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Microinjection of *Escherichia coli* UvrA, B, C and D proteins into fibroblasts of xeroderma pigmentosum complementation groups A and C does not result in restoration of UV-induced unscheduled DNA synthesis

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Summary

The UV-induced unscheduled DNA synthesis (UDS) in cultured human fibroblasts of repair-deficient xeroderma pigmentosum complementation groups A and C was assayed after injection of identical activities of either Uvr excinuclease (UvrA, B, C and D) from *Escherichia coli* or endonuclease V from phage T4. Under conditions where the T4 enzyme was able to induce repair synthesis in both XP complementation groups in agreement with earlier observations (de Jonge et al., 1985), no effect of the UvrABCD excinuclease could be observed either when the enzymatic complex was injected into the cytoplasm, or when it was delivered directly into the nucleus. In addition, no effect of the *E. coli* excinuclease was found on the repair ability of normal repair-proficient human fibroblasts. We conclude that the UvrABCD excinuclease may not work on DNA lesions in human chromatin.

Our current knowledge of DNA-repair processes in humans is limited. The majority of what is known is derived from studies of cell cultures established from patients with cancer-prone diseases such as xeroderma pigmentosum (XP), an autosomal recessive disorder. Patients suffering from XP develop atrophic skin (in areas exposed to sunlight) with pigmentation abnormalities and malignant growth (reviewed by Lehmann and Karran, 1981; Kraemer, 1983). In addition XP is often associated with neurological abnormalities and mental retardation. It was demonstrated that most patients suffering from this syndrome have a defect in the excision-repair pathway (Cleaver, 1968). Until now 9 excision-deficient complementation groups (XP-A through I) have been identified (de Weerd-Kastelein et al., 1972; Fisher et al., 1985). The XP-variant group is deficient in the post-replicational repair process but has normal excision repair (Lehmann et al., 1975). In spite of many efforts in several laboratories it has not yet been possible to clone and characterize the genes responsible for this hereditary disease.

An alternative approach to investigate the intricate process of excision repair in human cells is to

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introduce well-characterized prokaryotic or eukaryotic DNA-repair enzymes into human cells deficient in the excision-repair pathway. Tanaka et al. (1975, 1977) and Hayakawa and coworkers (1981) reported the introduction of bacteriophage T4 endonuclease V into XP cells (complementation groups A through F) by permeabilizing the cells with Sendai virus. Using the microneedle injection technique, de Jonge et al. (1985) injected *Micrococcus luteus* endonuclease into XP-fibroblasts (XP-A through I). Both the T4 and the *Micrococcus luteus* UV endonuclease are pyrimidine dimer specific and consist of one polypeptide, having a glycosylase- and an apurinic/apyrimidinic endonuclease activity (Nakabeppu and Sekiguchi, 1981; Grafstrom et al., 1982). In all XP groups tested a (partial) restoration of the excision-repair defect was observed upon microinjection of the prokaryotic enzyme. Using the same technique Zwetsloot et al. (1986) have investigated the effect of the photoreactivating enzyme (PRE) from yeast on the residual UDS of injected XP fibroblasts. A significant reduction of UDS was found in some XP complementation groups but not in others.

In *Escherichia coli* the excision-repair pathway is mediated by the UvrA, B and C gene products. In contrast to the dimer-specific enzymes mentioned above, these proteins act on a broader spectrum of DNA lesions, for instance damage caused by *N*-acetoxy-acetyl-aminofluorene, psoralen plus light, mitomycin C, MNNG, platinum compounds as well as UV-induced lesions such as pyrimidine dimers and 6–4 photoproducts (reviewed by Friedberg, 1985). From this point of view prokaryotic and eukaryotic excision repair systems resemble each other since the excision-repair system in human cells also acts on a large panel of different DNA adducts.

Many *Escherichia coli* genes involved in the repair of damaged DNA have been cloned and studied in detail (Friedberg, 1985). Insertion of these genes into high expression vectors and overproduction of the corresponding proteins in *Escherichia coli* have facilitated the purification of these enzymes (Sancar et al., 1983; Yeung et al., 1983; this paper). From in vitro studies with the purified proteins it was established that the UvrABC excinuclease incises the damaged DNA strand by a concerted action 7 nucleotides 5′ and 3–4 nucleotides 3′ of the pyrimidine dimer (Sancar and Rupp, 1983). More recently Kumura et al. (1985) presented evidence that in vitro incision of UV-irradiated DNA by UvrA, B and C is stimulated by the UvrD gene product. It has been suggested that UvrD, which has a DNA unwinding activity (= helicase II), functions in releasing the 12–13 base oligonucleotide containing the cyclobutane adduct from the incised DNA.

It is of interest to investigate whether the UvrABCD system, when introduced into human cells, is able to remove UV-induced lesions from the DNA of these cells and correct the DNA-repair defect of cell lines established from XP patients. This paper describes such experiments.

Materials and methods

Bacterial strains and plasmids

Strains CS4281 (Backendorf et al., 1983) and JM105 (Yanisch-Perron et al., 1985) have been described. Plasmids pCI857 and pKM-tacII were obtained from Dr. E. Remaut (Ghent) and Dr. H. de Boer (Genentech, San Francisco) respectively.

Overproduction and purification of UVR proteins

(A) UvrA

Strain CS4281 harbouring plasmids pPL-A9 (Amp^R) and pCI857 (Kan^R) was used. pPL-A9 harbours the *uvrA* gene under the control of the P_L promoter of phage λ (Backendorf et al., 1983) and pCI857 contains the gene for a thermosensitive cI repressor. Cells were grown at 28°C in L broth until $A_{655} = 0.3–0.35$ was obtained, after which the temperature was raised to 42°C and the culture was incubated for 2 h in order to induce the synthesis of UvrA protein. Cells were suspended in buffer A (50 mM Tris–HCl pH 7.5, 100 mM KCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, 25% glycerol), frozen at −80°C, thawed and sonicated (5 × 30 sec at maximum amplitude) in a MSE sonifier. The homogenate was centrifuged in a Ti50 rotor at 35 000 rpm during 1.5 h. The supernatant was used for further purification (Table 1). 10 mg UvrA protein was obtained per gram of wet cells with a purity of > 95%.

TABLE 1

CHROMATOGRAPHIC STEPS USED IN UvrA, B AND C PURIFICATIONS

	Matrix	Buffer/gradient	Elution
UvrA	phosphocellulose	buffer A	not retained
	single-stranded DNA cellulose	buffer A	0.35–0.4 M KCl
UvrB	DEAE cellulose	buffer A 0.1–0.5 M KCl	0.3 M KCl
	single-stranded DNA cellulose	buffer A	not retained
	affi-gel blue	buffer A 0.1–0.5 M KCl	0.3 M KCl
	phenylsepharose	buffer A 0–60% ethylene glycol	50% ethylene glycol
UvrC	phosphocellulose	buffer C	0.4 M KCl
	phenylsepharose	buffer C *	60% ethylene glycol
	single-stranded DNA cellulose	buffer C 0.1–1.0 M KCl	0.5 M KCl

buffer C * = buffer C without glycerol.

(B) UvrB

Strain CS4281 harbouring plasmids pPL-B2 (Amp^R) and pCI857 (Kan^R) were used. pPL-B2 contains the structural *uvrB* gene from plasmid pNP12 (van den Berg et al., 1981) under the control of the P_L-promoter of phage λ (van den Berg, 1983). Induction of UvrB protein and preparation of cell extracts were as described for UvrA. An ammonium sulphate precipitate from 30–50% saturation was resuspended in buffer A and processed as described in Table 1. 0.5 mg UvrB protein was obtained per gram wet cells with a purity > 95%.

(C) UvrC

Strain JM105 containing plasmid pBL12 (Amp^R) was used. pBL12 is derived from the high expression vector pKM-tacII (de Boer et al., 1983) and harbours the *uvrC* gene from plasmid pCA32 (van Sluis and Dubbeld, 1983) under the control of the hybrid tac promoter. Cells were grown in Vogel–Bonner medium supplemented with 0.1% glucose, 0.2% casamino acids and 3×10^{-4} M thiamine. At an $A_{655} = 0.3$ isopropyl–thio-D-galactoside was added to a final concentration of 80 mg/l. After an induction period of 4 h the cells were collected and lysed as described above using buffer B (100 mM phosphate buffer pH 7.5, 1 mM EDTA, 10 mM DTT, 10% sucrose), whereafter the protein preparation was dialysed against buffer C (= 100 mM phosphate buffer pH 7.5, 1 mM EDTA, 1 mM DTT, 25% glycerol). Further purification steps are described in Table 1. 2.5 mg UvrC protein was obtained per gram wet cells with a purity of > 98%.

(D) UvrD

UvrD protein was purified by a combination of published procedures. The lysate of 200 g *E. coli* cells, containing the multicopy *uvrD* plasmid pHMA16, was subjected to ammonium sulphate fractionated in 20 mM Tris · HCl pH 7.6, 0.1 mM EDTA, 0.5 mM EGTA, 50 mM 2-mercaptoethanol, 20% glycerol (Abdel Monem et al., 1979). The final pellet was resuspended in and dialysed against the same buffer supplemented with 50 mM NaCl prior to chromatography through a P11 column. The UvrD protein (which eluted at 180 mM NaCl) was then further purified through columns of heparin agarose and DNA agarose (Hickson et al., 1983). ATPase activity was assayed as described previously (Hickson et al., 1983) and found to be 10^7 units/mg where one unit of ATPase activity is defined as the hydrolysis of 1 nmole ATP in 20 min at 35°C. ATP hydrolysis was strictly dependent on the presence of single-stranded DNA. Purified UvrD protein also had ATP-dependent DNA helicase activity which was assayed as described by Hickson et al. (1983).

(E) T4 endonuclease

T4 endonuclease V was a gift from A.A. van Zeeland (State University of Leiden, The Netherlands). The enzyme was isolated from *E. coli* strain B67 infected with phage T4 *am*N82 (New England Biolabs). Cells were disrupted by passing the frozen cells through an Eaton press. Following centrifugation (60 min, 50 Ti rotor, 45 000 RPM) and dialysis against 10 mM Tris–HCl pH = 8, 10 mM ethylene glycol, T4 endonuclease V was purified by chromatrography using a DEAE-cellulose column as described (Seawell et al., 1981).

In vitro incision assay of the UvrA, B, C proteins

Incision of UV-irradiated plasmids by UvrABC

was carried out in 50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 1 mM ATP, 1 mM DTT, 100 ng of each of two supercoiled plasmids (differing in size). Generally the larger plasmid was irradiated with 200 J/m^2 and the smaller unirradiated plasmid served as an internal control. 1 pmole of each of the 3 Uvr proteins was added and the reaction was carried out at 37°C for 20 min in a final volume of 20 μl. The reaction was stopped by adding EDTA to a final concentration of 20 mM. Samples were electrophoresed on a 1% agarose gel and photographed under a short-wave UV lamp.

T4 endonuclease incision assay

The incision reaction with T4 endonuclease V was performed in 20 μl of buffer (50 mM Tris–HCl pH 8, 10 mM EDTA, 100 mM NaCl) containing 100 ng UV-irradiated (UV^+) (200 J/m^2) and 100 ng non-irradiated (UV^-) supercoiled plasmid DNA. After addition of T4 endonuclease V the reaction mixture was incubated at 37°C during 30 min. Samples were then treated as described for the UvrA, B, C proteins.

Microinjection assay

Cell strains used for the microinjection assay were C5RO (a repair-proficient wild-type strain), XP25RO (excision-repair-deficient strain belonging to XP complementation group A (Kraemer et al., 1975)) and XP21RO (excision-repair-deficient, belonging to xeroderma pigmentosum group C (Kleijer et al., 1973)). All strains are primary fibroblasts. The cells were cultured in Ham's F10 medium supplemented with 7% fetal (FCS) and 7% newborn calf serum (NCS), penicillin 100 I.E./ml and streptomycin 100 μg/ml. Prior to microinjection cells were fused using inactivated Sendai virus (thus generating homopolykaryons, de Weerd-Kastelein et al., 1972). Homopolykaryons do not enter S-phase 3 or more days after fusion (thereby avoiding confusion of labelling due to UV-induced UDS with that due to early or late S-phase DNA replication) and are easily identified. In the case of cytoplasmic injection only homopolykaryons were injected; nuclear injections were performed in monokaryons as well as in polykaryons (1 nucleus per polykaryon).

The purified enzymes were injected with the aid of a microneedle (Graessmann et al., 1980; de Jonge et al., 1983). After microinjection cells were washed with PBS and UV-irradiated (15 J/m^2; Philips TUV 15-W lamp). UDS was performed during 2 h in culture medium supplemented with 10 μCi/ml [^{3}H]thymidine (^{3}H-TdR; spec. act. 46 Ci/mmole) and fluorodeoxyuridine (1 μM). After incubation in radioactive medium, cells were fixed, the slides were covered with stripping film (Kodak stripping plate AR10) and kept in the dark for 5 days at 4°C for autoradiographic exposure (de Jonge et al., 1983). To analyse the effect of injection of enzymes on the UDS of injected cells the number of autoradiographic grains per nucleus (25–50 nuclei of injected cells) was counted, the average was calculated and compared to the mean number of grains of non-injected cells on the same slide and repair-proficient fibroblasts on a parallel slide (see de Jonge et al., 1983, for further details on the procedures).

Results

The UvrA, B, C and D proteins were purified as described in Materials and Methods. All 4 proteins were > 95% pure as could be judged by SDS-PAGE analysis (Fig. 1). The specificity of the UvrABC catalysed incision of UV-irradiated DNA has been analysed in Fig. 2. Incision is monitored by the conversion of the supercoiled into the relaxed form of the UV-irradiated plasmid (UV^+). In all experiments an unirradiated plasmid (UV^-), smaller in size than the irradiated plasmid, was added as an internal control. The results presented show that incision only occurs on irradiated DNA after incubation of the reaction mixture at 37°C in the presence of all 3 proteins (lanes 8, 9, 10). Keeping the reaction mixture at 0°C (lane 11) or incubation of the template in the presence of either only one (lanes 2, 3, 4) or two (lanes 5, 6, 7) Uvr components does not result in UV-specific incision. The unaffected ratio of supercoiled to relaxed form for the non-irradiated plasmid indicates that the effect observed on the UV-irradiated plasmid is UV-dependent and not due to contaminating aspecific nicking activities in (one of) the enzyme preparations. Lanes 12 and 13 show that the activity of a crude T4 endonuclease V extract can be monitored using the same method.

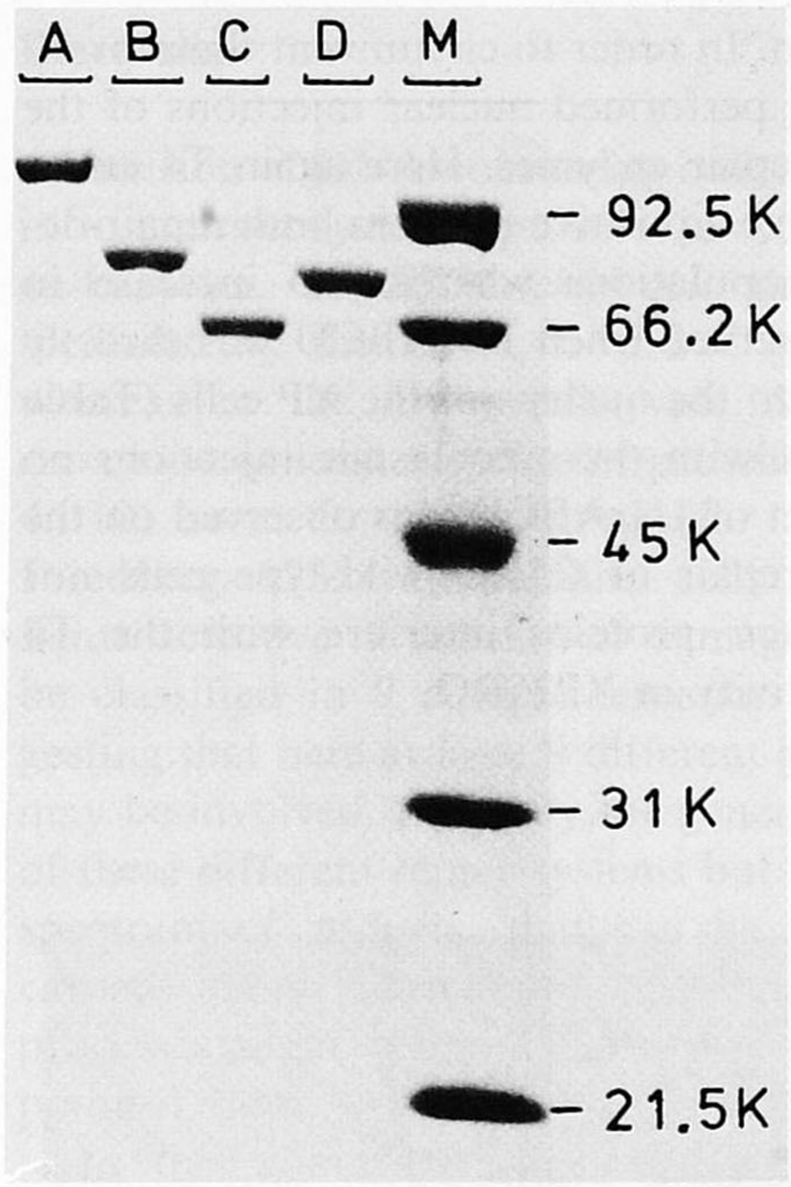

Fig. 1. SDS–PAGE electrophoresis of purified Uvr proteins: 10 pmoles of each of the 4 proteins were layered onto a 10% acrylamide gel. A, UvrA; B, UvrB; C, UvrC; D, UvrD; M, molecular weight markers.

Hence we were able to compare the activity of the UvrABC system to the activity of the T4 endonuclease.

In order to investigate whether the prokaryotic enzymes were able to induce unscheduled DNA synthesis (UDS) in repair-deficient XP cells the microneedle injection assay was used. Since Kumura et al. (1985) showed that the UvrD gene product (DNA helicase II) may be involved in the release of the UvrABC–oligonucleotide complex after DNA incision we have included the UvrD protein in all microinjection experiments at a molar ratio of A : B : C : D = 1 : 1 : 1 : 0.5. The release of the oligonucleotide–enzyme complex from incised DNA may be necessary in order to allow repair synthesis to occur.

In the initial experiments cytoplasmic microinjections were performed into fibroblasts from repair-deficient XP cells of complementation groups A (XP25RO) or C (XP21RO) or into repair-proficient fibroblasts. The results of these microinjection experiments are summarized in Table 2: T4 endonuclease V gave a positive response in XP-A as well as in XP-C but no detectable increase in UDS was observed after injection of the UvrABCD proteins, although both protein preparations had the same in vitro activity. The inability of the Uvr excinuclease to induce UDS in repair-deficient human cells is not due to an inhibitory factor present in (one of) the UvrABCD preparations as the positive effect of T4 endo V can still be monitored when the enzyme is coinjected with UvrABCD (Fig. 3). UvrABCD did not interfere

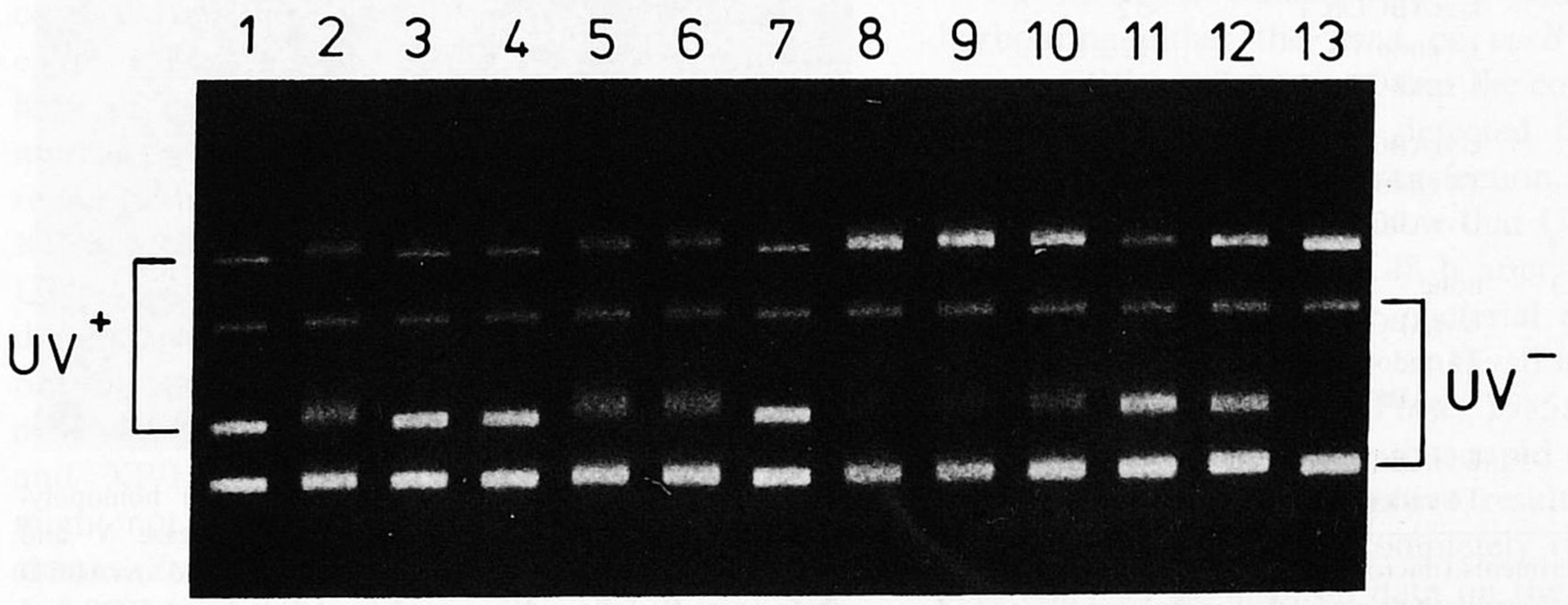

Fig. 2. UvrA, B, C specific incision of UV-irradiated DNA. The reaction mixture contains two plasmids, the larger plasmid is UV-irradiated with a UV fluence of 200 J/m^2 (UV^+) and the smaller one is not irradiated (UV^-). The following reactions were performed: lane 1: control, no proteins added; lane 2: 1 pmole UvrA protein; lane 3: 1 pmole UvrB; lane 4: 1 pmole UvrC; lane 5: 1 pmole UvrA + 1 pmole UvrB; lane 6: 1 pmole UvrA + 1 pmole uvrC; lane 7: 1 pmole UvrB + 1 pmole UvrC; lanes 8, 9, 10, 11: 1 pmole UvrA + 1 pmole UvrB + 1 pmole UvrC; lanes 12 and 13: 1 μl T4 endonuclease. Lanes 1–8 and lane 13; incubation during 30 min at 37°C; lane 9: 20 min at 37°C; lane 10: 10 min at 37°C; lanes 11 and 12: reaction mixture kept at 0°C for 30 min.

with the endogenous repair system of normal cells as no decrease in UDS of repair-proficient C5RO cells was observed after introduction of the *E. coli* proteins into the human cells. Nor did UvrABCD interfere with the correcting activity of the T4 enzyme in XP25RO or XP21RO as no significant decrease in T4 endonuclease induced UDS occurred when both activities were coinjected (Table 2).

The inability of the Uvr enzymes to induce unscheduled DNA synthesis in human repair-deficient cells might be due to the fact that after injection the proteins remain in the cytoplasm and cannot enter the nucleus. Indeed the *Escherichia coli* proteins have a considerable size (UvrA 114 kD, UvrB 80 kD, UvrC 67 kD and UvrD 75 kD) as compared to the molecular weight of 16 kD of the T4 protein. In order to circumvent these problems we have performed nuclear injections of the prokaryotic repair enzymes. Here again T4 endonuclease V gave a positive result in both repair-deficient cell populations whereas no increase in UDS was observed when UvrABCD was directly introduced into the nucleus of the XP cells (Table 2). Consistent with the cytoplasmic injections no negative effect of UvrABCD was observed on the endogenous repair of C5RO wild-type cells nor did the *E. coli* proteins interfere with the T4 correcting activity in XP25RO.

TABLE 2

MICROINJECTION OF THE *E. coli* UvrABCD PROTEIN COMPLEX INTO HUMAN REPAIR-PROFICIENT AND XP-FIBROBLASTS (XP-A AND XP-C)

Cell strain	Injected enzymes	UDS ± SEM % of control
C5RO (repair-proficient)	none	100 ± 7
	UvrABCD(C)	100 ± 6
	UvrABCD(N)	95 ± 6
XP25RO(XP-A)	none	< 2
	UvrABCD(C)	< 2
	T4 endo V(C)	22 ± 2
	UvrABCD + T4(C)	16 ± 1
	UvrABCD(N)	< 2
	T4 endo(N)	11 ± 1
	UvrABCD + T4(N)	7 ± 1
XP21RO(XP-C)	none	16 ± 1
	UvrABCD(C)	15 ± 1
	T4 endo(C)	36 ± 4
	UvrABCD + T4(C)	37 ± 1
	UvrABCD(N)	16 ± 1
	T4 endo(N)	30 ± 2

In control experiments (micro-injection of the uvrABCD complex without UV-irradiation) no UDS was measured in any of the tested lines.
C denotes cytoplasmic injection, N nuclear injection.
Control (repair-proficient) cells display 100–120 grains per nucleus. UDS is calculated as a percentage of UDS of the non-injected C5RO cells. The average per nucleus is determined by counting 25–50 nuclei.

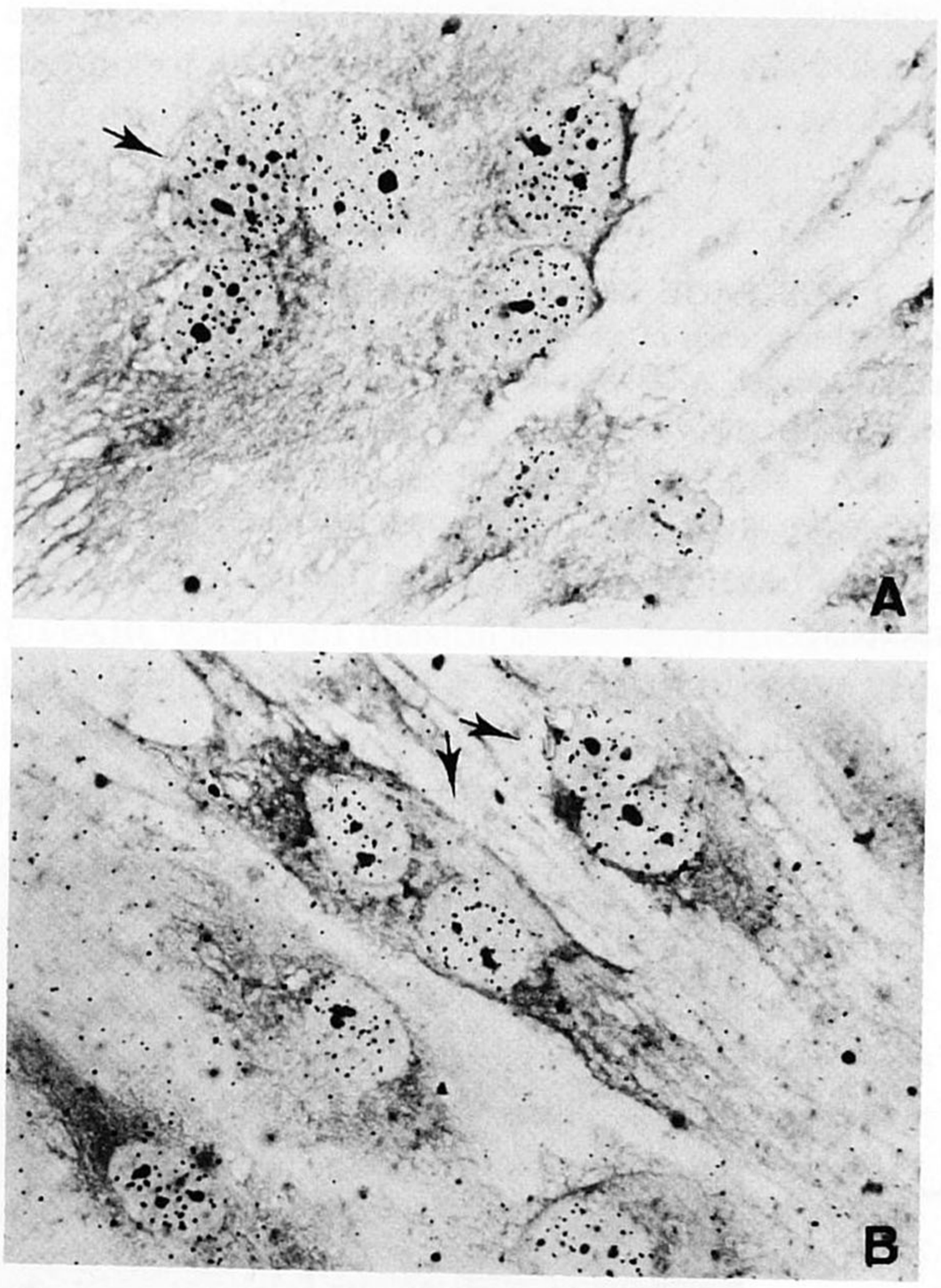

Fig. 3. Photomicrographs of XP21RO (XP-C) homopolykaryons after microinjection of T4 endonuclease V and uvrABCD (1 : 1 mixture, coinjected) (part A) and uvrABCD alone (part B) followed by assay for UV-induced UDS and autoradiography. Silver grains above nuclei indicate UDS. The homopolykaryon containing 5 nuclei (arrow, part A) and the two dikaryons (arrows, part B) were injected, the monokaryons were not injected. Microneedle injection was done in the cytoplasm. See Materials and Methods for experimental details.

Discussion

Incision of DNA-containing bulky base damage is a multi-enzymatic process in both pro- and eu-karyotes. In *Escherichia coli* this process is mediated by the UvrA, B and C gene products whereas in the lower eukaryote *Saccharomyces cerevisiae* at least 5 different proteins (rad1, 2, 3, 4, 10) seem to be involved (for a review see Friedberg, 1985). In humans, cells from XP patients defective in incision of damaged DNA, can be classified in 9 complementation groups, suggesting that here at least 9 different gene products may be involved. Not only the genetic complexity of these different repair systems but also the large spectrum of adducts, that can be repaired, has caused many authors to postulate that these processes might well be similar from a mechanistic point of view.

In this communication we have investigated this last point: the microneedle injection technique was used to introduce purified UvrABCD excinuclease into cultured normal and repair-deficient human cells in order to study the effect of the bacterial enzymes on the repair ability of the human cells in situ. Such experiments have been carried out using UV endonucleases from either phage T4 or the bacterium *Micrococcus luteus* and have shown that the repair defects of all 9 incision-deficient XP-complementation groups can be corrected by these (small) enzymes (Hoeijmakers et al., 1983; de Jonge et al., 1985). Recently, we have probed the ability of yeast PRE to monomerize pyrimidine dimers from normal and repair-deficient fibroblasts belonging to different XP complementation groups with high residual UDS levels. The yeast enzyme was able to remove dimers in XP-C, XP-F, XP-I and in wild-type fibroblasts, but no decrease in residual UDS was observed in fibroblasts from XP-A, XP-D, XP-E and XP-H, indicating that the photoproducts might not be equally well accessible in both XP classes (Zwetsloot et al., 1985, 1986).

In the present study we have used one XP strain from each of the two classes, namely XP-A (XP25RO) and XP-C (XP21RO) and a repair-proficient strain (C5RO) to probe the ability of the bacterial Uvr excinuclease to remove DNA damage from human chromatin in situ. In contrast to the T4 endo V and PRE experiments described above no effect on the UDS level of either one of the XP fibroblasts or the repair-proficient cell line was observed. T4 endonuclease with the same in vitro activity injected as a control in the same experiment did induce UDS in both XP groups. Furthermore, from coinjection experiments with T4 endonuclease and from injections in repair-proficient cells it appeared that the bacterial protein preparations did not contain any inhibiting factor interfering with correction or with the endogenous repair system. At present we can advance several explanations for the negative results obtained with UvrABCD:

(1) Rapid degradation or disruption of the Uvr excinuclease or one of the components after microinjection

After microneedle injection of the UvrABCD proteins (tested in vitro prior to injection) the cells are UV-irradiated and immediately probed for unscheduled DNA synthesis in the next 2 h. This would mean that if degradation occurs it has to occur very rapidly. The positive results obtained with T4 endo V, *Micrococcus luteus* extracts, PRE and human cell extracts (Hoeijmakers et al., 1983; de Jonge et al., 1983; Zwetsloot et al., 1985) assayed in some experiments more than 8 h after injection argue against a rapid degradation of injected proteins in our system.

Furthermore, transfections of shuttle vectors harbouring either the *uvrA* or *uvrB* genes into simian COS-1 cells showed that the corresponding bacterial proteins can be detected immunologically even 70–90 h after transfection. In the case of UvrA we were able to show that UvrA protein isolated from COS-1 cells 48 h after transfection had the size of the native bacterial enzyme and was still able to bind to single-stranded DNA (Backendorf and Van de Putte, 1985). These experiments make it unlikely that rapid degradation is the reason for the negative results obtained. Nevertheless, we cannot completely rule out this possibility as we have no data on the stability of the UvrC and D components.

(2) The action of the Uvr excinuclease on human chromatin cannot be visualized by an increase in UDS

In vitro studies with purified UvrABCD have

shown that the bacterial excinuclease can remove a fragment of 12–13 bp containing the base damage from irradiated DNA (Sancar and Rupp, 1983). If this small gap is subsequently filled in by endogenous human polymerases, it is possible that such a short-patch type of repair is not detected in our UDS assay. However, in that case one might expect a decrease in UV-induced UDS in repair-proficient cells after introduction of the UvrABCD proteins. Since such a decrease has not been observed we believe that this possibility is unlikely.

(3) The Uvr excinuclease is unable to reach or act on the lesions in human chromatin

The UvrABCD excinuclease is a bulky enzymatic complex with a total molecular weight of about 350 kD. It is possible that the ABCD excinuclease, which is involved in the removal of lesions from prokaryotic DNA, is unable to penetrate into the complex chromatin structure in eukaryotic cells and reach UV damage. Experiments with DNAase coupled to (bulky) carriers have shown that large molecules can penetrate less deeply into chromatin than small molecules (Burgoyne and Skinner, 1981). Endogenous eukaryotic repair systems might have to deal with similar problems. Indeed, recently it has been shown that actively transcribed chromatin, which is believed to have a more open structure, is more efficiently repaired than non-transcribed DNA sequences (Bohr et al., 1985). Furthermore, unscheduled DNA synthesis might be confined to the nuclear matrix (McCready and Cook, 1984). It is not likely that the "bulky" Uvr excinuclease would fit into such a structure in order to be able to deal with DNA lesions.

Finally, it is possible that the UvrABCD excinuclease can penetrate into eukaryotic chromatin structures but that it is unable to incise the damaged DNA strand due to e.g. specific proteins attached to the lesion or a modified DNA structure not recognized by the prokaryotic enzyme.

Recent in vitro studies performed by E. Seeberg and H. Krokan (in preparation) have shown that the UvrABC excinuclease is unable to incise UV-irradiated SV40 minichromosomes. Although isolated minichromosomes might not be an accurate reflection of in vivo chromatin organization, these experiments indicate, however, that chromatin structure might be the reason for the inability of the Uvr excinuclease to incise eukaryotic DNA, in agreement with the in vivo results reported here.

Although we realize that negative results are not always very conclusive we consider our inability to detect an effect of microinjected UvrABCD excinuclease on the repair synthesis of XP-A, XP-C and normal cells significant. If both UvrABCD and T4 endo V have the same opportunity to act on a lesion in the human chromatin of repair-deficient cells an increase in UDS to the same level would be expected in both cases as identical activities of both protein preparations have been injected. The negative results reported here suggest that the *Escherichia coli* "repairosome" is too different from the equivalent human system to be able to complement or bypass defective steps in xeroderma pigmentosum.

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