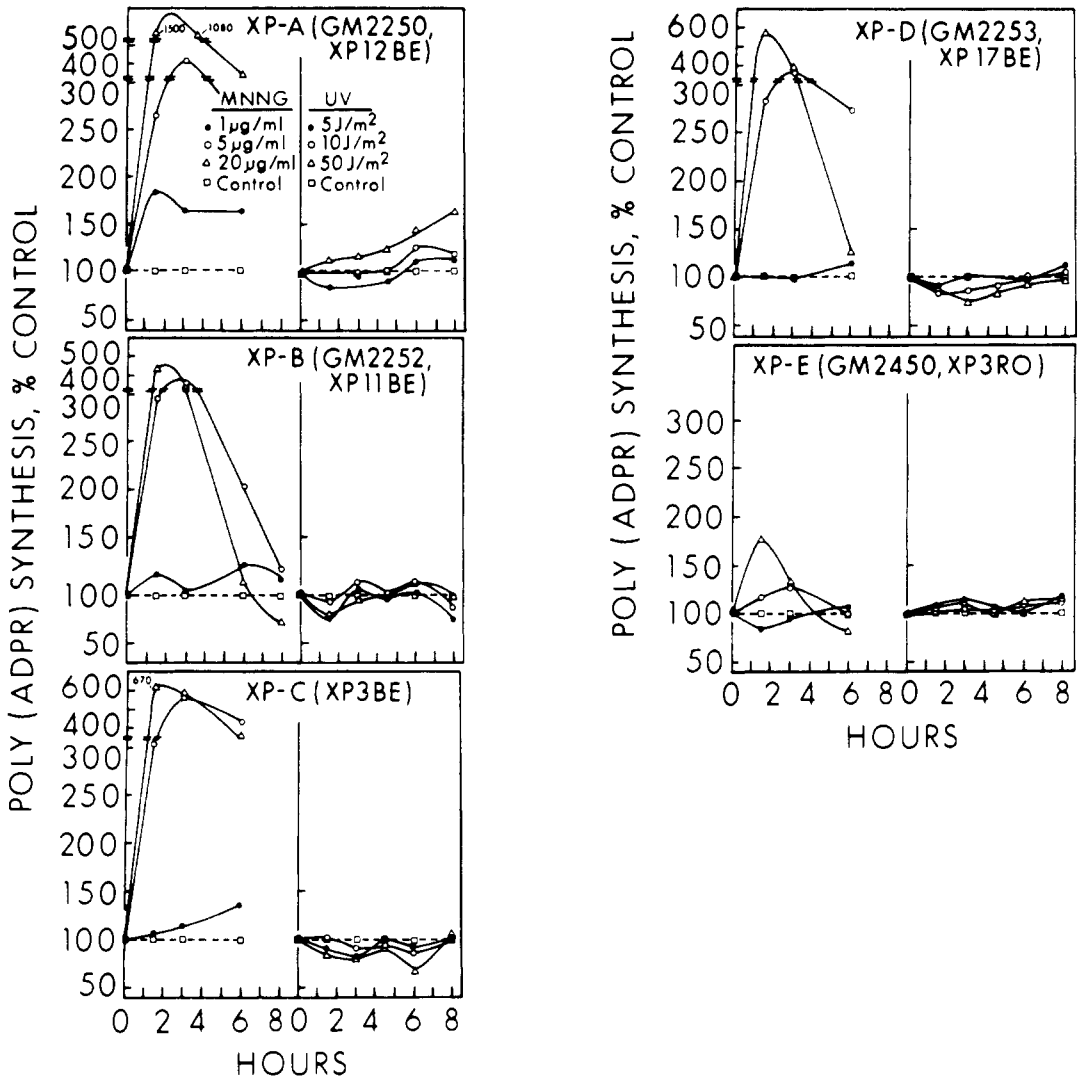

FIGURE 2: Effect of treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or UV irradiation on synthesis of poly(ADPR) in long-term lymphocyte cell lines derived from patients with xeroderma pigmentosum. The number and complementation group of each cell line are included across the top of each set of figures. Treatment with DNA-damaging agents was at 0 h. At each time point, poly(ADPR) synthesis is expressed as a percentage of the control levels measured simultaneously in cells that were mock treated as described under Methods. Left-side panels represent cells treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine which was added to final concentrations of 1 μg/mL (●), 5 μg/mL (○), 20 μg/mL (△), or no drug in the case of control cultures (□). Right-side panels represent cultures UV irradiated to final doses of 5 J/m² (●), 10 J/m² (○), 50 J/m² (△), or mock irradiated in the case of the control cells (□). The ordinates are linear scales; however, note the break between 300 and 400% and the change in the ordinate scale above 400% in each of the top four panels. The highest values in the upper left panel for XP-A cells treated with MNNG are 1500 and 1080%. The initial levels of poly(ADPR) synthesis (dpm/10⁶ cells) in control XP cells for the MNNG experiments were the following: XP-A, 6100; XP-B, 10800; XP-C, 10300; XP-D, 14-700; XP-E, 22500. The levels in control cells for the UV experiments were the following: XP-A, 13100; XP-B, 5100; XP-C, 16100; XP-D, 14500; XP-E, 13500. In XP-A and XP-C cells, where the response to MNNG was off scale, the maximum values achieved are indicated next to the appropriate points.

When the irradiated and mock-irradiated normal cells were treated with DNase, they all showed increases in poly(ADPR) synthesis. The XP cells showed essentially no increase in poly(ADPR) synthesis in response to UV irradiation. However, when the irradiated and mock-irradiated XP cells were treated with DNase, they all showed increases in levels of poly(ADPR) synthesis. The levels of poly(ADPR) synthesis in the DNase-treated, irradiated XP cells were similar to those obtained in the DNase-treated control XP cells. Thus, while XP cells do not increase their synthesis of poly(ADPR) in response to UV irradiation, they are still capable of doing so in response to other types of DNA damage such as that caused by treatment with DNase.

Discussion

We have recently shown that peripheral blood lymphocytes derived from normal human donors show an abrupt increase in both poly(ADPR) synthesis and DNA repair synthesis in response to treatment with MNNG or UV irradiation (Berger et al., 1979b). The present studies demonstrate that long-term lymphocyte cell lines derived from normal donors show a similar increase in poly(ADPR) synthesis in response to MNNG and UV irradiation. These treatments also stimulated unscheduled DNA synthesis in the long-term lymphocyte cell lines (data not shown). In contrast, long-term lymphocyte cell lines derived from XP patients in five different complementation groups all showed a normal to exaggerated poly(ADPR) synthesis response to treatment with MNNG but an abnormal to absent response to UV irradiation.

It has been proposed that the synthesis of poly(ADPR) in response to DNA damage may serve to modify chromatin structure so that the enzymes of DNA repair can gain access to the damaged DNA (Miller, 1975; Cleaver, 1978; Berger et al., 1979b). Poly(ADPR) is uniquely suited to perform the proposed chromatin modifications since it is a negatively charged polynucleotide, sometimes covalently linked to chro-

matin proteins, that can be rapidly synthesized and degraded (Hayaishi & Ueda, 1977; Sudhakar et al., 1979). The enzyme poly(ADPR) polymerase exists in large reserve quantities bound to chromatin, its activity is markedly stimulated by many different types of DNA damage, and the increase in its activity is not affected by inhibitors of protein synthesis (Berger et al., 1979b).

In order to synthesize poly(ADPR) in response to DNA damage, cells must possess poly(ADPR) polymerase, appropriate quantities of the substrate NAD^+, and a mechanism to sense the DNA damage and/or its consequences. Since some poly(ADPR) molecules have been found covalently attached to chromosomal protein acceptors (Hayaishi & Ueda, 1977; Sudhakar et al., 1979), it is also possible that the response to a particular type of DNA damage requires the presence of specific chromosomal acceptors.

All XP cells were able to synthesize poly(ADPR) appropriately after DNA damage induced by MNNG or DNase treatment, indicating that there is no intrinsic defect in poly(ADPR) polymerase or its ability to respond to DNA damage. Since these assays were conducted in permeable cells supplied with an excess of NAD^+, the defect in poly(ADPR) synthesis in XP cells cannot be due to lack of substrate. Moreover, the experiments with added DNase indicate that it is unlikely that any component of the poly(ADPR) synthesis system is inactivated by UV irradiation.

The failure of XP cells to show significant increases in poly(ADPR) synthesis in response to UV irradiation could be due to defects in the cells' mechanism for sensing DNA damage or failure of the cells to convert the DNA damage to the appropriate signal to stimulate poly(ADPR) synthesis. Since poly(ADPR) synthesis has been shown to increase in response to DNA strand breaks (Miller, 1975; Halldorsson et al., 1978; Berger et al., 1979a), it is possible that its failure to increase in XP cells in response to UV irradiation is linked to the defective ability of XP cells to excise UV-induced pyrimidine dimers. Tanaka et al. (1975) showed that the UV repair defect in XP cells could be corrected by supplying cells with T4 endonuclease V which specifically incises DNA near pyrimidine dimers. The ability of this enzyme to correct the defect in DNA repair provides strong support for the proposal that XP cells are defective in their ability to perform the incision step of DNA repair. Thus, the failure of UV irradiation to stimulate poly(ADPR) synthesis in XP cells may be secondary to the failure of these cells to create DNA strand breaks in the region of UV-induced DNA damage. These observations with the normal and XP cells suggest that the introduction of DNA strand breaks at the site of DNA damage may act as a stimulus for poly(ADPR) synthesis.

It is clear that more direct evidence is required to determine whether poly(ADPR) synthesis is an integral part of the DNA repair process or whether it reflects some independent process which occurs simultaneously with the induction and resealing of DNA strand breaks. However, these experiments are consistent with a pathway for DNA repair in which DNA damage results in breaks of the DNA backbone which stimulates the synthesis of poly(ADPR) which in turn produces alterations in the chromatin structure such that the DNA damage can be excised and the region repaired. Subsequent degradation of poly(ADPR) would then allow the chromatin to return to its original conformation.

Acknowledgments

We thank Dr. Allen Andrews for the XP3BE cell line.

References

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

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

Berger, N. A., Adams, J. W., Sikorski, G. W., Petzold, S. J., & Shearer, W. T. (1978a) *J. Clin. Invest. 62*, 111–118.

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

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

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

Cheng, W.-S., Tarone, R. E., Andrews, A. D., Whang-Peng, J. S., & Robbins, J. K. (1978) *Cancer Res. 38*, 1601–1609.

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

Cleaver, J. E. (1973) *Cancer Res. 33*, 362–369.

Cleaver, J. E. (1978) in *The Metabolic Basis of Inherited Disease* (Stanbury, J. B., Wyngaarden, J. B., & Fredrickson, D. S., Eds.) 4th ed., pp 1072–1095, McGraw-Hill, New York.

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

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

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

Jacobson, M. K., & Jacobson, E. L. (1978) *J. Supramol. Struct., Suppl. 2*, 74.

Kraemer, K. H., Coon, H. G., Petinga, R. A., Barrett, S. F., Rahe, A. E., & Robbins, J. H. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 59–63.

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

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

Sudhakar, S., Tew, K. D., Schein, P. S., Wooley, P. W., & Smulson, M. E. (1979) *Cancer Res. 39*, 1411–1417.

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