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Induction of plasminogen activator by UV light in normal and xeroderma pigmentosum fibroblasts

(DNA repair/SOS function/xeroderma pigmentosum heterozygotes)

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ABSTRACT **Normal and DNA repair-deficient human fibroblasts have been used to study induction of plasminogen activator (PA) by DNA damage. UV light induced the synthesis of PA in skin fibroblasts of all types of xeroderma pigmentosum (XP) in XP heterozygotes and in human amniotic cells. Enzyme induction was, however, not observed in fibroblasts of normal adults. In classical XP, which are deficient in excision repair, PA synthesis occurred in a narrow range of low-UV fluences. In such strains, the level of enzyme produced was correlated with the extent of repair deficiency. UV fluences required for PA induction in XP variants and XP heterozygotes were at least 10 times those inducing enzyme synthesis in excision-deficient XP. Maximum enzyme induction occurred 48 hr after irradiation, and the highest levels of enzyme produced were 15–20 times those of PA baseline levels. Electrophoretic analysis showed that UV irradiation enhances the synthesis of the M_r 60,000 human urokinase-type PA, which is present in low amounts in untreated cells. Our results suggest that PA induction in human cells is caused by unrepaired DNA damage and represents a eukaryotic SOS-like function. In addition, PA induction may provide a sensitive assay for detection of cellular DNA repair deficiencies and identification of XP heterozygotes.**

DNA damage induces in bacteria the coordinate expression of a set of diverse responses collectively called SOS functions. These functions include the appearance of repair and mutagenic activities, inhibition of septation, induction of certain prophages, and production of large amounts of the *recA* protein (for review, see ref. 1). Induction of SOS functions appears to involve the protease activity of the *recA* protein, which cleaves cellular repressors of SOS functions including its own repressor, the *lexA* gene product (2–5).

Several observations suggest that SOS-like activities are also induced in mammalian cells in response to DNA damage (for reviews, see refs. 6 and 7). Thus, analogous to Weigle reactivation of bacteriophage, the survival of certain UV-irradiated viruses is enhanced by exposing the mammalian host cells to DNA-damaging agents prior to infection (6, 7). Some studies indicate that this enhanced reactivation is accompanied by viral mutagenesis (8, 9). Other possible manifestations of the SOS response in mammalian cells are the induction of infectious viruses in transformed cell lines (10) and the enhancement of postreplication repair in Chinese hamster and human cells (11, 12) after UV irradiation or treatment with chemical carcinogens. The possible involvement of SOS-like functions in carcinogenesis has been discussed by several authors (1, 13, 14).

Miskin and Reich (15) have recently reported that physical and chemical agents that damage DNA induce in embryonic fibroblasts of several vertebrates the synthesis of plasminogen activator (PA). PA is a highly specific serine protease closely associated with cellular transformation, neoplasia, and tumor promotion (for reviews, see refs. 16, 17). In view of the central role attributed to proteolysis in the regulation of SOS functions in bacteria and because of the possible relation of both PA and SOS-like functions with neoplastic transformation, we further explored the induction of PA in human cells by UV irradiation.

In the present work, we studied the role of DNA repair in PA induction by skin fibroblasts from xeroderma pigmentosum (XP) patients. XP belongs to a class of repair-deficient human autosomal recessive disorders that have in common predisposition to cancer and chromosome instability (18, 19). XP is clinically characterized by abnormally enhanced sensitivity to sunlight and the appearance of carcinomas in exposed areas of the skin. Two classes of XP patients can be biochemically identified: those whose cells show defective excision repair of UV light-induced damage to DNA (excision-deficient XP) and patients whose cells are impaired in postreplication repair but exhibit normal excision repair (XP variants) (20–22).

We have tested skin fibroblasts of different excision-deficient and variant XP strains and cells of XP heterozygotes and normal donors for UV-induced PA synthesis. We found, unexpectedly, that, in contrast to embryonic cells, PA synthesis was not induced in normal adult skin fibroblasts. PA induction occurred, however, in fibroblasts of all the XP strains tested and in XP heterozygotes. Our results show that PA induction in human cells is associated with deficient DNA repair and exhibits properties expected of an SOS-type response.

MATERIALS AND METHODS

Cell Cultures and Irradiation. The cell strains used are shown in Table 1. Cells were obtained as skin fibroblast cultures and grown in Ham's F-10 medium or Dulbecco's modified Eagle's minimal essential medium, supplemented with 15% fetal bovine serum (GIBCO), penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were used between passages 5 and 17 of subcultivation.

Cultures were seeded into a series of 60-mm dishes at 2–2.5 × 10^5/dish. After 24 hr, cells were washed twice with phosphate-buffered saline and irradiated in a thin layer of this saline with a Hanovia low-pressure mercury lamp at a fluence rate of 0.15 $J \cdot m^{-2} \cdot sec^{-1}$. For fluences >10 J/m^2 a Mineralight lamp model R51 at 1.5 $J \cdot m^{-2} \cdot sec^{-1}$ was occasionally used. After irradiation, cells were washed twice with medium and incubated with 4 ml of fresh medium. Control cultures were subjected to identical treatment but were not irradiated.

Quiescent cells in G_1 (G_0) phase were obtained by limiting the serum concentration. Experiments were initiated by plating 2.5 × 10^5 cells per dish as described above. The following day the medium was changed to Ham's F-10/1% fetal bovine serum

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Abbreviations: PA, plasminogen activator; XP, xeroderma pigmentosum.

Table 1. Cell strains and UV fluences required for maximum PA synthesis

Strain	Complementation group	UV fluence,* J/m²	Source of strain
XP1EG	A	1	Camden GM2990
XP4LO	A	1–1.5	A. Lehmann
XPHF4LO	Father of XP4LO	15	A. Lehmann
XPHM4LO	Mother of XP4LO	>15	A. Lehmann
XP2BE	C	2	ATCC CRL1166
XP102LO	D	3–4	F. Giannelli
XP1BR	D	2–4	A. Lehmann
XP1WI	Unassigned	3–4	Camden GM1630
XPHF1WI	Father of XP1WI	>18	Camden GM1631
XP4BE	Variant	>15	ATCC CRL1162
XP115LO	Variant	ND	F. Giannelli
XP30RO	Variant	15	A. Lehmann
XP7TA	Variant	15	A. Lehmann
Control TA		ND	Hadassah
Control DR		ND	Hadassah
Control OY		ND	Hadassah
Control KM		ND	Hadassah

* Camden, Human Genetic Mutant Cell Repository, Camden, NJ; Hadassah, Cell Repository, Human Genetics, Hadassah Hebrew University Medical Center, Jerusalem; ND, nondetectable.

and cultures were incubated for 24–48 hr. After irradiation, cells were reincubated in 4 ml of medium containing 1% serum. Cells were judged to have been arrested in G_1 (G_0) phase by testing for the cessation of DNA synthesis.

Assays of Plasminogen Activator. Forty-eight hr after irradiation cells were collected, stored and lysed as previously described (15, 23) and assayed for PA activity in the fibrin plate assay as specified by Soreq and Miskin (24).

Briefly, tested samples were added to wells containing insoluble ^{125}I-labeled fibrin and human plasminogen. Proteolysis was monitored by measuring radioactivity of soluble ^{125}I-labeled peptides after 4 and 6 hr of incubation. Urokinase (Leo pharmaceutical products, Denmark) was added to each assay (0.25–3 ploug milliunits per well), and results were expressed as milliunits of Urokinase per mg of sample protein. Protein was determined by the method of Lowry *et al.* (25). Control assays from which plasminogen was omitted did not show any fibrinolysis, indicating an absolute dependence on plasminogen of the activity tested. Secreted PA could not be detected in conditioned media even after acid treatment to inactivate serum protease inhibitors. This was probably due to residual inhibitors (15).

Electrophoretic analysis of intracellular PA of the different cell strains was performed as described by Heussen and Dowdle (26) with the following modifications: the thickness of the gel was 0.5 mm, gelatin was replaced by casein (vitamin free, 0.1% final concentration per gel), the concentration of human plasminogen (27) was 20 μg/ml, and gels were incubated overnight to express proteolysis. Counterpart gels from which plasminogen was omitted did not show any active bands.

RESULTS

PA Induction in Excision-Deficient XP. Induction of PA synthesis by UV irradiation in two different skin fibroblast strains of XP complementation group A is shown in Fig. 1. Induction occurred in a narrow range of low UV fluences (0.5–2 J/m²) and resulted in a 15- to 20-fold increase of PA baseline levels. The decrease in PA synthesis at fluences >1–1.5 J/m² may be due to the pronounced reduction in survival of XP group A cells at these doses as a consequence of their inability to carry out excision repair (28). In contrast to XP cells, PA synthesis was not enhanced in any of the normal cell strains tested even if the cells were exposed to UV fluences of up to 25 J/m².

As the fluences that induce maximum induction of PA in XP group A fibroblasts also severely inhibit DNA synthesis and in view of the critical role attributed to the inhibition of DNA replication in bacterial SOS induction (1), we determined whether induction of PA synthesis occurs in nonreplicating cells. As shown in Fig. 1A, serum-starved cells, which do not synthesize DNA, produced significant amounts of PA in response to UV light.

PA synthesis by irradiated XP cells was inhibited by cycloheximide at concentrations (2 μg/ml) that only partially inhibited overall protein synthesis (Fig. 1A). Maximum induction of PA synthesis occurred 48 hr after treatment, and a similar delayed response was observed in other XP strains. At no time after UV irradiation (15 J/m²) was any evidence for the induction of PA synthesis by normal cells obtained (not shown).

Cell fusion experiments have shown the existence of seven complementation groups of XP, and fibroblasts of these groups differ in residual repair of UV-light damage (21). To determine the role of unexcised damage in PA synthesis, we compared enzyme induction as a function of dose in XP cells of complementation groups A, C, and D. The strains used have been shown to perform <5%, 16%, and 25%, respectively, of normal repair synthesis (29–31). We found that, the more severe the repair deficiency of XP, the higher the levels of PA induced (Fig. 1B). Furthermore, the fluences required for induction increased as a function of residual repair capacity. XP group A required the lowest inducing fluences for maximum enzyme induction and XP group D required the highest. Low levels of PA were also induced in an additional XP group D strain and in XP1WI, a strain not yet assigned to a complementation group.

PA Induction in XP Heterozygotes. As we were unable to detect PA induction in cells of normal donors, it was of interest

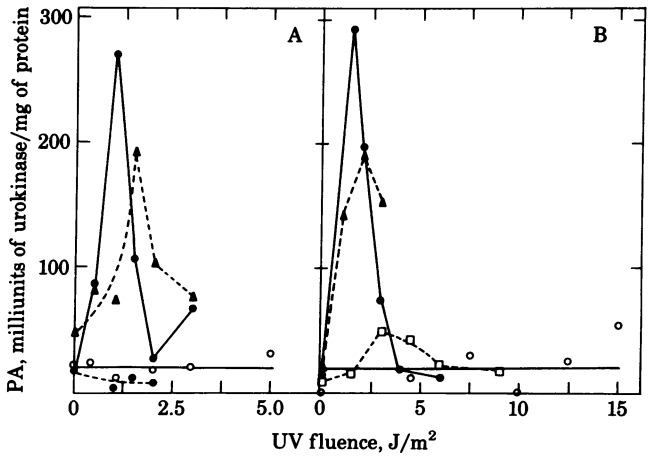

FIG. 1. Effect of UV irradiation on intracellular PA synthesis in skin fibroblasts of excision-deficient XP and normal adult donors. Cells were exposed to various fluences of UV light. After irradiation, cells were incubated for 48 hr and assayed for PA under standard conditions. (*A*) XP1EG complementation group A fibroblasts (●——●), the same cell strain incubated in the presence of cycloheximide (2 μg/ml) after irradiation (●– – –●), serum-starved XP1EG cells (▲), fibroblasts of a normal male donor (○). (*B*) XP4LO cells (XP group A) (●——●); XP2BE (XP group C) (▲); XP102LO (XP group D) (□); normal male donor (○).

to determine whether UV light induces PA synthesis in XP heterozygotes. As shown in Fig. 2, fibroblasts of both the mother and the father of patient XP4LO responded to UV light by induction of PA synthesis above a threshold dose of 7.5 J/m^2 and inducing fluences for maximum enzyme synthesis were more than 10-fold higher than those producing similar enzyme levels in the corresponding XP homozygote (Table 1). Consistently, the levels of PA produced by XPHM4LO (the mother) were higher than those of XPHF4LO (the father). Experiments with cells of XPHF1WI, the father of an XP patient of unassigned complementation group, are also included in Fig. 2. PA induction in the heterozygote strains tested may be a consequence of their presumed partial repair deficiency.

XP Variants. Four different XP variant strains were tested, and all but one (XP115LO) showed enzyme induction (Fig. 3 and Table 1). Maximum PA synthesis occurred at high fluences ($\geq$15 J/m^2), and the ultimate level of PA produced in some XP variant strains was similar to that induced by fluences 1/10 those required in the most excision-deficient XP.

Type of Basal and Induced PA in XP Fibroblasts and Human Amniotic Cells. It has been observed (32) that human PA exhibits two immunochemically distinct molecular forms, the so-called urokinase-like enzyme, which has species of apparent M_r 60,000 and 40,000, and the melanoma-like PA, which has a major species of apparent M_r 70,000. To test whether UV light induces the synthesis of a new molecular form of PA or enhances the synthesis of the species of the enzyme already present, the lysates of all the XP cell strains, before and after irradiation, were electrophoretically analyzed. Fig. 4 shows that the PA of both treated and untreated cells comigrates with the M_r 60,000 species of commerical human urokinase. Furthermore, a clear enhancement of this type of PA was observed after irradiation. In a few cases, a minor band that comigrates with the M_r 40,000 species of urokinase can be observed. However, in no case was any activity of the M_r 70,000 melanoma-type enzyme detected. Further support for the similarity between the basal and the induced PA and the urokinase-like enzyme was obtained by using sera raised against purified urokinase and melanoma PA (unpublished results).

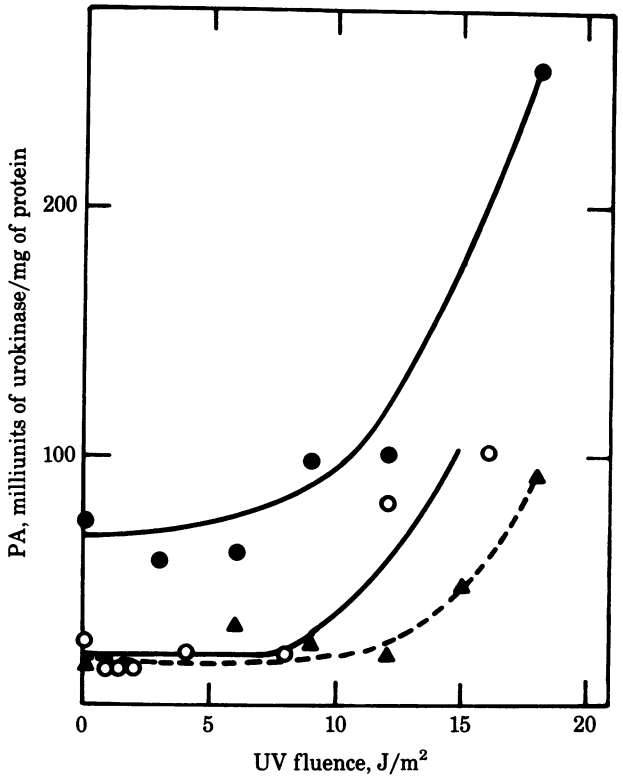

FIG. 2. UV-induced PA synthesis in fibroblasts of XP heterozygotes. Cells exposed to various fluences of UV light were incubated for 48 hr and assayed for intracellular PA. ●, XPHM4LO; ○, XPHF4LO; ▲, XPHF1WI.

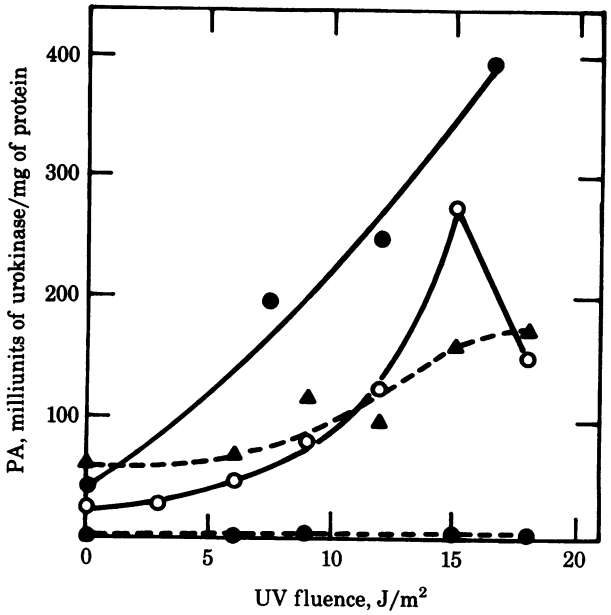

FIG. 3. PA induction in fibroblasts of XP variants. Cells were treated as described in the legend to Fig. 2. ●——●, XP4BE; ○, XP30RO; ▲, XP7TA; ●- - -●, XP115LO.

The lack of UV-induced PA enhancement in adult skin fibroblasts was in contrast to the positive response observed with embryonic human, rodent, and chicken fibroblasts (15). To test whether this response is characteristic of embryonic cells, we assayed human amniotic cells for enzyme induction and observed that UV light enhances enzyme production in these cells (not shown). Gel electrophoresis (Fig. 4) shows that the basal and induced PA are of the urokinase type also in amniotic cells.

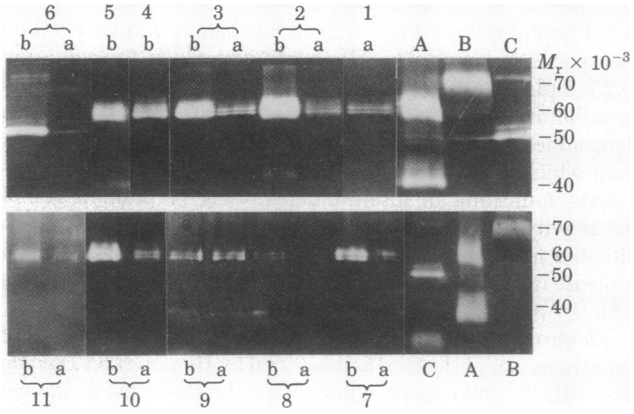

FIG. 4. Electrophoretic analysis of PA in skin fibroblasts of XP patients and human amniotic cells before and after irradiation. Six-milliliter samples of lysates of various cells were analyzed on NaDodSO$_4$/polyacrylamide gels. Lanes: 1, normal adult skin fibroblasts; 2, XP4BE (variant); 3, XP30RO (variant); 4, XP1EG (XP A); 5, XP2BE (XP C); 6, hamster embryo fibroblasts; 7, XP4LO (XP A); 8, XPHF4LO (XP heterozygote); 9, XPHM4LO (XP heterozygote); 10, XP102LO (XP D); 11, amniotic cells; A, commercial urokinase; B, conditioned medium of Bowes melanoma cells; C, mouse urine. a, Unirradiated cells; b, cells irradiated at UV fluences that result in maximum induction of PA; amniotic cells were irradiated at 18 J/m^2.

DISCUSSION

The results presented here show that DNA repair deficiency predisposes human cells to produce enhanced levels of PA in response to UV-light damage. The most striking effect of repair deficiency on PA induction was observed in excision-deficient XP. In these strains, induction occurred in a narrow dose range and resulted in an up to 20-fold increase in PA baseline levels. The fluences required for induction were 1/10th of those required for production of comparable levels of PA in XP variants, XP heterozygotes, and embryonic cells (15). These results imply that unexcised damage, left in DNA as a consequence of inefficient repair, is responsible for PA induction in human cells. Further support for this conclusion is provided by the experiments shown in Fig. 1*B*, which show that, among excision deficient-XP, cells of complementation group A (the most repair-deficient strain) produced the highest levels of PA and induction occurred at lower fluences than in XP strains of higher residual repair capacity.

In repair-deficient bacteria, unexcised damage causes hyperinducibility of a cluster of SOS functions (1, 33). Comparison of PA induction with UV-enhanced mutagenesis (28) and induced viral reactivation (34) in XP cells shows that all these functions exhibit a similar inverse relationship between residual repair capacity and inducing fluence. Enhanced viral reactivation (34) and induced PA synthesis occur in XP group A strains in the same low dose range and both exhibit a pronounced delayed expression; maximum levels of PA induction were observed 48 hr after irradiation. This time course seems to be characteristic for DNA damage-induced PA synthesis; similar induction kinetics were observed in human, rodent, and chicken embryonic cells after treatment with various physical and chemical DNA-damaging agents (15). It differs, however, from PA induction by hormones, oncogenic viruses, and tumor promoters, which elicit maximum enzyme levels 6–8 hr after treatment (23, 35–37).

As expected for an inducible function, synthesis of PA in XP cells after UV irradiation was completely inhibited by cycloheximide. A requirement for *de novo* protein synthesis has also been implicated in induction of UV mutagenesis, viral reactivation, and enhanced postreplication repair in mammalian cells (12, 34), as well as for the induction of SOS functions in bacteria (1).

Electrophoretic migration experiments showed that, in all cell strains tested, the basal and induced PA are of the same type and comigrate with the M_r 60,000 species of commercial urokinase, the human urinary PA. No evidence for induction of the immunochemically distinct melanoma type PA of M_r 70,000 was obtained. These results indicate that UV light enhances expression of the same PA that is constitutively formed in low amounts. A similar quantitative increase in gene expression has also been observed for the *recA* (38) and a set of *din* (damage-inducible) genes in *Escherichia coli* (39).

It has been proposed that disruption of DNA synthesis by DNA damage is responsible for the induction of SOS functions in bacteria (1) and for enhanced viral reactivation in mammalian cells (40). Our experiments show that PA induction occurs in quiescent XP group A fibroblasts that do not synthesize DNA. These results are compatible with the demonstration that enhanced postreplication repair is induced in Chinese hamster cells in G_2 phase (41) and, as PA induction occurs in the absences of excision, they suggest that the enzyme is produced as a consequence of persistent DNA damage. It is of interest that UV light also induces PA synthesis in XP variants that are proficient in excision repair but deficient in cellular events involved in bypassing DNA damage (postreplication repair). This suggests that enzyme induction is an SOS-like response to DNA lesions not dealt with by either excision or postreplication repair.

Cells of XP heterozygotes, in contrast to skin fibroblasts of normal donors, also respond to UV irradiation by elevated PA levels. Induction is observed, however, only above a threshold fluence of 7.5 J/m^2. These results support the assumption that XP heterozygotes are partially repair deficient and that induction is initiated when the levels of repair enzymes become rate limiting and this leads to accumulation of unexcised pyrimidine dimers. It will be important to determine whether PA induction occurs in the parents of XP patients from all the various complementation groups and whether this induction could be used as an assay for detection of heterozygotes; such XP heterozygotes comprise 0.5% of the general population and show a significantly higher incidence of skin cancer than normal individuals (42).

Among all cell types tested in this study, human adult skin fibroblasts are unique in their lack of PA inducibility. These results were unexpected because DNA-damage-induced PA synthesis occurs in human embryonic fibroblasts (15) and, as shown in Fig. 4, also in human amniotic cells. Several explanations may be offered to account for this difference. To mention a few: (*i*) embryonic cells may have an as yet undetermined repair deficiency that causes induction, (*ii*) adult skin fibroblasts differ from embryonic fibroblasts and XP cells in their content of intracellular modulators which regulate PA synthesis, and (*iii*) DNA damage-induced PA synthesis is an embryonic property that persists in XP patients.

Our results support the conclusion that the induction of PA synthesis is one manifestation of the pleiotropic SOS-like response of human cells to DNA damage. Furthermore, they suggest that PA induction may serve as a relatively easy and convenient measure to identify XP cells and possibly other repair deficiencies and, more importantly, XP heterozygotes.

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