Regulation of DNA Repair in Serum-stimulated Xeroderma Pigmentosum Cells

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ABSTRACT The regulation of DNA repair during serum stimulation of quiescent cells was examined in normal human cells, in fibroblasts from three xeroderma pigmentosum complementation groups (A, C, and D), in xeroderma pigmentosum variant cells, and in ataxia telangiectasia cells. The regulation of nucleotide excision repair was examined by exposing cells to ultraviolet irradiation at discrete intervals after cell stimulation. Similarly, base excision repair was quantitated after exposure to methylmethane sulfonate. WI-38 normal human diploid fibroblasts, xeroderma pigmentosum variant cells, as well as ataxia telangiectasia cells enhanced their capacity for both nucleotide excision repair and for base excision repair prior to their enhancement of DNA synthesis. Further, in each cell strain, the base excision repair enzyme uracil DNA glycosylase was increased prior to the induction of DNA polymerase using the identical cells to quantitate each activity. In contrast, each of the three xeroderma complementation groups that were examined failed to increase their capacity for nucleotide excision repair above basal levels at any interval examined. This result was observed using either unscheduled DNA synthesis in the presence of 10 mM hydroxyurea or using repair replication in the absence of hydroxyurea to quantitate DNA repair. However, each of the three complementation groups normally regulated the enhancement of base excision repair after methylmethane sulfonate exposure and each induced the uracil DNA glycosylase prior to DNA synthesis. These results suggest that there may be a relationship between the sensitivity of xeroderma pigmentosum cells from each complementation group to specific DNA damaging agents and their inability to regulate nucleotide excision repair during cell stimulation.

Eucaryotic DNA repair pathways function to conserve the genetic information encoded in nucleotide sequences in cellular DNA. Two major excision repair pathways have been characterized in human cells. In nucleotide excision repair, which is responsible for the removal of pyrimidine dimers and other bulky lesions from DNA, the initial enzymatic action is endonucleolytic (29). Subsequently, the modified nucleotides are removed within an oligonucleotide fragment. In base excision repair, the initial enzymatic action is that of a DNA glycosylase (41). The glycosylase cleaves the base sugar glycosyl linkage removing the modified base and leaving an apurinic or apyrimidinic site in DNA. Recent studies suggest that mammalian cells actively regulate each DNA repair pathway during the defined pattern of gene expression observed during cell proliferation (4, 46). As compared with basal levels in quiescent cells, proliferating cells (*a*) have a greater capacity for DNA repair synthesis after exposure to ultraviolet irradiation (25, 26, 28, 40, 47, 56), ionizing radiation (39), or alkylating agents (25, 26, 28, 56); (*b*) have higher specific activities of the DNA repair enzymes uracil DNA glycosylase (1, 10, 19, 23, 58), 3-methyladenine DNA glycosylase (15, 23), and the O^6-methylguanine methyltransferase in the absence of cellular insult (49, 50) or during toxicity-induced cellular hyperplasia (48); and (*c*) increase the rates of excision of DNA adducts as a function of their proliferative state (20, 22, 34).

We have begun to examine whether normal human cells regulate nucleotide excision repair and base excision repair as they are stimulated to proliferate. In these studies two basic approaches were used. First, we examined the regulation of specific DNA repair enzymes relative to that induction observed for DNA polymerase during cell stimulation. In these

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studies, human cells were not exposed to any DNA damaging agent nor to any agents that inhibited their stimulatory response. Second, we examined the enhancement of DNA repair processes by exposing cells to DNA damaging agents at specific intervals after cell stimulation. Cell populations were untreated prior to exposure and thus cells examined at separate intervals had progressed to different stages of their normal stimulatory response. In serum stimulated WI-38 human embryonic lung fibroblasts and in serum stimulated normal human skin fibroblasts, we demonstrated that nucleotide excision repair after ultraviolet irradiation and base excision repair after methylmethane sulfonate exposure were enhanced during cell growth (25, 26, 28). The base excision repair enzyme uracil DNA glycosylase was induced after serum stimulation of quiescent normal human cells (25, 26, 28). The increase in the specific activity of this DNA repair enzyme during growth as cell number increased suggested that this pattern of gene expression was a normal event during cell proliferation (62). Using monoclonal antibodies directed against the human placental uracil DNA glycosylase, we suggested that this enhancement of the glycosylase may be due to the selective expression of individual excision repair gene families (3). Subcellular localization studies demonstrated that this increase in glycosylase activity was solely due to an increase in the activity of the nuclear enzyme as the activity of the mitochondrial enzyme remained constant during cell growth (27). The uracil DNA glycosylase removes uracil residues that may be formed in DNA by the mutagenic deamination of cytosine bases (31) in DNA or by the incorporation of deoxyuridine 5′-monophosphate during DNA replication (6).

Xeroderma pigmentosum (XP)[1] is an autosomal recessive syndrome that is characterized clinically by a pronounced increase in rates of skin cancer and by cellular hypersensitivity to ultraviolet irradiation (2, 21, 54, 57). Recent evidence indicates that several complementation groups exist within this human syndrome (37). Individuals within each complementation group are characterized by increased rates of mutagenesis (44) and transformation (7), and by reduced levels of nucleotide excision repair (35, 37, 38, 51). In confluent cells, the capacity for nucleotide excision repair can be severely diminished. XP fibroblasts, complementation group A, have only 1% of the nucleotide excision repair level observed in normal human cells (51). Alternatively, other complementation groups contain substantial repair capacity. XP fibroblasts, complementation group D, and cells from complementation group E, possess between 25–55% and between 40–60% of normal repair capacity, respectively (35, 38). In contrast, quiescent cells from individuals with the variant form of XP have normal levels of nucleotide excision repair capacity yet are also cancer prone (9, 13, 55) and have increased rates of mutagenesis (45). As we had previously determined that normal human cells enhanced their capacity for nucleotide excision repair and for base excision repair during cell stimulation, we sought to examine this regulation of repair pathways in XP cells. We now report that XP cells from three different complementation groups were unable to enhance nucleotide excision repair during cell stimulation yet normally enhanced base excision repair. In contrast, xeroderma pigmentosum variant cells and ataxia telangiectasia cells normally enhanced both excision repair pathways after serum stimulation.

[1] *Abbreviations used in this paper:* HU, hydroxyurea; J, joule; XP, Xeroderma pigmentosum.

MATERIALS AND METHODS

Cell Culture: Human diploid fibroblasts (WI-38, CCL 75), xeroderma pigmentosum fibroblasts (XP12BE, CRL 1223; XP8BE, CRL 158, and XP5BE; CRL 1150 corresponding to complementation groups A, C, and D), xeroderma pigmentosum variant fibroblasts (CRL 1258), and ataxia telangiectasia fibroblasts (CRL 1347) were purchased from the American Type Culture Collection (Rockville, MD). Cells were grown either in Eagle's minimum essential medium or Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 12 mM sodium bicarbonate, penicillin (100 U/ml), and streptomycin (100 μg/ml). WI-38 cells were serially subcultured every 5–6 d and used before their 25th passage. XP fibroblasts, ataxia telangiectasia fibroblasts, and normal human skin fibroblasts were serially subcultured every 8–9 d with fresh media added every 3 d between subcultures and were used prior to their 20th passage. For synchronous growth, cells were kept in medium containing 0.5% serum for 5 d then stimulated to proliferate by the addition of fresh medium containing 20% fetal calf serum. The absence of mycoplasma contamination was confirmed by the thymidine incorporation test (61). DNA synthesis was measured by the incorporation of [^{3}H]thymidine (6.7 Ci/mmol; 20 μCi/culture) for 30 min before collection. In parallel cultures, cells were harvested and cell pellets were used to determine total DNA polymerase and uracil DNA glycosylase activities in parallel cultures as previously described (25, 26, 58).

Enzyme Assays: Uracil DNA glycosylase was measured by determining the release of ethanol soluble radioactivity from a DNA substrate containing [^{3}H]uracil. Uracil containing DNA was prepared in a DNA polymerase assay using *Escherichia coli* DNA polymerase I, and DNase-activated calf thymus DNA as a template. The reaction mixture contained 50 μM [^{3}H]dUTP, 50 μM dGTP, 50 μM dATP, and 50 μM dCTP as deoxynucleosidase triphosphate precursors. After 60 min at 37°C, the polymerase reaction was phenol-extracted and then dialyzed against 4 × 4 liters of 1 M NaCl in 50 mM Tris-HCl (pH 8.0) followed by dialysis against 4 × 4 liters of 50 mM Tris-HCl (pH 8.0).

Uracil DNA glycosylase activity was measured in a reaction mixture (total volume 100 μl) that contained 100 mM Tris-HCl (pH 8.0), 5 mM dithiothreitol, 10 mM dipotassium EDTA, 2 μg of [^{3}H]uracil-labeled calf thymus DNA (280 cpm/pmol), and varying amounts of cell-free extract. Incubations were performed at 37°C for 30 min. Reactions were terminated by adding sequentially 300 μl of cold ethanol, 60 μl of 2 M NaCl, and 100 μl of denatured calf thymus DNA (1 mg/ml). After a minimum of 60 min at −20°C, the mixture was centrifuged at 2,300 *g* for 10 min. The ethanol supernatant (200 μl) was removed and the radioactivity counted in a liquid scintillation counter.

DNA polymerase was assayed in reaction mixtures (total volume 100 μl) that contained 50 mM Tris-HCl (pH 8.0); 10 mM $MgCl_2$; 50 μM dATP; 50 μM dCTP; 50 μM dGTP; 50 μM [α^{32}P]dTTP (200–1,000 cpm/pmol); 7.5 μg activated calf thymus DNA; 4 μg BSA; and 1 mM dithiothreitol. The reaction mixture was incubated for 60 min at 37°C. Reactions were terminated by addition of 1 ml of 10% trichloroacetic acid and 100 μl of heat denatured calf thymus DNA (1 mg/ml). Acid precipitable radioactivity was measured in a liquid scintillation counter. Both DNA polymerase and uracil DNA glycosylase enzyme activities were determined using the identical cell free extracts to quantitate each activity.

DNA Repair Assays: For quantitation of unscheduled DNA synthesis cells were synchronized by serum deprivation then stimulated to proliferate by the readdition of serum. Unscheduled DNA synthesis was measured at specific intervals during cell stimulation by treating cells at that interval with freshly prepared 10 mM hydroxyurea (HU) to inhibit semiconservative DNA synthesis and thus to stop cell stimulation at that specific interval. Prior to the addition of HU, cells were untreated and were only responding to the readdition of serum. Other cell cultures remained untreated and thus continued to respond to serum stimulation. These cultures would be treated with HU at later intervals to measure unscheduled DNA synthesis at those later intervals during the response of quiescent cells to serum stimulation. Before ultraviolet irradiation with 2.5–20 joule (J)/m^2 at 254 nm, the media was removed and cultures were washed once with Hank's balanced salt solution (HBSS). Unirradiated cultures containing only 10 mM HU served as controls. Methylmethane sulfonate (MMS) (final concentration 2 mM) was added to cells preincubated with 10 mM HU as described. After 15 min, [^{3}H]thymidine (20 μCi/culture; 40–50 Ci/mmol) was added and cells incubated for an additional 2 h. Control samples were treated identically except no methylmethane sulfate was added. Unscheduled DNA synthesis was determined by the incorporation of [^{3}H]-thymidine into acid precipitable material and quantitated in a liquid scintillation spectrometer. Unscheduled DNA synthesis in the presence of 10 mM HU is expressed as the difference in [^{3}H]thymidine incorporation after exposure to

either ultraviolet irradiation or to methylmethane sulfonate and that [^{3}H]-thymidine incorporation quantitated in cell cultures preincubated at each interval with 10 mM HU and pulsed with [^{3}H]thymidine without exposure to either DNA damaging agent. The ability of the cells to perform repair replication in the absence of HU was also determined. In this study 1×10^7 cells were preincubated for 60 min with 5'-bromodeoxyuridine (5 μg/ml) and fluorodeoxyuridine (0.5 μg/ml) and then exposed to either MMS (2 mM) or ultraviolet irradiation (20 J/m^2). Repair replication was determined as described previously (25, 26, 28).

RESULTS

Regulation of DNA Repair in Normal Human Cells

Synchronous cultures of WI-38 embryonic lung fibroblasts were used to examine the regulation of DNA repair in normal human cells. Cells were synchronized by serum depletion then stimulated to proliferate by the readdition of serum. At specific intervals after serum addition, cells were pulsed with [^{3}H]thymidine to measure the induction of DNA replication or, at identical intervals, were exposed to specific DNA damaging agents to measure their DNA repair potential at that interval after serum addition. A typical experiment with WI-38 cells is shown in Fig. 1. Serum addition resulted in the stimulation of quiescent cells as evidenced by the induction of DNA synthesis. As shown in Fig. 1*A* in this experiment, DNA replication started to increase 20 h after serum stimulation, was maximal at 24 h, and diminished thereafter. The regulation of the base excision repair enzyme uracil DNA glycosylase and of DNA polymerase was then examined. Each

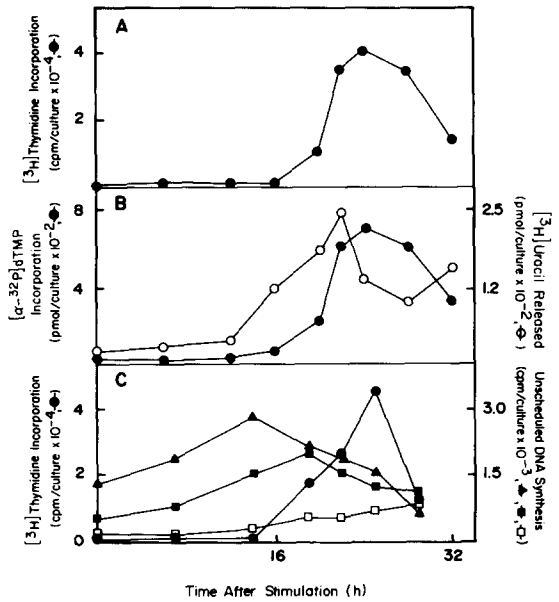

FIGURE 1 Serum stimulation of normal human WI-38 fibroblasts. Cells were synchronized by serum depletion then stimulated to proliferate by the readdition of serum as described in Materials and Methods. Cells were pulsed for 30 min with [^{3}H]thymidine (1 μCi/culture) at each indicated interval. The identical cell free extracts were used to examine the regulation of uracil DNA glycosylase and DNA polymerase. Unscheduled DNA synthesis of 10 mM HU was used to examine nucleotide excision repair and base excision repair. (*A*) Induction of DNA replication after serum readdition; (*B*) regulation of the uracil DNA glycosylase and of DNA polymerase; (*C*) regulation of nucleotide excision repair and base excison repair. ▲, UDS after ultraviolet irradiation in the presence of 10 mM HU (net counts per minute); ■, UDS after MMS exposure in the presence of 10 mM HU (net counts per minute); □, incorporation with 10 mM HU alone (gross counts per minute).

enzyme activity was measured using the identical cells used to quantitate the induction of DNA replication. As shown in Fig. 1*B*, glycosylase activity was increased 13.8-fold during cell stimulation. Enzyme activity started to increase 16 h after serum addition and was maximal at 22 h. Previous experiments demonstrated that cells kept in low serum for equivalent intervals showed no increase in basal levels of this DNA repair enzyme observed immediately after the readdition of serum to quiescent cells (25). The regulation of DNA polymerase was examined using the same cell extracts. As shown in Fig. 1*B*, DNA polymerase activity was also increased. Polymerase activity started to increase at 20 h after cell stimulation and reached its maximal level at 24 h. The temporal sequence of polymerase stimulation was concomitant with the induction of DNA replication. In contrast, glycosylase activity peaked prior to the maximal increases in both the rate of DNA synthesis and the induction of a DNA replicative enzyme. Further, glycosylase activity was diminished when both DNA replication and polymerase enhancement were at their peak.

To examine the regulation of DNA repair WI-38 cells were exposed to DNA damaging agents at specific intervals after serum stimulation. A typical experiment is shown in Fig. 1*C*. The capacity for nucleotide excision repair was examined by irradiating cells with 20 J/m^2 of ultraviolet light. The capacity for base excision repair was determined by exposing cells to 2 mM methylmethane sulfonate. Each agent produces distinct lesions in DNA repaired by the respective excision repair pathways (29, 41). As shown in Fig. 1*C*, basal levels of nucleotide excision repair and of base excision repair were observed in cells examined immediately after the addition of serum at 0 h. This result is in accord with numerous studies demonstrating that confluent eucaryotic cells contain detectable repair capacity. Cell stimulation resulted in an enhancement of each repair pathway prior to the induction of DNA replication. These findings are similar to our previously reported results using WI-38 cells (25, 26) and are similar to our results previously reported using synchronous populations of normal human skin fibroblasts (28). Further, proliferating normal human cells enhanced their DNA repair capacity as cell number increased in a 5-day growth period (results not shown).

Regulation of DNA Repair in Xeroderma Pigmentosum Cells

The capacity of xeroderma pigmentosum cells to regulate nucleotide excision repair, base excision repair, and to induce the uracil DNA glycosylase was then examined. XP cells from complementation groups A and C as well as XP variant fibroblasts were used. As shown in Fig. 2*A*, the readdition of serum to quiescent XP cells, complementation group A, resulted in cell stimulation. DNA synthesis started to increase 21 h after serum addition, was maximal at 28 h, and diminished thereafter. Similarly, base excision repair after methylmethane sulfonate exposure was enhanced 4.6-fold reaching this maximal level in cultures exposed to methylmethane sulfonate 21 h after serum addition. Base excision repair declined thereafter. In contrast to their enhancement of base excision repair and of DNA replication, XP cells, complementation group A, did not enhance nucleotide excision repair after serum stimulation. Using a dose of either 5 J/m^2 or a dose of 20 J/m^2, nucleotide excision repair did not

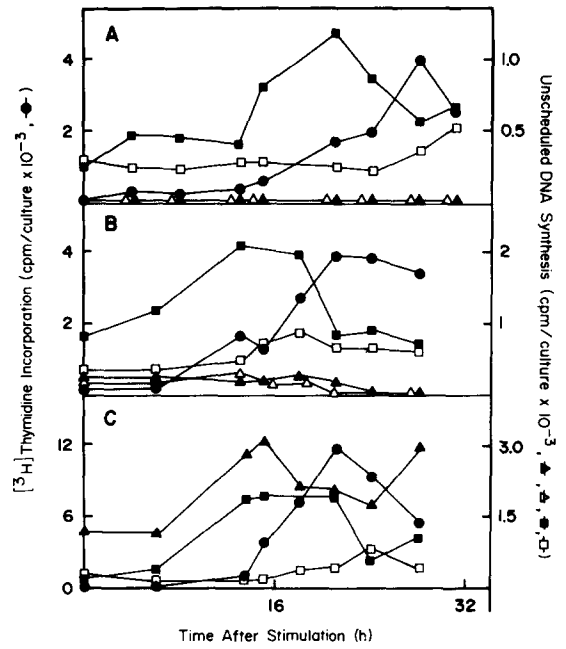

FIGURE 2 Serum stimulation of xeroderma pigmentosum cells. The experiments were performed as described in the legend to Fig. 1 and as described in Materials and Methods. The regulation of DNA repair in xeroderma pigmentosum cells was examined using unscheduled DNA synthesis in the presence of 10 mM HU. The absolute hours at which each activity was enhanced in each of the xeroderma pigmentosum cell strains should not be compared as each of the experiments were performed independently and used different serum samples. □, 10 mM HU alone (gross counts per minute); ▲, UDS after ultraviolet irradiation (net counts per minute); ■, UDS after MMS exposure net counts per minute. (*A*) Regulation of DNA repair in xeroderma pigmentosum cells, complementation group A; (*B*) regulation of DNA repair in xeroderma pigmentosum cells, complementation group C; (C) regulation of DNA repair in the xeroderma pigmentosum variant.

increase above background levels at any interval examined during cell proliferation. Similar results were observed in XP-A cells as cell number increased in proliferating cultures over a 5-d growth period (results not shown). This extremely low level of nucleotide excision repair agrees with previously published reports relating the severe decrease in repair capacity in this complementation group (51).

In contrast, in XP cells, complementation group C, nucleotide excision repair, although diminished, was easily detectable in cells pulsed with either 5 J/m^2 or a dose of 20 J/m^2 ultraviolet light immediately after the readdition of serum at 0 h (Fig. 2*B*). However, XP-C cells did not increase this basal level of repair capacity in cells that were ultraviolet at any interval examined. In serum stimulated XP-C cells, DNA synthesis started to increase 13 h after serum addition and was maximal at 21–24 h. Similarly, base excision repair after methylmethane sulfonate exposure was increased 2.5-fold above basal levels observed at 0 h.

To determine whether this pattern of repair capacity during cell proliferation was characteristic of the XP syndrome, the regulation of DNA repair was examined in the XP variant. A typical experiment is shown in Fig. 2*C*. In the XP variant, nucleotide excision repair after ultraviolet irradiation was increased 2.4-fold in cultures irradiated at 15 h after cell stimulation. The 2.4-fold increase in nucleotide excision repair in XP variant cells was comparable with the 2.1-fold increase in nucleotide excision repair observed in normal human cells. Nucleotide excision repair then declined and was diminished during S phase. Base excision repair after methylmethane sulfonate exposure was increased 8.6-fold in XP variant cells. This increase in repair capacity was similar to the 3.9-fold increase in base excision repair capacity observed in normal human cells (Fig. 1*C*). Thus, in the xeroderma pigmentosum variant, the extent of nucleotide excision repair and base excision repair enhancement was comparable with that observed in normal human cells. In contrast, in two xeroderma complementation groups, A and C, only the enhancement of base excision repair was similar. As measured by unscheduled DNA synthesis, each of the complementation groups was unable to increase their respective levels of nucleotide excision repair capacity at any interval during cell stimulation. This result is identical to that previously observed with the D complementation group (26).

Regulation of the Uracil DNA Glycosylase and DNA Polymerase in XP Cells

The regulation of the base excision repair enzyme uracil DNA glycosylase relative to the induction of DNA polymerase was then examined in two XP complementation groups and in the XP variant. After serum addition cells were collected at specific intervals and each enzyme activity was determined. As shown in Table I, a similar enhancement of glycosylase regulation was observed in each XP cell strain. In XP cells, (complementation group A) in this experiment uracil DNA glycosylase activity was increased 2.1-fold at 14 h after serum addition. DNA polymerase activity was increased 2.2-fold reaching this level at 19 h after cell stimulation. In XP cells, complementation group C, a similar pattern was observed. In this experiment, the glycosylase was increased 2.7-fold at 20 h while polymerase was maximally increased 3.0-fold at 24-h. Similarly, as shown in Table I, in the XP variant, glycosylase activity was stimulated 5.5-fold in cultures collected at 18 h in that experiment. DNA polymerase was increased 21-fold reaching that stimulation 21 h after serum addition.

TABLE I

Induction of DNA Polymerase and Uracil DNA Glycosylase in Xeroderma Pigmentosum Cells

Cell type	DNA polymerase [α-^{32}P]-dTMP incorporation	Fold increase	Uracil DNA glycosylase [^{3}H]uracil released	Fold increase
	pmol/culture		*pmol/culture*	
XP-A (CRL 1223)	*90:197 (19)‡	2.2	500:1017 (14)	2.1
XP-C (CRL 1158)	54:163 (24)	3.0	182:484 (20)	2.7
XP-variant (CRL 1258)	9:194 (21)	21.0	11:61 (18)	5.5

XP cells were synchronized by serum depletion, stimulated by the readdition of serum, and collected at discrete intervals thereafter. Cells were not exposed to either ultraviolet irradiation or to methylmethane sulfonate. Both DNA polymerase and uracil DNA glycosylase activities were examined using the identical cell free extracts to quantitate the level of each enzyme during cell proliferation.

* The first number in each series represents the enzyme activity observed at 0-h interval; the second number in each series represents the maximum enzyme activity after serum stimulation.

‡ The number in parenthesis represents that interval (hours after serum stimulation) at which the enzyme activity was maximally induced in that experiment.

These results are in accord with our previous suggestion that there may be an equivalence between the increase of DNA replicative enzyme activity and the increase in DNA repair enzyme activity as a function of cell stimulation. In particular, in two XP cell strains, the extent of glycosylase stimulation was comparable with that observed for DNA polymerase. The sole exception was that of the XP variant in which the enhancement of polymerase activity was greater than that observed for the uracil DNA glycosylase.

Repair Replication as a Measure of DNA Repair in XP Cells

Although we have determined that 10 mM HU by itself has no effect on the capacity of mammalian cells to perform base excision repair (36), others have suggested that 10 mM HU may affect the DNA polymerase or DNA ligase step(s) of nucleotide excision repair (14). Our results in Fig. 2, *A* and *B* demonstrating that XP cells, complementation groups A and C, were unable to enhance their nucleotide excision repair capacity during cell stimulation was examined using unscheduled DNA synthesis in the presence of 10 mM HU to quantitate nucleotide excision repair. Thus, to verify these results as detected by unscheduled DNA synthesis, repair replication into parental DNA in the absence of 10 mM HU was used as a technique to examine the modulation of DNA repair in XP cells. As previously reported, the regulation of DNA repair in normal human WI-38 cells could be observed using repair replication instead of unscheduled DNA synthesis to quantitate DNA repair. A typical experiment is shown in Table II. In contrast, as measured by repair replication, XP cells complementation group D, failed to increase their capacity for nucleotide excision repair during cell growth. At comparable intervals during serum stimulation in which WI-38 cells increased repair replication after ultraviolet irradiation, the levels of nucleotide excision repair in XP-D cells remained at basal levels. However, XP-D cells did exhibit an enhancement of base excision repair reaching a maximum at 16 h after serum stimulation. Thus, the inability of XP cells from a third complementation group to enhance nucleotide excision repair can be detected using repair replication in the absence of HU as it can be detected using unscheduled DNA synthesis in the presence of 10 mM HU as a technique to quantitate DNA repair.

TABLE II

Induction of Repair Replication in Human Cells

	Nucleotide excision repair			Base excision repair		
Cell type	Time after stimulation	Repair replication	Fold increase	Time after stimulation	Repair replication	Fold increase
	h	*cpm/μg DNA*		*h*	*cpm/μg DNA*	
WI-38	0	37	1.0	0	41	1.0
	14	162	4.4	16	47	1.2
	16	40	1.1	20	86	2.1
	24	22	0.6	24	37	0.9
XP-D	0	34	1.0	0	26	1.0
	14	32	0.9	14	26	1.0
	16	27	0.8	16	50	1.9
	22	23	0.7	22	22	0.9

At the indicated intervals in the cell cycle, 1×10^7 WI-38 cells or 1×10^7 XP cells (XP 5 BE; CRL 1160) were preincubated for 60 min with 5 μg/ml of 5′-bromodeoxyuridine. Cells were then exposed to ultraviolet light at 254 nm (5 or 20 J/m^2) to measure nucleotide excision repair or were exposed to 2 mM methylmethane sulfonate to quantitate base excision repair. Cells were then incubated with [^{3}H]thymidine (100 μCi/culture, 52 Ci/mmol) in medium containing 5 μg/ml of 5′-bromodeoxyuridine for 2 h. Equilibrium density analysis was performed according to the protocol of Pettijohn and Hanawalt (52). DNA was isolated according to the procedures of Goth-Goldstein (24) and sedimented on a neutral cesium chloride gradient to separate parental DNA (ρ = 1.71) from replicated DNA (ρ = 1.75) as previously described (25). Parental DNA was then further purified through an alkaline cesium chloride equilibrium density gradient. The specific activity of parental DNA was then determined. In the second gradient, [^{3}H]thymidine incorporated into parental DNA varied between 500–2,000 total cpm in different gradients. The absolute values of DNA repair in WI-38 cells and XP-D cells are not comparable as these experiments were not performed simultaneously.

Regulation of DNA Repair in Ataxia Telangiectasia Cells

To examine the potential relationship between the pattern of repair regulation in XP cells to their respective cellular hypersensitivities, the regulation of DNA repair was examined in ataxia telangiectasia cells. Although AT cells show normal sensitivity to either ultraviolet irradiation or to methylmethane sulfonate exposure, they are hypersensitive to ionizing radiation and individuals with ataxia telangiectasia are cancer prone (2, 19, 52). As shown in Fig. 3*A*, ataxia telangiectasia cells regulated both nucleotide excision repair and base excision repair during cell stimulation. In this experiment using ataxia telangiectasia cells, nucleotide excision repair after ultraviolet irradiation (20 J/m^2) was increased 5.2-fold in cultures irradiated at 16 h after serum addition. Base excision repair after 2 mM methylmethane sulfonate exposure was enhanced 11.6-fold during cell stimulation. DNA replication was maximal at 24 h after serum addition and then declined. As shown in Fig. 3*B*, the uracil DNA glycosylase activity was also increased after serum addition. In this experiment, the glycosylase activity was increased 6.3-fold reaching this increase at 20 h after cell stimulation. Thus, the regulation nucleotide excision repair, base excision repair,

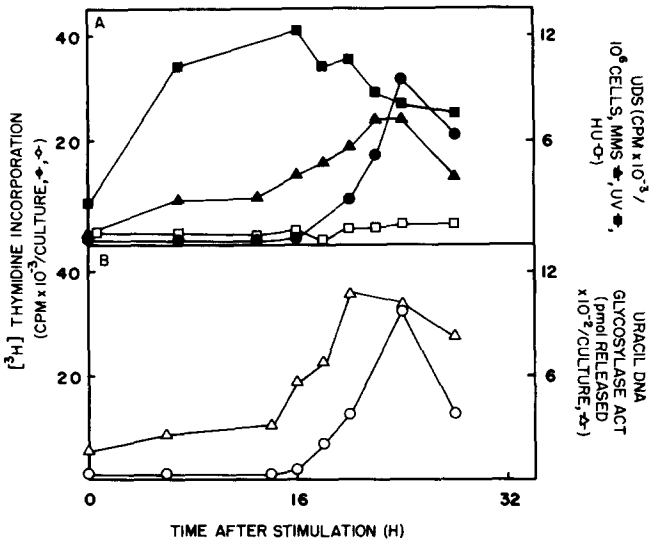

FIGURE 3 Serum stimulation of ataxia telangiectasia cells. The experiment was performed as previously described in the legend to Fig. 1 and in Materials and Methods. Using unscheduled DNA synthesis in the presence of 10 mM HU to examine DNA repair. □, 10 mM HU alone (gross counts per minute); ▲, UDS after ultraviolet irradiation (net counts per minute); ■, UDS after MMS exposure (net counts per minute). (*A*) Regulation of nucleotide excision repair and base excision repair; (*B*) regulation of the uracil DNA glycosylase.

and the enhancement of uracil DNA glycosylase activity in ataxia telangiectasia cells was equivalent to that observed in normal human cells as well as in the variant form of xeroderma pigmentosum.

DISCUSSION

Numerous studies have established that eucaryotic cell proliferation requires a temporal program of gene expression as quiescent cells are stimulated to proliferate (4, 46). This requires an inherent selectivity in the specific regulation of singular structural genes. Thus, with respect to the regulation of a specific gene as a function of cell proliferation, three types of regulation may occur: (*a*) the increased expression of a gene with the concomitant increase in its product; (*b*) no change in its expression with no increase in its product; or (*c*) a decrease in its expression with the subsequent decrease in its product. Alternatively, allosteric interactions within multienzyme complexes could alter enzyme activity without modulation of that specific structural gene. Recent evidence demonstrates that eucaryotic cells use each regulatory mechanism with respect to the modulation of nucleic acid enzymes during cell proliferation. Thus, some nucleic acid enzymes are increased during cell growth. These include DNA polymerase α (5, 12, 16, 42, 43), thymidine kinase (8), thymidylate kinase (8), as well as each eucaryotic RNA polymerase (32). In contrast, other enzyme activities remain constant during cell proliferation. These include GMP kinase (43), dGMP kinase (43), DNA topoisomerase I (11, 18), and DNA ligase II (60). Other activities may be diminished. In particular, during liver cell proliferation induced by partial hepatectomy, thymidine triphosphate dephosphorylase activity is diminished (33). This decrease in dTTP dephosphorylase activity as a function of cell proliferation emphasizes the inherent specificity of this genetic program. Further, association of individual enzymes within multienzyme complexes can affect enzyme activity. As described by Reddy and Pardee (1953), formation of the DNA replitase complex rendered thymidylate synthetase sensitive to inhibition by specific inhibitors of ribonucleotide reductase (HU), topoisomerase (novobiocin), and to DNA polymerase α (aphidicolin). In contrast, thymidylate synthetase activity was unaffected by each inhibitor when the enzyme was assayed in soluble extracts (53).

In this report we provide evidence to suggest that human cells actively regulate DNA repair pathways during cell stimulation. Similar enhancements of repair activity were observed in xeroderma pigmentosum variant fibroblasts and in ataxia telangiectasia cells. This normal regulation of repair capacity in these cancer prone human cells is in accord with their normal levels of nucleotide excision repair capacity and of base excision repair capacity. We have also presented evidence that three xeroderma pigmentosum complementation groups normally enhanced their capacity for base excision repair and normally enhanced uracil DNA glycosylase activity after serum stimulation. In contrast, each of the three different complementation groups was characterized by an identical inability to enhance nucleotide excision repair capacity during cell stimulation. In particular, although each complementation group was characterized by variable levels of basal nucleotide excision repair capacity, these respective basal levels were unaltered. Two separate techniques were utilized to quantitate the regulation of DNA repair pathways during cell stimulation. Although unscheduled DNA synthesis in the presence of 10 mM HU has been routinely used in studies of DNA repair, it had been suggested that HU, by itself, could alter DNA repair processes (17). In contrast, other studies suggest that HU has no effect on DNA repair (30, 59). Further, it should be noted that the enhancement of repair synthesis during cell stimulation would not be due to changes in deoxyribonucleoside triphosphate pool sizes during cell growth. In particular, in the presence of 10 mM HU, the relative pool size remained constant in concanavalin A stimulated lymphocytes as compared with unstimulated cells (56). We repeated these studies using serum stimulated WI-38 cells. Quantitation of DNA synthesis as previously described (56) were performed using [^{3}H]thymidine of varying specific activities (20-fold differences). These studies demonstrated that measurements of unscheduled DNA synthesis in the presence of 10 mM HU using this radioactive precursor were also independent of any possible changes in deoxyribonucleoside triphosphate pool size during cell proliferation (Gupta, P. K., and M. A. Sirover, unpublished observations). We demonstrated that HU did not inhibit the excision of 7-methylguanine over a 72 h-interval nor did it inhibit the activity of the uracil DNA glycosylase for 24 h (36). Further, in this study, we demonstrated that identical results were observed using either unscheduled DNA synthesis in the presence of HU or repair replication in the absence of HU. In particular, using either technique we could demonstrate (*a*) the normal regulation of nucleotide excision repair in normal human cells; and (*b*) the inability of xeroderma pigmentosum cells to enhance nucleotide excision repair during cell stimulation. Thus, irrespective of any putative effects of HU on DNA repair processes, there was no effect on our capacity to examine the enhancement, or lack of enhancement, of specific DNA repair pathways during cell stimulation.

It may be argued that one would not expect any of the XP complementation groups to demonstrate any regulation of the nucleotide excision repair pathway after serum stimulation. Each of the complementation groups that have been identified have reduced constitutive levels of nucleotide excision repair. Thus, since these cells are genetically defective in their basal levels of nucleotide excision repair, one should not expect these cells to regulate a DNA repair system that is already beyond their capacity to regulate. In support of this argument are our results using XP cell complementation group A, in which the basal levels of nucleotide excision repair were at background levels at every interval examined during cell proliferation. These results are in accord with other studies on the low level of nucleotide excision repair in this complementation group. On the other hand, other complementation groups contain significant amounts of this repair capacity. In our studies, we were able to easily quantitate repair levels in XP-C and XP-D cells. These results are in accord with previous studies demonstrating that cells from some XP complementation groups may contain from 33–65% of that nucleotide excision repair capacity observed in normal human cells. One may thus presume that cells from these complementation groups also contain a comparably reduced, but still substantial, level of regulatory capacity with respect to the recognition, transcription, and translation of the nucleotide excision repair genome. Thus, one would expect to see regulatory responses in XP complementation groups proportional to the amount of repair regulatory capacity remaining in each complementation group. It may be of significance that this expectation was not fulfilled and that cells from each complemen-

tation group exhibited only their characteristic basal levels of nucleotide excision repair during cell stimulation. Further studies are thus required to attempt to resolve this apparent inconsistency.

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