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Specific action of T4 endonuclease V on damaged DNA in xeroderma pigmentosum cells *in vivo*

(excision repair/incision/hemagglutinating virus of Japan/4-nitroquinoline 1-oxide/UV irradiation)

KIYOJI TANAKA*, HIROSHI HAYAKAWA†, MUTSUO SEKIGUCHI†, AND YOSHIO OKADA*‡

* Department of Animal Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan; and † Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

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ABSTRACT **The specific action of T4 endonuclease V on damaged DNA in xeroderma pigmentosum cells was examined using an *in vivo* assay system with hemagglutinating virus of Japan (Sendai virus) inactivated by UV light. A clear dose response was observed between the level of UV-induced unscheduled DNA synthesis of xeroderma pigmentosum cells and the amount of T4 endonuclease V activity added. The T4 enzyme was unstable in human cells, and its half-life was 3 hr. Fractions derived from an extract of *Escherichia coli* infected with T4v_1, a mutant defective in the endonuclease V gene, showed no ability to restore the UV-induced unscheduled DNA synthesis of xeroderma pigmentosum cells. However, fractions derived from an extract of T4D-infected *E. coli* with endonuclease V activity were effective. The T4 enzyme was effective in xeroderma pigmentosum cells on DNA damaged by UV light but not in cells damaged by 4-nitroquinoline 1-oxide. The results of these experiments show that the T4 enzyme has a specific action on human cell DNA *in vivo*. Treatment with the T4 enzyme increased the survival of group A xeroderma pigmentosum cells after UV irradiation.**

Xeroderma pigmentosum (XP) is a rare hereditary disease caused by mutation of an autosomal recessive gene. It is characterized by the development of pigmentation abnormalities and numerous malignancies after exposure of the skin to sunlight. XP cells have been classified into five complementation groups (A, B, C, D, and E) by the cell fusion technique (1). These cells have defects in the mechanism of excision repair of damaged DNA (2), and they show low levels of UV-induced unscheduled DNA synthesis (3). Another class of XP patients has also been described and designated as the XP variant. XP variant cells are believed to have defects in the postreplication repair process (4). Recently, we reported (5) that UV-induced unscheduled DNA synthesis in these five complementation groups of XP cells is restored to the normal level through the introduction of a UV-specific endonuclease (T4 endonuclease V) fraction derived from *Escherichia coli* infected with T4 bacteriophage. This was accomplished in an *in vivo* system by the use of hemagglutinating virus of Japan (HVJ; Sendai virus) (6, 7). The present study confirms that this restoration is due to the specific action of T4 endonuclease V itself. We also demonstrate that XP cells can acquire UV resistance when the T4 enzyme is introduced.

MATERIALS AND METHODS

Cells. Strain UNK is a strain of human fibroblasts derived from normal skin. Strains XP10OS and XP27OS are from Japanese patients and belong to complementation group A (8). Strains CRL1199 (XP11BE), CRL1161 (XP9BE), and CRL1160 (XP5BE), belonging to groups B, C, and D, respectively, were obtained from the American Type Culture Collection. Gm708 (XP2RO), belonging to group E, was obtained from the Institute for Medical Research, Camden, NJ. These fibroblasts were grown in Eagle's minimum essential medium supplemented with 15% fetal calf serum (Flow Lab.). They were maintained under 5% CO_2 in air in an incubator at 37°.

Enzymes. T4 endonuclease V was prepared from T4D-infected *E. coli* 1100, as described (9, 10). A crude extract (fraction I) was subjected to phase partition in dextran-500 and polyethylene glycol-6000 and the resulting supernatant was dialyzed against PEME buffer (10 mM potassium phosphate/10% ethylene glycol/10 mM 2-mercaptoethanol/2 mM EDTA at pH 6.5) (fraction II). The fraction was applied to a column of carboxymethyl-Sephadex C-25 and eluted with a linear gradient of 0–0.5 M KCl and PEME buffer (fraction III). The purity of the resulting preparation was about 400-fold of that of the crude extract. In the experiments related to the acquisition of UV resistance, this preparation was dialyzed against a balanced salt solution (0.14 M NaCl/5.4 mM KCl/0.34 mM Na_2HPO_4/0.44 mM KH_2PO_4/2.4 mM $CaCl_2$, buffered with 10 mM Tris·HCl at pH 8.0) for 1 hr at 4° before use, in order to remove 2-mercaptoethanol. Colony formation in human fibroblasts is inhibited by 2-mercaptoethanol. For the experiments described in the second part, enzyme fractions were prepared from T4D-infected and T4v_1-infected *E. coli* 1100 by a slight modification of this procedure: the enzyme was eluted from the carboxymethyl-Sephadex C-25 column with 0.5 M KCl/PEME buffer, instead of a linear gradient of KCl and PEME buffer, and six 5-ml fractions were collected (fraction III-1 to III-6). The enzyme activity was determined by measuring degradation of ^{32}P-labeled, UV-irradiated T4 DNA in the presence of T4v_1-infected cell extract. One unit of endonuclease V was defined as the activity that releases 24 nmol of DNA nucleotides as acid-soluble material in 20 min at 37° (9, 10). Protein was determined by the method of Lowry *et al.* (11).

HVJ (Sendai Virus). HVJ, Z strain, propagated in embryonated eggs, was partially purified by differential centrifugation and suspended in balanced salt solution. The virus was inactivated with UV-light before use.

Estimation of Unscheduled DNA Synthesis. Fibroblasts were grown as a monolayer in petri dishes (35 mm in diameter) containing coverslips (15 mm in diameter). The dishes were washed twice with Dulbecco's phosphate-buffered saline and then either irradiated with 30 J/m^2 of UV light or treated with 3.3 μM 4-nitroquinoline 1-oxide (4NQO) dissolved in minimum essential medium for 60 min at 37°. After this treatment, the cells were washed, and two drops of a 1:1 mixture of 2000 hemagglutinating units (HAU) of UV-inactivated HVJ and T4 enzyme preparation were put on each coverslip. The coverslips were incubated for 15 min on ice, followed by 15 min at 37°. Then 1 ml of minimum essential medium supplemented with 15% fetal calf serum containing 10 μCi/ml of [^3H]thymidine

Abbreviations: HVJ, hemagglutinating virus of Japan, (synonym: Sendai virus); HAU, hemagglutinating unit; XP, xeroderma pigmentosum; 4NQO, 4-nitroquinoline 1-oxide; PEME buffer, phosphate/ethyleneglycol/mercaptoethanol/EDTA buffer.

‡ To whom correspondence should be addressed.

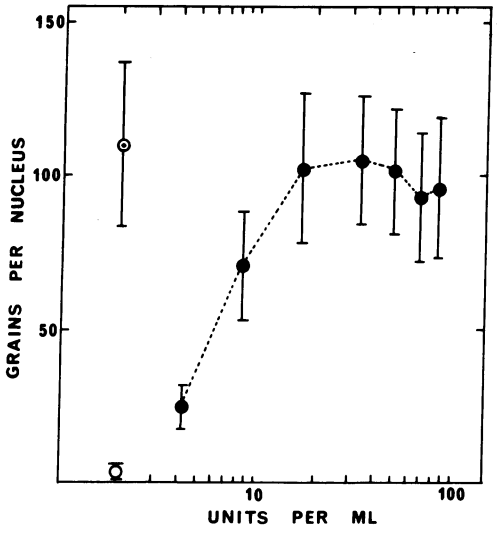

FIG. 1. A dose response of T4 endonuclease V activity to the level of UV-induced unscheduled DNA synthesis of XP cells. XP10OS cells were irradiated with UV light at 30 J/m^2 and treated with various concentrations of T4 endonuclease V (abscissa) plus 2000 HAU of UV-inactivated HVJ. Then they were labeled with [^{3}H]thymidine for 3 hr at 37° and grain number/nucleus were estimated autoradiographically (●). The level of UV-induced unscheduled DNA synthesis of XP10OS cells treated with HVJ alone (○) and of normal UNK cells treated without T4 enzyme or HVJ (⊙). The bars show standard deviations of means.

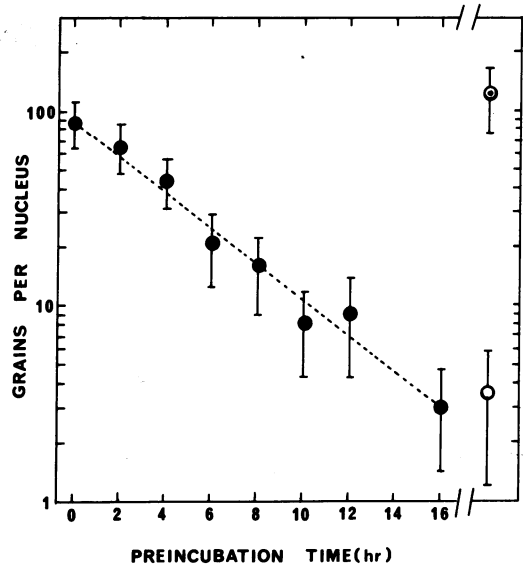

FIG. 2. Stability of T4 endonuclease V activity in XP cells. XP10OS cells were treated with 8.5 units/ml of T4 endonuclease V plus HVJ before UV irradiation. Then they were incubated for the indicated times (abscissa), washed with phosphate-buffered saline, and UV-irradiated at a dose of 30 J/m^2. Then they were labeled with [^{3}H]thymidine for 3 hr at 37° and grain number/nucleus were estimated autoradiographically (●). The level of UV-induced unscheduled DNA synthesis of XP10OS cells treated with HVJ alone (○) and of normal UNK cells treated without T4 enzyme or HVJ (⊙). The bars show standard deviations of means.

was added without washing and the cells were cultured for 3 hr at 37°. After further incubation with 5 μg/ml of unlabeled thymidine for 1 hr, cells on the coverslips were washed and fixed with methanol. Acid-soluble material was removed with 5% trichloroacetic acid at 4°, and the coverslips were dipped in Kodak NTB 3 nuclear emulsion. They were developed after 7–14 days of exposure, and cells were stained with Giemsa. In autoradiographic preparations, "lightly labeled" nuclei showing UV-induced unscheduled DNA synthesis were easily identified from "heavily labeled" nuclei in S phase. Silver grains over "lightly labeled" nuclei of both fused and nonfused cells were counted at random. The average number of grains per nucleus and the standard deviation were calculated from the number on 100 nuclei. Less than 2% of the total number of "lightly labeled" nuclei were contaminated by early S and late S phase nuclei under the experimental conditions described above.

UV Sensitivity of Cells. Fibroblasts were seeded into Falcon plastic dishes (35 mm in diameter) containing three coverslips (15 mm in diameter) at a cell density of 1.5×10^4 cells per dish. After incubation for 6 hr at 37°, the coverslips were washed once with phosphate-buffered saline and irradiated with UV light at a rate of 0.13 J/m^2 per sec. Then 2 drops of the enzyme/HVJ mixture were placed on each coverslip, and the coverslips were incubated for 15 min on ice followed by 15 min at 37°. Two milliliters of culture medium were added to each dish and incubation was continued for 11–17 hr at 37°. Then the cells were harvested carefully by trypsinization and replated in 60- or 100-mm Falcon dishes. The cells were cultured for 9–14 days; then the number of colonies that developed in each plate was determined.

RESULTS

UV-induced unscheduled DNA synthesis

Effect of T4 Endonuclease V. XP10OS (group A) cells were irradiated with UV light at 30 J/m^2 and treated with various concentrations of T4 endonuclease V in the presence of 2000 HAU of UV-inactivated HVJ. A clear relationship was observed between the amount of T4 enzyme added and the level of unscheduled DNA synthesis of the XP cells; the level increased with higher enzyme concentrations (from 2 to 20 units/ml), reaching a plateau at concentrations of over 20 units/ml (Fig. 1).

Next, the stability of T4 endonuclease V activity in XP cells was investigated. XP10OS cells were treated with 8.5 units/ml of the T4 enzyme plus 2000 HAU of UV-inactivated HVJ, and then incubated for various times in culture medium at 37°. After incubation, the cells were washed with phosphate-buffered saline, irradiated with UV light at 30 J/m^2, and labeled with [^{3}H]thymidine (10 μCi/ml) for 3 hr at 37°. As shown in Fig. 2, the level of restoration of unscheduled DNA synthesis decreased exponentially with an increase in the time of preincubation. The half-life of the activity was about 3 hr, and after incubation for 16 hr the level of unscheduled DNA synthesis was as low as without the addition of T4 enzyme.

Involvement of T4v_1 Gene Product. Synthesis of T4 endonuclease V in *E. coli* infected with bacteriophage T4 is controlled by the *v* gene of T4 (12). T4v_1 is a UV-sensitive strain, with a mutation in the *v* gene of T4, and it cannot produce the T4 enzyme in *E. coli*. Extracts from *E. coli* infected with T4D (a wild strain) or T4v_1 were fractionated as described in *Materials and Methods*. Table 1 shows the UV-specific endonuclease activities estimated by assay *in vitro*, the protein contents, and the specific activities of these fractions. Fractions I, II, III-2, III-3, and III-4 from T4D-infected cells showed high activity of UV-specific endonuclease, whereas the fractions from T4v_1-infected cells all showed low activity.

The effects of these fractions in restoring UV-induced unscheduled DNA synthesis in the five complementation groups (A, B, C, D, and E) of XP cells were tested, using HVJ. Table 2 shows that fractions III-2, III-3, and III-4 from T4D-infected *E. coli* were effective, whereas other fractions, including fractions III-2, III-3, and III-4 from T4v_1-infected *E. coli*, had little or no effect. These results indicate that the *v* gene product,

Table 1. UV-specific endonuclease V activity in various fractions of T4D-infected and T4v_1-infected *E. coli* estimated by assay *in vitro*

Fraction	Activity, units/ml	Protein, mg/ml	Specific activity, units/mg
Phage T4D			
I	21.5	23.5	0.9
II	8.9	4.2	2.1
III-1	0.1	0.1	—
III-2	29.7	2.4	12.4
III-3	29.0	1.0	28.4
III-4	2.3	0.3	8.0
III-5	1.2	0.1	—
III-6	0.3	0.04	—
Phage T4v_1			
I	0.6	22.8	0.03
II	0.6	4.2	0.14
III-1	0.0	0.1	—
III-2	0.5	2.6	0.19
III-3	0.35	1.1	0.33
III-4	0.15	0.2	0.63
III-5	0.25	0.1	—
III-6	0.15	0.08	—

T4 endonuclease V, is indeed responsible for restoring UV-induced unscheduled DNA synthesis in XP cells.

In these experiments, we found that although fractions I and II from T4D-infected *E. coli* had high UV-specific endonuclease activities, they actually inhibited UV-induced unscheduled DNA synthesis in XP cells instead of restoring it. Further analysis showed that on exposure of human fibroblasts to these crude extracts for 15 min on ice and for 15 min at 37° with or without HVJ, their DNA synthesis (scheduled and unscheduled) was inhibited when measured autoradiographically. Fraction I was more inhibitory than fraction II, and the inhibition was removed by further purification. Crude extracts of T4v_1-infected *E. coli* caused a similar degree of inhibition (Table 2).

4NQO-induced unscheduled DNA synthesis

Effect of T4 Endonuclease V. Friedberg (13) and Ito and Sekiguchi (14) found that T4 endonuclease V incises DNA damaged by UV irradiation but not by 4NQO. On the other hand, it has been shown that in human fibroblasts unscheduled DNA synthesis is induced by 4NQO as well as by UV irradiation. The level of 4NQO-induced unscheduled DNA synthesis in XP cells is lower than that in normal cells; it is lowest in complementation groups A and B and is higher (in ascending order) in groups C, D, and E (K. Tanaka, H. Takebe, and Y. Okada, unpublished data). This order is the same as that of UV-induced unscheduled DNA synthesis. From these results we expected that when the enzyme was introduced into XP cells by the action of UV-inactivated HVJ it would not be able to restore unscheduled DNA synthesis induced by 4NQO if the substrate specificity of T4 enzyme had not been altered in human cells.

Table 3. Effects of T4 endonuclease V on unscheduled DNA synthesis in XP cells exposed to UV light and to 4NQO*

Cells	4NQO	UV
UNK (normal)	78 ± 20	118 ± 35
XP10OS (group A)		
− Endonuclease†	10 ± 4	3 ± 2
+ Endonuclease‡	11 ± 5	121 ± 31

* Results are given as grains per nucleus (mean ±SEM).
† Cells were treated with 1000 HAU of HVJ.
‡ Cells were treated with a mixture of 19 units/ml of T4 enzyme and 2000 HAU of HVJ.

To test this we treated XP10OS cells with both T4 endonuclease V (19 units/ml) and UV-inactivated HVJ (2000 HAU) immediately after UV irradiation (30 J/m^2) or 4NQO treatment (3.3 μM). As shown in Table 3, 4NQO-induced unscheduled DNA synthesis of XP cells was not restored at all by this treatment, while UV-induced unscheduled DNA synthesis was restored to the normal level. This result clearly shows that T4 endonuclease V exhibits a specific action in human cells.

Acquisition of UV resistance on introduction of T4 endonuclease V

The above findings confirmed that T4 endonuclease V can compensate for the defect in the excision repair of UV-damaged DNA when introduced into XP cells. To further understand the enzyme activity of T4, it was important to know whether the restoration of UV-induced unscheduled DNA synthesis by this

Table 2. Effects of various fractions of T4D-infected and T4v_1-infected *E. coli* on UV-induced unscheduled DNA synthesis of XP cells*

Treatment	Exp. 1†		Exp. 2†		Exp. 3†
	XP10OS (group A)	CRL1160 (group D)	CRL1199 (group B)	CRL1161 (group C)	Gm708 (group E)
HVJ alone	4 ± 2	33 ± 11	6 ± 5	9 ± 5	57 ± 14
HVJ and T4D					
Fraction I	1 ± 1	3 ± 2	—	—	9 ± 5
Fraction II	3 ± 2	14 ± 6	—	—	34 ± 12
Fraction III-2	171 ± 48	154 ± 40	69 ± 16	64 ± 16	81 ± 24
Fraction III-3	154 ± 41	157 ± 36	69 ± 20	67 ± 17	83 ± 25
Fraction III-4	92 ± 26	89 ± 29	36 ± 9	34 ± 13	57 ± 19
HVJ and T4v_1					
Fraction I	2 ± 1	5 ± 2	—	—	7 ± 3
Fraction II	3 ± 2	11 ± 4	—	—	21 ± 8
Fraction III-2	10 ± 5	25 ± 11	5 ± 3	11 ± 4	53 ± 12
Fraction III-3	7 ± 4	27 ± 13	7 ± 4	13 ± 6	59 ± 13
Fraction III-4	5 ± 2	23 ± 11	5 ± 3	8 ± 4	56 ± 15

The same lot of samples indicated in Table 1 was mixed with HVJ and applied to cells.
* Results are given as grains per nucleus (mean ±SEM).
† Treatment of strain UNK (normal cells) with HVJ alone gives the following results: Exp. 1, 182 ± 59; Exp. 2, 82 ± 30; Exp. 3, 92 ± 24.

Table 4. Acquisition of UV resistance by XP27OS cells on treatment with T4 endonuclease V plus HVJ

Treatment	Mean no. of colonies per plate*	(1)/(2)	A/B, %
A. UV irradiated			
(1) Treated with T4 enzyme and HVJ	26.8 ± 10.0†	4.8	6.4
(2) Treated with HVJ alone	5.6 ± 3.5	1.0	1.3
(3) Not treated with T4 enzyme or HVJ	5.0 ± 3.2	—	—
B. Not UV irradiated			
(1) Treated with T4 enzyme and HVJ	420 ± 21	0.96	100
(2) Treated with HVJ alone	437 ± 16	1.0	100

XP27OS (group A) cells that had been irradiated with 1.95 J/m^2 of UV light or not irradiated were treated with a mixture of equal volumes of 30 units/ml of T4 endonuclease V and 2000 HAU of inactivated HVJ, or with 1000 HAU of inactivated HVJ alone, or not treated with them, and the abilities to form colonies were observed (see *Materials and Methods*).

* Twenty plates were used for each sample and the mean number of colonies per plate is indicated.

† Standard deviation.

enzyme resulted in increased survival of XP cells after exposure to UV light.

First, XP10OS cells were irradiated with UV light at various doses and then treated either with T4 endonuclease V plus UV-inactivated HVJ or with UV-inactivated HVJ alone. Then each sample's capability to form colonies was determined. At all UV doses used, the number of colonies of samples treated with enzyme plus HVJ was greater than that of samples treated with HVJ alone. The difference between them increased with increasing UV dose from 0.65 to 1.95 J/m^2 and then decreased at a dose of 2.6 J/m^2. The ratios of the number of colonies of samples treated with T4 enzyme to those not treated with the enzyme were 1.02 at 0.65 J/m^2, 1.33 at 1.3 J/m^2, 4.50 at 1.95 J/m^2, and 2.29 at 2.6 J/m^2, as the mean of two separate experiments.

Next, XP27OS cells (group A) were tested again. The cells were UV irradiated at a dose of 1.95 J/m^2 or not irradiated and then treated with both T4 enzyme (30 units/ml) and UV-inactivated HVJ (2000 HAU) or with UV-inactivated HVJ alone or not treated with them. The results are summarized in Table 4. When the cells were not UV irradiated, no difference in the numbers of colonies per plate appeared between the samples treated with both T4 enzyme and HVJ and with HVJ alone. When cells were UV irradiated, the number of colonies decreased greatly in both samples; however, a clear difference (4.8:1.0) was observed between the samples treated with T4 enzyme and those not treated with the enzyme. The number of colonies in the sample that was UV irradiated alone was the same as that UV irradiated and then treated with HVJ. These results show that XP27OS cells acquired UV resistance on introduction of T4 endonuclease V; the restoration of UV-induced unscheduled DNA synthesis in XP cells by this enzyme was related to increased survival of the cells after UV irradiation. The treatment with UV-inactivated HVJ inhibited the efficiency of colony formation a little under the present conditions, mainly because of fusion of a number of cells. But T4 enzyme itself did not affect the colony formation of cells that had not been UV irradiated. Thus, the presence of the T4 enzyme itself does not complicate the demonstration of its effect on cell survival after UV irradiation.

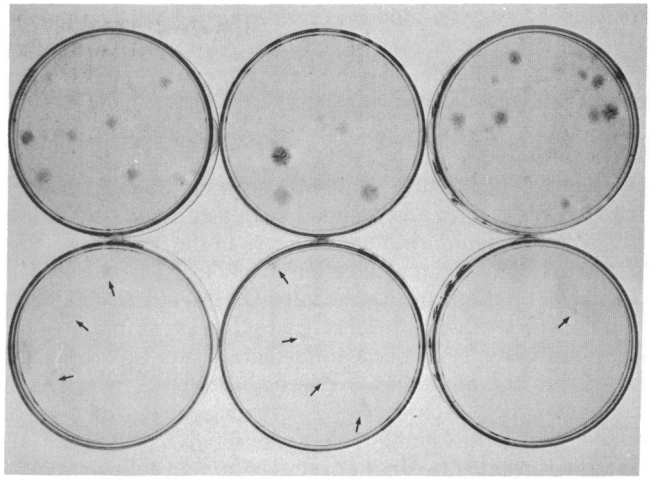

FIG. 3. Acquisition of UV resistance by XP cells on treatment with T4 endonuclease V plus UV-inactivated HVJ. XP10OS cells were treated with UV light (1.95 J/m^2) and then treated with T4 endonuclease V (51 units/ml) plus UV-inactivated HVJ (2000 HAU) (*upper row*) or with UV-inactivated HVJ alone (*lower row*). The cells were replated (see *Materials and Methods*) and cultured for 14 days. (*Upper*) Many colonies appeared, some of which were large in size, compact with high cell densities, and visible macroscopically. (*Lower*) A small number of colonies appeared, all of which were small, sparse in cell density, and difficult to detect macroscopically (arrows).

Important evidence for the above conclusion was also provided by the morphology of the colonies. As shown in Fig. 3, colonies in samples treated with the T4 enzyme were compact with high cell densities, and some were large in size compared with those of XP cell colonies that had not been UV irradiated. Control colonies that had been UV irradiated and treated with HVJ were sparse and small and were not detected macroscopically, as in the case of samples that had been UV irradiated alone.

DISCUSSION

Previously, we reported that the UV-induced unscheduled DNA synthesis in the five complementation groups of XP cells was restored to a normal level by treatment with T4 endonuclease V plus UV-inactivated HVJ. We suggested that this enzyme was introduced into XP cells by the action of HVJ and that it incised UV-damaged XP cell DNA, thereby restoring the UV-induced unscheduled DNA synthesis of XP cells to a normal level. It seems likely that XP cells are defective in the incision step of excision repair and that the incision step in human cells is controlled by at least five different genes. The present work was undertaken in an effort to confirm this hypothesis by demonstrating a specific action of T4 endonuclease V on DNA in XP cells *in vivo*.

A clear relationship was observed between the T4 endonuclease V activity of the fractions estimated *in vitro* and their ability to restore the UV-induced unscheduled DNA synthesis of XP cells. No activity to restore the UV-induced unscheduled DNA synthesis of XP cells was found in fractions derived from extracts of *E. coli* infected with T4v_1. These results indicate that the T4 enzyme interacted with XP cell DNA to restore the unscheduled DNA synthesis.

T4 endonuclease V incises DNA containing pyrimidine dimers (10), but not DNA treated with 4NQO (13, 14) or methylmethanesulfonate (15). It also fails to incise DNA containing 5-methyl-5,6-dihydrothymine or an adduct of thymine and psoralen (F. Makino, H. Tanooka, and M. Sekiguchi, unpublished data). Thus, T4 endonuclease V is strictly specific for cyclobutane-type dimers. The results reported above, showing

that the T4 enzyme induced unscheduled DNA synthesis in UV-damaged XP cells but not in 4NQO-damaged ones, are consistent with the idea that the T4 enzyme has a specific effect on XP cell DNA *in vivo*.

The most striking characteristics of XP cells are their high UV sensitivity and their low level of unscheduled DNA synthesis (16). The high UV sensitivity seems to be related to the inability to perform unscheduled DNA synthesis. Thus, it was important to demonstrate an increase in the survival of UV-irradiated XP cells through the introduction of T4 enzyme. The T4 enzyme restored the unscheduled DNA synthesis of XP cells to a normal level. This level refers to the rate of unscheduled DNA synthesis per unit time immediately after UV irradiation but not to the net value of unscheduled DNA synthesis. A continuation of unscheduled DNA synthesis over 1 day is to be expected in normal human cells under standard conditions (17, 18), but the half-life of T4 enzyme in human cells was only 3 hr. Moreover, cell survival may require complete repair of damaged sites specific for cell growth. If 190,000 sites of the DNA of an XP cell were damaged by UV light at a dose of 1.9 J/m^2, and if 99% of the sites are restored by the introduction of the T4 enzyme, 1900 damaged sites would still remain. One or more of these sites may quite possibly be essential for cell growth. Thus, it is highly unlikely that all of the XP cells will survive the UV damage despite introduction of the enzyme under the limited conditions of this experiment. However, it is important to know whether some of the UV-irradiated XP cells are repaired by introduction of T4 enzyme. The present results show clear increases in the survival rate of irradiated cells through introduction of the enzyme.

Other defects in XP cells have been reported. These include reduced levels of a photoreactivation enzyme (19) and alteration of apurinic site-specific endonuclease (20). The relationship between these findings and ours is not clear.

The excision repair system of human cells seems to be more complex than that of *E. coli*, and the latter is more complex than that of bacteriophage T4. One could consider that development of repair system(s) of genomes damaged by sunlight was essential for the appearance of organisms on the earth. Over time, the repair systems presumably differentiated along with independent evolution of the organisms, probably resulting in a variety of excision repair systems. From the results of this series of experiments, T4 endonuclease V may be a primitive form of the enzymes participating in the incision step of the excision repair system. It is controlled by only one gene, and it can identify all sites of pyrimidine dimers on a DNA strand. In human cells, the incision step is more complex and seems to be controlled by five or more genes. Normal human cells can repair excisable DNA damage induced by various chemicals, such as *N*-acetoxy- and *N*-hydroxy-2-acetylaminofluorene (2), 8-methoxypsoralen and long-wave UV light (2), 4NQO (2), 7-bromomethylbenz[*a*]anthracene (21, 22), 1,3-bis(2-chloroethyl)-1-nitrosourea (2), K-region epoxide of benz[*a*]anthracene (2), and nitrous acid (23). However, XP cells cannot. This may suggest an evolutionary differentiation of the system involving the incision step of excision repair in human cells.

The results of this series of experiments may introduce new approaches to the study of the evolution of macromolecular systems in organisms. The important technique is the artificial introduction of macromolecules into viable cells. For this purpose, several kinds of methods have been established, including liposome methods (24, 25) and erythrocyte ghost/HVJ methods (26, 27). The method used here is the most simple and applicable method for the introduction of rather small macromolecules of about 20,000 daltons.

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