Complementation of the Xeroderma Pigmentosum DNA Repair Defect in Cell-Free Extracts

Richard D. Wood, Peter Robins, and Tomas Lindahl
Imperial Cancer Research Fund
Clare Hall Laboratories
South Mimms, Hertfordshire
EN6 3LD, England

Summary

Soluble extracts from human lymphoid cell lines that perform repair synthesis on covalently closed circular DNA containing pyrimidine dimers or psoralen adducts are described. Short patches of nucleotides are introduced by excision repair of damaged DNA in an ATP-dependent reaction. Extracts from xeroderma pigmentosum cell lines fail to act on damaged circular DNA, but are proficient in repair synthesis of ultraviolet-irradiated DNA containing incisions generated by Micrococcus luteus pyrimidine dimer-DNA glycosylase. Repair is defective in extracts from all xeroderma pigmentosum cell lines investigated, representing the genetic complementation groups A, B, C, D, H, and V. Mixing of cell extracts of group A and C origin leads to reconstitution of the DNA repair activity.

Introduction

The relevance of DNA repair in counteracting carcinogenesis has been highlighted by studies of the disease xeroderma pigmentosum (XP). This autosomally recessive disorder occurs worldwide, with a frequency of about 1 in 250,000 in the United States and Europe (Andrews, 1983). Clinically, the principal manifestation of the disease is a hypersensitivity to the ultraviolet (UV) component of sunlight, resulting in numerous skin and eye lesions including cancers. The syndrome was first described by Kaposi (1882), who noted the exceptional appearance of multiple skin tumours in young patients. He also pointed out a hereditary disposition for the disease, since two pairs of patients were siblings.

Cells from XP patients exhibit reduced levels of DNA repair synthesis in response to UV light (Cleaver, 1968). XP cells have deficiencies in removal of pyrimidine dimers (cyclobutane dimers and pyrimidine [6–4] pyrimidone adducts) induced by UV light (Zelle and Lohman, 1979; Mitchell et al., 1985) and in removal of chemical adducts generated by mutagens such as acetylaminofluorene, psoralen plus near-UV light, and benzpyrene (Amacher and Lieberman, 1977; Kaye et al., 1980; Yang et al., 1980). These DNA damaging agents introduce lesions that cause major distortions in the double helix structure, apparently with a sharp bend or kink in the helix as a unifying feature (Pearlman et al., 1985; Rao and Kollman, 1985). Such damage is normally removed by DNA excision repair. Because of the repair defect, XP cells are hypermutable by UV light and several chemical mutagens (Maher et al., 1976).

An unexpected genetic heterogeneity in XP was demonstrated when several complementation groups were defined by cell fusion experiments (de Weerd-Kastelein et al., 1972). A total of ten complementation groups have been identified to date, termed XP-A through XP-I and XP-V (reviewed by Kraemer et al., 1987). Studies of the kinetics of complementation suggest that the different groups are deficient in different proteins (Giannelli et al., 1982). Nevertheless, it seems that cells from most or all XP complementation groups have a defect in the initial incision at DNA damage (Fornace et al., 1976; Thielmann et al., 1985).

Little is known about the molecular mechanisms of nucleotide excision repair in human cells. In contrast, the biochemistry of excision repair of UV-irradiated DNA in E. coli is now well understood. Incision at pyrimidine dimers requires the functions of the *uvrA*, *uvrB*, and *uvrC* genes (Boyce and Howard-Flanders, 1964). Initial studies by Seeberg et al. (1976) on an ATP-dependent DNA incision activity allowed the partial characterization of the UvrA, UvrB, and UvrC gene products. Subsequently, the *uvrA*, *uvrB*, and *uvrC* genes were cloned and sequenced, and the protein products overexpressed and purified to homogeneity. The multisubunit UvrABC nuclease has been shown to cut a damaged DNA strand on each side of the lesion in an ATP-dependent reaction (Sancar and Rupp, 1983; Yeung et al., 1986).

Certain parallels suggest that it is reasonable to approach the problem of DNA excision repair of UV damage in human cells in a way similar to that used for E. coli. First, a number of human DNA repair enzymes have been identified, such as several DNA glycosylases and O^6-methylguanine-DNA methyltransferase, which serve to correct DNA base damage caused by deamination, alkylation, ring saturation, and ring fragmentation. All of these activities closely resemble enzymes previously found in E. coli (Lindahl, 1982). Second, studies with permeabilized human cells indicate that damage-specific incision of DNA after exposure to UV light requires ATP (Dresler and Lieberman, 1983; Kaufmann and Briley, 1987) as it does in E. coli. Third, pyrimidine dimers in the DNA of human cells are excised within short oligonucleotides (LaBelle and Linn, 1982; Weinfeld et al., 1986), again as in E. coli. Fourth, the presence of multiple complementation groups in XP suggests the existence of a multisubunit repair nuclease analogous to the E. coli UvrABC enzyme.

We set out to develop a soluble system based on human cell extracts that can perform UV-dependent DNA excision repair and reflect the differences between normal and XP cells. The basic approach is to monitor the formation of repair patches in exogenously added, UV-irradiated plasmid DNA.

Results

Removal of Pyrimidine Hydrates from UV-Irradiated DNA

Mammalian cells contain a readily detectable enzyme that can incise UV-irradiated DNA at sites different from pyrimidine dimers (Bacchetti and Benne, 1975; Doetsch et

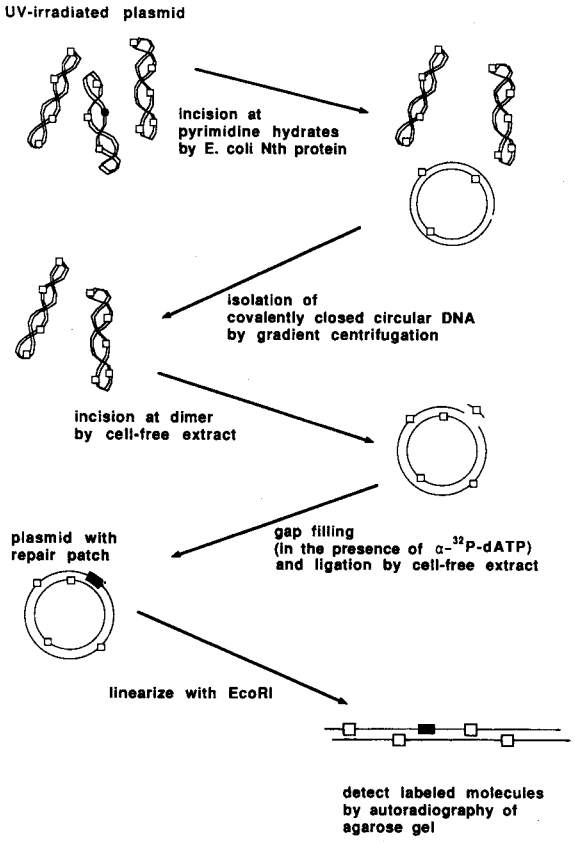

Figure 1. Scheme for Detection of DNA Repair Replication In Vitro

A UV-irradiated plasmid substrate, free of sites sensitive to pyrimidine hydrate-DNA glycosylase, is utilized. A major UV photoproduct (cyclobutane or [6–4] pyrimidine dimer) is represented by an open square, and a lesion sensitive to pyrimidine hydrate-DNA glycosylase by a filled circle.

al., 1986). This nicking activity has been found in extracts from all mammalian cell types tested, including normal human and XP cells. The activity is due to a DNA glycosylase that catalyzes release of hydrated and ring-fragmented pyrimidines (Breimer, 1983; Hollstein et al., 1984; Lee et al., 1987). The enzyme also cleaves DNA chains at apyrimidinic and apurinic sites, apparently by promoting β-elimination (Bailly and Verly, 1987), and it does not require either Mg^{2+} or ATP as a cofactor. The protein has been given several names including pyrimidine hydrate–DNA glycosylase, thymine glycol–DNA glycosylase, urea-DNA glycosylase, redoxynuclease, UV endonuclease, X-ray endonuclease, and (in E. coli) endonuclease III. The E. coli enzyme is encoded by the *nth* gene (Cunningham and Weiss, 1985).

In order to avoid detecting repair events initiated by the pyrimidine hydrate-DNA glycosylase, UV-irradiated DNA was prepared in which sites of action for the enzyme had been eliminated by pretreatment with purified E. coli Nth protein (endonuclease III). An outline of the procedure is shown in Figure 1. DNA from plasmid pAT153 was UV-irradiated with 450 J/m^2. This fluence produced an average of about 12 pyrimidine dimers and 0.9 Nth protein sensitive sites per molecule. The irradiated plasmids were treated with an excess of Nth protein and the remaining covalently closed circular DNA was isolated by gradient centrifugation. These purified plasmid DNA molecules were no longer sensitive to cleavage by Nth protein, and were used as the UV-irradiated DNA substrate in the work described below. Greater than 99% of the circles could be converted to the nicked form by treatment with pyrimidine dimer-DNA glycosylase from Micrococcus luteus; this bacterial enzyme incises UV-irradiated DNA at the sites of cyclobutane pyrimidine dimers (Grafstrom et al., 1982).

Repair Replication of UV-Irradiated DNA in Cell-Free Extracts

A method was devised to detect DNA repair events in the UV-irradiated plasmid in a reaction mixture containing an appropriate human cell extract. This scheme (Figure 1) efficiently detects repair by monitoring incorporation of radioactive material into repair patches. In the reaction mixture (which includes [α-^{32}P]dATP), components in the cell extract incise damaged plasmids and promote formation of a repair patch. Most of these patches are joined into closed circular plasmid molecules by DNA ligases in the extract (see below, Figure 6, lane 6). As an internal control, unirradiated pBR322 was included in each reaction mixture. During incubation, many topoisomers of the plasmids are formed by the action of topoisomerases I and II, including large catenated networks. In order to simplify analysis of repair replication activity in the extracts, the products of the reaction were linearized with EcoRI (which cuts pAT153 and pBR322 only once each) prior to gel electrophoresis (Figure 2A).

The DNA repair synthesis in irradiated plasmids is shown in Figure 2B. Increasing amounts of cell extract from the lymphoid line GM1953, derived from a healthy individual, were employed. Incorporation of radioactive nucleotide residues into UV-irradiated plasmid was approximately 10-fold higher than with unirradiated plasmid DNA. The degree of incorporation was proportional to protein concentration in the range 10–200 μg protein per 50 μl reaction mixture.

Also shown in Figure 2 are results for an extract from the XP-A lymphoid cell line GM2345. This extract is clearly deficient in repair replication of UV-irradiated DNA (compare lanes in the figure showing the same amount of protein in the normal and XP-A extracts).

UV-dependent repair replication catalyzed by extracts from normal cells was found to be critically dependent on the method of preparation of the cell extract and the reaction conditions. The clearest results were obtained with whole cell extracts of the type frequently employed for in vitro transcription experiments (Manley et al., 1980). Such extracts incubated with DNA at 30°C promoted repair incorporation on UV-irradiated DNA that increased in an approximately linear manner between 30 min and 6 hr, with little increase after that time. On the other hand, cytosolic extracts of the type used to promote plasmid replication from the SV40 origin (Li and Kelly, 1984) and a number of other types of crude cell extracts contained background nuclease activities that largely obscured the repair. The

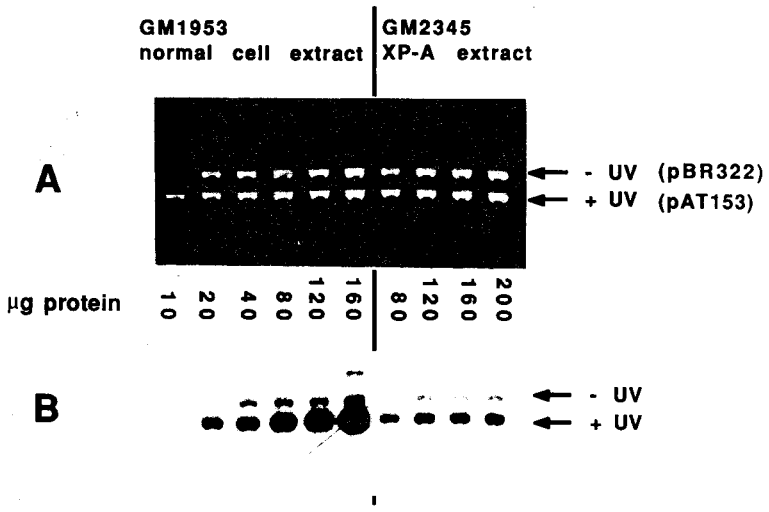

Figure 2. Repair Replication Performed by Human Cell Extracts

Three hundred nanograms each of pBR322 (not UV-irradiated), and pAT153 (UV-irradiated, treated with Nth protein, and repurified as outlined in Figure 1) were incubated together under standard reaction conditions with increasing amounts of extract from normal lymphoid cell line GM1953 (first six lanes), or XP group A line GM2345 (last four lanes). The plasmids were linearized with EcoRI and separated on an agarose gel. (A) Photograph of the ethidium bromide-stained gel. (B) Autoradiograph of the gel.

specificity of the reaction for UV-irradiated DNA was most marked with a KCl concentration in the range 40–100 mM. In the presence of less than 20 mM KCl, some XP extracts appeared to have increased residual repair activity on UV-irradiated DNA, which may reflect increased leakiness of altered XP proteins in a low salt environment. At KCl concentrations above 100 mM, incorporation of radioactive material into irradiated DNA was strongly reduced in extracts derived from both normal and XP cells.

Several types of experiments showed that the radioactive material associated with irradiated plasmid molecules was covalently incorporated in small patches, essentially at random sites. The radioactive label remained associated with full-length single-stranded plasmid molecules after linearization and heating to 95°C or incubation at pH 12.0. In addition, the plasmid was fully sensitive to digestion by the restriction enzyme DpnI. This shows that E. coli *dam* methylation was maintained, which would not have been the case if large segments of the plasmid were replicated in the extract. Each DpnI fragment was labeled in proportion to its length, suggesting random repair. More importantly, when the repair replication reaction was carried out in the presence of 5-Br-dUTP in place of TTP, and the products were banded on alkaline CsCl gradients, only a very small shift to higher density relative to unrepaired plasmid was observed, indicating heterogeneous patch sizes of less than 150 nucleotides in length. From this patch size estimate and from the number of ^{32}P-dAMP residues incorporated in a standard repair reaction with 80 μg protein from GM1953 normal cells, we estimate that about 1% of the irradiated pAT153 molecules undergo a repair event. This low efficiency may be typical of certain cell-free systems; replication of SV40 origin-containing plasmids in cell extracts initiates on less than 2% of the input molecules (Li and Kelly, 1984; Stillman and Gluzman, 1985).

ATP Dependence

Repair replication promoted by the cell extracts is strongly dependent on the presence of ATP and an ATP regenerating system (phosphocreatine and creatine phosphokinase). Figure 3 (A and B) shows that incorporation of radioactive material into both unirradiated and UV-irradiated plasmids is reduced by >99% in the absence of ATP; this is most likely due to dephosphorylation and depletion of the deoxynucleoside triphosphates. However, when 2 mM ATP is added to the reaction mixture in the absence of a regenerating system, the characteristic background incorporation into unirradiated plasmid is observed, whereas the incorporation of radioactive material into UV-irradiated plasmid is substantially reduced (compare Figure 3A with 3C and 3D). This indicates that UV-dependent repair replication in the extract requires maintenance of the ATP level.

The specific requirement for ATP in repair of UV-irradiated DNA is associated with the incision step and/or oligonucleotide displacement, rather than later events. As noted, background incorporation into unirradiated DNA occurs without ATP regeneration. Moreover, when a plasmid containing a single-stranded gap of approximately 25 nucleotides is added to the reaction mixture, replicative

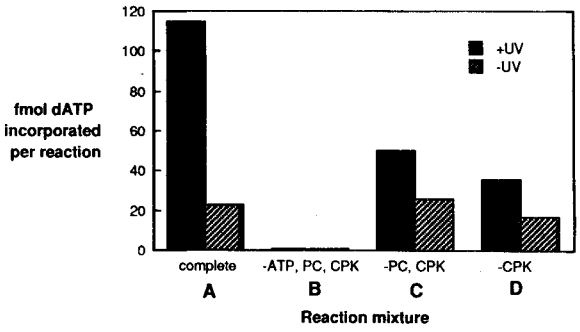

Figure 3. UV-Stimulated Repair Replication Dependent on ATP (Column B) and an ATP Regenerating System (Columns C and D)

The complete reaction mixture (column A) included ATP, phosphocreatine (PC), and creatine phosphokinase (CPK). All reactions were carried out with 80 μg of GM1953 normal cell extract. Data were quantitated by densitometric scanning of an autoradiograph similar to that shown in Figure 2.

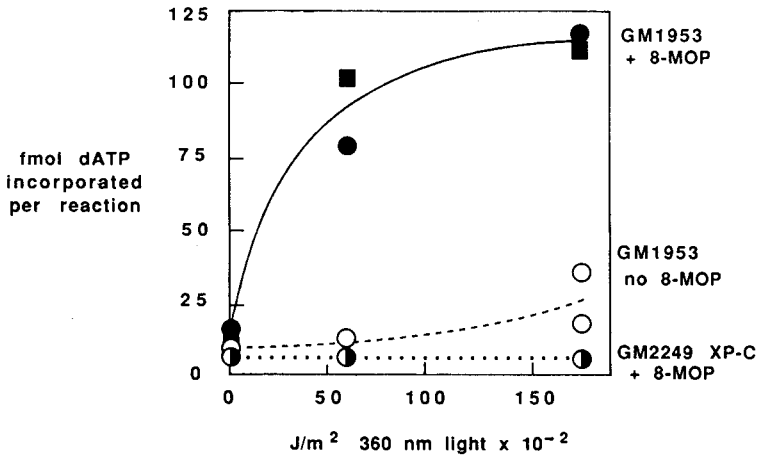

Figure 4. Stimulation of Repair Replication by 8-MOP DNA Adducts

pAT153 plasmid in the presence (solid symbols) or absence (open symbols) of 8-methoxypsoralen (8-MOP) was irradiated with near UV light (peak output at 360 nm) as indicated and used in standard repair reactions with GM1953 extract (circles and squares) or GM2249 XP-C extract (half-filled circles). A fluence of 1.1×10^4 J/m^2 induced the formation of an average of approximately one alkali-resistant psoralen cross-link per plasmid DNA molecule. Incorporation of radioactive material was quantitated by densitometric scanning of autoradiographs. Circles and squares represent independent experiments.

gap filling (30% of complete system) occurs in the absence of ATP and a regenerating system, although repair patches are not ligated in this case (data not shown).

Repair of Helix-Distorting Adducts

The E. coli UvrABC nuclease can incise DNA containing many types of helix-distorting base adducts; comparisons in vivo between normal and xeroderma pigmentosum cells indicate that a function of similar broad specificity is present in human cells (Cleaver, 1983). Here, in an extract from GM1953 cells, DNA repair incorporation could be stimulated not only by 254 nm UV irradiation but also by covalently attached psoralen adducts (Figure 4). When photoactivated with 360 nm near UV light, 8-methoxypsoralen reacts with DNA to produce interstrand cross-links, principally between thymine bases, as well as about 10 monoadducts for every cross-link (Hearst et al., 1984). A substantial repair signal was observed using DNA containing one to two cross-links per molecule. Irradiation with near UV light alone does not generate characteristic 254 nm UV pyrimidine dimer photoproducts, and did not stimulate repair (Figure 4). Activity on psoralen-damaged DNA was not present in extracts from the line GM2249, representative of xeroderma pigmentosum group C (Figure 4), or in GM2345, from group A.

It was of interest to determine whether DNA single-strand interruptions or damage causing only minor DNA helix distortion could initiate a significant repair replication event. For this reason, plasmids were treated with pancreatic DNAase I to introduce an average of one nick per circle, or heated at pH 5.0 to introduce an average of one apurinic site per molecule (apurinic sites are nicked with high efficiency in human cell extracts). Very little stimulation of repair incorporation occurred as a consequence of such treatments. Thus, reaction mixtures containing 300 ng of undamaged plasmid incorporated 140 dpm from [α-^{32}P]dATP in a standard reaction, whereas reactions containing DNAase I–nicked plasmids, or plasmids with an apurinic site, had incorporated 260 dpm or 170 dpm, respectively. In contrast, UV-irradiated plasmid substrate (450 J/m^2) contained 1200 dpm of radioactive material.

Defective Repair in Xeroderma Pigmentosum Cell Extracts

Extracts from a number of different human cell lines of lymphoid origin have been surveyed for proficiency in UV-stimulated repair. A strong repair signal is shown by extracts from the normal cell lines GM1953 and GM0892, as well as GM3403, derived from a patient with Bloom's Syndrome (Figure 5). HeLa cells also yielded similarly active

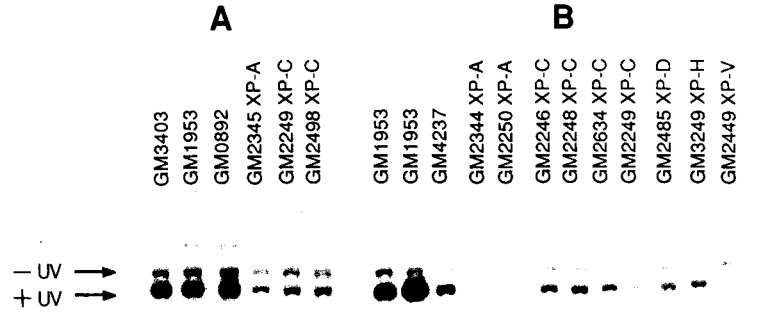

Figure 5. DNA Repair Signal in Various Lymphoid Cell Lines (Autoradiographs of Agarose Gels)

(A) Standard reactions containing 80 μg protein each. Lane 1, GM3403 (Bloom's Syndrome); lane 2, GM1953 (normal individual); lane 3, GM0892 (normal); lane 4, GM2345 XP-A; lane 5, GM2249 XP-C; lane 6, GM2498 XP-C.

(B) Standard reactions containing 50 μg protein each. Lanes 1 and 2, GM1953 (two separately prepared extracts); lane 3, GM4237 (phenotypically normal parent of GM2246 XP-C); lanes 4 through 12, cell lines as indicated in this figure.

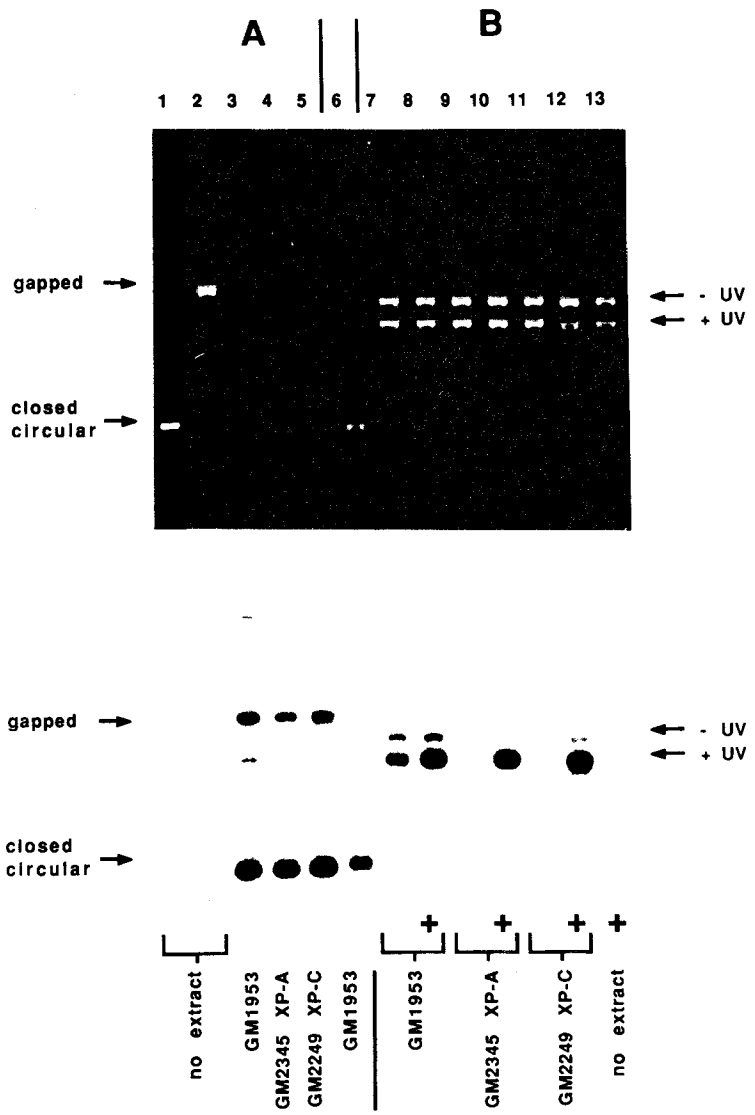

Figure 6. Completion of Repair Events by XP Cell Extracts: Ability to Fill Small Gaps, and Stimulation of Repair Replication by Pyrimidine Dimer-DNA Glycosylase from M. luteus

(Top) Photograph of agarose gel cast and run in buffer containing ethidium bromide. (Bottom) Autoradiograph of gel.

(A) Closed circular pAT153 (lane 1) was treated with pancreatic DNAase I and E. coli exonuclease III to create plasmids containing a small randomly placed gap of about 25 nucleotides (lane 2). The gapped plasmid was incubated in a standard reaction with 100 μg of extract from GM1953 normal cells (lane 3); GM2345 XP-A cells (lane 4); or GM2249 XP-C cells (lane 5). DNA was extracted from the reaction mixture and loaded onto the gel without EcoRI cleavage. In lane 6, UV-irradiated pAT153 was incubated with GM1953 extract and loaded without EcoRI cleavage to show that most of the radioactive material is present in closed circular molecules.

(B) Lanes 7 through 13 include a mixture of unirradiated pBR322 and UV irradiated pAT153, linearized with EcoRI before loading. Reaction mixtures contained GM1953 extract (lanes 7 and 8), GM2345 XP-A extract (lanes 9 and 10), or GM2249 XP-C extract (lanes 11 and 12). Lanes 8, 10, and 12 were supplemented with 0.1 μg cyclobutane pyrimidine dimer-DNA glycosylase from M. luteus, indicated by "+"; lane 13 contained the standard reaction mixture and M. luteus enzyme but no cell extract.

extracts. In contrast, preparations from XP cell lines are consistently deficient in UV-stimulated repair, compared to control lines. Figure 5 shows results for XP-A lines GM2345, GM2344, and GM2250; XP-C lines GM2249, GM2498, GM2246, GM2248, and GM2634; XP-D line GM2485, XP-H line GM3249, and XP-V line GM2449. Line GM4237 was derived from an unaffected heterozygous parent of the patient providing XP-C line GM2246. Extracts from GM4237 appeared to show repair intermediate between that of XP extracts and normal extracts. In addition to the results shown, extracts from a second XP-V line, GM1646, and an XP-B line, GM2252, were examined and both also proved deficient in repair replication in vitro.

An approximately 2-fold variation in repair activity occurred between different preparations of extracts from the same line (see the three separate GM1953 extracts in Figure 5). Moreover, the extracts of normal cells showed slightly higher "background repair" of nonirradiated DNA than the XP extracts (Figure 5). The latter observation may be related to the finding of low but significant ATP-dependent incision activity on nondamaged plasmid DNA by the purified E. coli UvrABC nuclease (Van Houten and Sancar, 1987).

Ability of XP Extracts to Complete Repair Events

A number of experiments were performed that suggest that the deficiency in XP extracts is related to inefficient initiation of repair. When plasmids containing a small single-stranded gap (approximately 25 nucleotides long) were employed instead of UV-irradiated DNA as substrate in the standard reaction mixture, efficient DNA repair synthesis and ligation of the strand interruptions were observed. Extracts from normal, XP-A, and XP-C cells were equally active in this regard (Figure 6A). Thus, the XP repair defect detected in our cell-free standard assay (Figure 5) would be expected to occur prior to the DNA gap-filling step in the excision-repair pathway.

Another type of experiment made use of pyrimidine

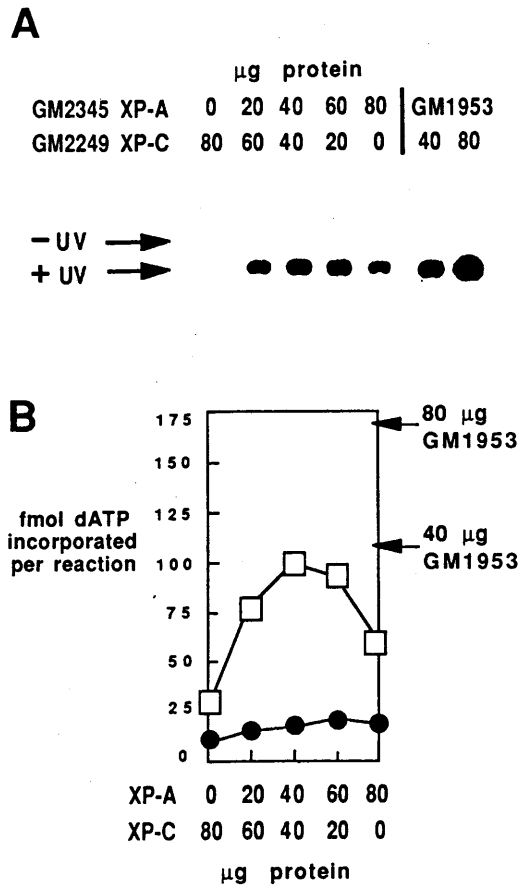

Figure 7. In Vitro Complementation by Mixing Extracts (Total 80 μg Protein Per Reaction) from Cell Lines GM2345 (XP-A) and GM2249 (XP-C)

(A) Autoradiograph of gel. Also shown are results obtained with 40 μg and 80 μg of GM1953 normal cell extract. (B) Incorporation of radioactive material quantified by densitometric scanning of the autoradiograph. Squares: pAT153, +UV; circles: pBR322, −UV. The level of incorporation seen with 40 μg and 80 μg of GM1953 extract is indicated.

dimer-DNA glycosylase from M. luteus. This low molecular weight enzyme introduces nicks into UV-irradiated DNA at the sites of cyclobutane pyrimidine dimers, producing a break with a protruding 5′ pyrimidine dimer and a 3′ baseless sugar (Grafstrom et al., 1982). Mammalian cells apparently do not normally incise near pyrimidine dimers in this fashion (La Belle and Linn, 1982). However, several investigators have introduced the M. luteus enzyme or the similar T4 bacteriophage *denV*-encoded enzyme into repair deficient XP cells, and have shown that UV-induced repair replication is stimulated (Tanaka et al., 1977; de Jonge et al., 1985; Arrand et al., 1987). In an analogous approach, we added M. luteus pyrimidine dimer-DNA glycosylase to the standard reaction mixture in the presence of human cell extract. Repair replication was dramatically stimulated in XP cell extracts, showing that they contain all components necessary to complete a repair patch initiated by the M. luteus enzyme (Figure 6B).

In Vitro Complementation of DNA Repair by Mixing XP Cell Extracts

Repair-deficient extracts from different complementation groups of XP have been mixed in attempts to restore activity. Figure 7 shows an experiment demonstrating biochemical complementation. A marked enhancement of repair activity was observed when two repair-deficient extracts, GM2345 (XP-A) and GM2249 (XP-C) were combined. When equal amounts of protein from the XP-A and XP-C extracts were used, incorporation of radioactive material was approximately 50% of the level attained by normal cell extracts (Figure 7). Little repair replication was promoted by either XP extract alone, although the XP-A extract exhibited somewhat more residual repair activity than the XP-C extract. Similar reconstitution of activity was also achieved when GM2345 (XP-A) extracts were mixed with extracts from another XP-C cell line, GM2498. Mixing two different XP-A extracts, GM2250 and GM2345, or two XP-C extracts, GM2249 and GM2498, did not result in detectable complementation.

Discussion

Repair of UV-Irradiated DNA In Vitro

The results reported here demonstrate that normal human cell extracts can promote repair synthesis on UV-irradiated and psoralen damaged plasmid DNA, and that such repair is defective using extracts from XP cells. Moreover, reconstitution of the repair activity can be achieved by mixing extracts from two different XP complementation groups. Previous efforts to detect excision repair in a cell-free system and to demonstrate an associated defect in XP cell extracts have been generally unsuccessful, apparently because of several technical barriers. Most investigators have searched for a UV-specific nicking activity in crude cell extracts. As a result, pyrimidine hydrate-DNA glycosylase has been repeatedly rediscovered. This problem has been circumvented in the present study by removing pyrimidine hydrates from the UV-irradiated DNA prior to the repair assay. An alternative approach has been to use DNA irradiated with a very low fluence, so that pyrimidine hydrates are rare, but the resulting lower sensitivity of detection has given unsatisfactory results (Waldstein et al., 1979).

The repair reaction depends on the presence of ATP and a regenerating system, and this dependence seems to be associated with early steps of repair. We have attempted to detect directly the ATP-dependent introduction of single strand breaks at dimers, but have found that a difficulty with such an assay is the often extensive ATP-dependent concatenation of plasmid molecules in human cell extracts (unpublished data). These catenated networks do not enter agarose gels and can be retarded on nitrocellulose membrane filters used in standard nicking assays (Holden and Low, 1985). Furthermore, in a crude cell extract, the incision reaction is likely to be rate-limiting, as it is in vivo (Fornace et al., 1976; Erixon and Ahnström, 1979). Once the initial incision has occurred, gap filling and ligation should proceed rapidly in the pres-

ence of ATP, making a nicked intermediate difficult to detect. In the strategy presented in this paper, repair events are instead measured as radioactively labeled patches in plasmid molecules.

Another new approach used here has been to prepare cell extracts for DNA repair by methods previously used to produce extracts active in transcription. The procedure for extract preparation is crucial if complex biological processes are to be carried out in vitro (Manley et al., 1980; Li and Kelly, 1984). The human cell extracts employed in this work apparently contain, in soluble form, all necessary protein factors for ATP-dependent incision and repair replication at lesions in DNA introduced by exposure to UV light or photoactivated psoralen. Preparation of such extracts has been greatly aided by the availability of EB virus–immortalized lymphoid cell lines of normal and XP origin (Andrews et al., 1974), which can be grown in suspension culture to provide large numbers of cells for biochemical experiments.

Defective Repair in Different XP Complementation Groups

Twelve different XP cell lines have been used in this work, representing all the more frequently detected complementation groups, and several of the rare ones. These include three independently derived cell lines from XP group A, four from group C, one each from groups B, D, and H, and two from group V. Cell extracts from all these lines show deficiencies in the in vitro repair reaction, although some of the lines seem "leaky" under our experimental conditions. Thus, a consistent relationship has been observed between the XP phenotype seen in human patients and reduced repair replication of UV-irradiated DNA in our cell-free system.

The deficient excision repair shown by extracts of complementation group V ("variant") origin is surprising, because group V ("variant") cell lines have been regarded as being essentially proficient in excision repair, having instead an aberration in rejoining of nascent DNA strands after replication of a UV-damaged DNA molecule (Lehmann et al., 1975). However, two recent in vivo studies have described defects in excision repair in XP variants. In an analysis of a number of XP-V cell lines, Thielmann et al. (1985) demonstrated convincingly a defect in excision repair in XP variants that overlaps with that of several classical excision-defective groups such as group E. Similarly, Kondo et al. (1987) found that epidermal keratinocyte lines from group V show a diminished DNA repair synthesis indistinguishable from that exhibited by group E cells. These observations suggest that XP-V is a complementation group with a defect in excision repair. The considerable leakiness exhibited by XP complementation groups such as V, E, and D, when investigated by the standard in vivo assay for UV-stimulated unscheduled DNA synthesis (Andrews, 1983; Cleaver, 1983) is difficult to reconcile with the clinical phenotype. In this regard, the cell-free assay system described here appears to afford a more consistent distinction between the repair capacities of normal and XP cells.

Comparison with Other Cell-Free Systems

The repair deficiency described here for XP-derived cell extracts is different from results in previous systems that examined pyrimidine dimer excision from endogenous DNA in crude human cell extracts (Mortelmans et al., 1976; Kano and Fujiwara, 1983). In those studies, extracts from cells of all XP groups tested were apparently able to excise dimers from naked DNA, but not from chromatin, and the excision did not depend on added ATP. This would appear to be an atypical situation, since in addition to the ATP dependence of the repair described here, permeabilized human cell repair systems have been shown to require ATP (Dresler and Lieberman, 1983; Kaufmann and Briley, 1987). It is unclear to what extent exogenously added plasmid DNA remains "naked" in soluble whole cell extracts, but reconstitution into intact chromatin appears unlikely. Thus, our results do not provide support for the hypothesis that certain XP gene products may serve to make UV lesions in chromatin accessible to repair enzymes, although it remains possible that tenaciously DNA binding proteins present in the extracts might have to be actively displaced during the repair reaction.

The suggestion that all ten XP complementation groups may be defective in incision of UV-irradiated DNA at pyrimidine dimers indicates an unexpected complexity of the event in human cells. However, in E. coli, three gene products are required to form the incision complex (UvrA, UvrB, and UvrC) plus two (UvrD and PolI) to remove the damaged oligonucleotide (Husain et al., 1985). A greater complexity of enzymes involved in nucleic acid metabolism in mammalian cells is not without precedent; thus, mammalian RNA polymerases have nine to ten subunits, whereas E. coli RNA polymerase (core enzyme) has three subunits.

Future Directions

Efforts are underway in a number of laboratories to clarify human excision repair of UV damage by making use of XP cells. An interesting approach has been complementation of XP cells by microinjection of cell extracts. This method has allowed for the partial purification of the XP-A protein, although the painstaking assay has resulted in slow progress (de Jonge et al., 1983; Yamaizumi et al., 1986). Detergent-permeablized XP cells can also be converted to a repair-proficient state by the simultaneous addition of DNA polymerase δ and an XP-complementing factor from normal cells (Nishida et al., 1988). Alternatively, there have been many attempts to clone DNA repair genes by transformation of XP cells with DNA from normal cells (reviewed by Friedberg et al., 1987). This approach has proved to be remarkably difficult because of spontaneous reversion of XP cells (Royer-Pokora and Haseltine, 1984; Schultz et al., 1985) and the apparently very limited capability of human cells to take up and stably integrate large DNA fragments (Hoeijmakers et al., 1987). However, Bootsma and coworkers (Westerveld et al., 1984; van Duin et al., 1986) have successfully cloned a human DNA repair gene by transfer to a suitable UV-sensitive Chinese hamster cell recipient (Wood and Burki, 1982).

Characterization of the XP gene products would not only identify biochemical features of excision repair in human cells and the particular repair defects in xeroderma pigmentosum, but would greatly facilitate attempts to clone the corresponding genes. The in vitro complementation of extracts from two different XP groups (Figure 7) suggests an obvious strategy for purifying human proteins involved in excision repair. For example, it should be possible to assay and purify the XP-A gene product by identifying fractions from XP-C cell extracts that can complement deficient XP-A extracts, and such experiments are in progress.

Experimental Procedures

Cell Lines

Human lymphoid cell lines were obtained from the N. I. G. M. S. Human Genetic Mutant Cell Repository (Camden, NJ). All lines used were tested and shown to be free of Mycoplasma. Cultures were grown in suspension in RPMI 1640 medium supplemented with 15% or 20% fetal calf serum as required.

Cell-Free Extracts

Extracts were prepared essentially by the method of Manley et al. (1980). One liter cultures of cells in late exponential phase at $5–9\times10^5$ cells/ml were used for each preparation. The cell pellet (packed cell volume 1 ml) was rinsed twice in ice cold phosphate-buffered saline, and then resuspended in 4 ml of hypotonic buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, and 5 mM dithiothreitol). After 20 min at 0°C, protease inhibitors were added (0.5 mM phenyl methyl sulfonyl fluoride and 0.5 μg/ml each of leupeptin, pepstatin, and chymostatin), and the swollen cells were broken by 8 to 20 strokes in a Dounce homogenizer. Four ml of an ice cold solution containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 25% sucrose, and 50% glycerol were added slowly with stirring. One ml of neutralized saturated ammonium sulfate solution was then slowly added with gentle mixing. The extremely viscous lysate was stirred very gently for 30 min at 0°C and then carefully poured into polyallomer ultracentrifuge tubes and centrifuged for 3 hr at 42,000 rpm in an SW50.1 rotor at 2°C. The supernatant was withdrawn, leaving the last 2 ml behind; usually 6 ml super natant were recovered. Protein was precipitated by addition of 0.33 g/ml ammonium sulfate (neutralized with 10 μl of 1 M NaOH per g ammonium sulfate). The precipitate was collected by centrifugation, resuspended in dialysis buffer (1/20 the volume of the high-speed supernatant), and dialyzed for 12 hr in the cold against two 125 ml changes of 25 mM HEPES-KOH (pH 7.9), 0.1 M KCl, 12 mM $MgCl_2$, 1 mM EDTA, 2 mM dithiothreitol, and 17% glycerol. Insoluble material was removed by centrifugation and the clarified extract (~1 ml per 1 l starting culture) was quick-frozen in small aliquots. Extracts typically contained 15 mg/ml protein. The extracts were stable for over a year at 80°C and remained active after one thawing and refreezing.

Nth Protein

Nth protein (endonuclease III) was prepared from the Nth overproducer E. coli BW531 (MM294 [*endA hsdR*δ(*srl-recA*)306*thi*]/pRPC53 [*nth*$^+$]) kindly provided by Drs. R. P. Cunningham and B. Weiss. Twelve liter cultures were grown in a fermentor in the presence of 25 μg/ml ampicillin, and lysed by sonication. Purification followed the method of Breimer and Lindahl (1984), through fraction V. The enzyme preparation was >80% pure and free from contaminating nuclease activities. Activity was assayed by nicking of pAT153 irradiated with 1000 J/m^2; the activity was UV-dependent and sensitive to heat inactivation (10 min at 55°C). Assays were carried out in 40 mM HEPES-KOH (pH 8.1), 0.1 M KCl, 1 mM EDTA, and 0.5 mM dithiothreitol for 20 min at 37°C.

UV-Irradiated Plasmid DNA Preparation

pAT153 plasmid DNA was prepared from E. coli strain DH5 (*recA hsdR*) without chloramphenicol amplification. DNA was irradiated in a thin stirred layer at 50 μg/ml in TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) with 254 nm (peak) germicidal UV light at a fluence rate of 0.5 W/m^2; UV fluence rate was measured with a Latarjet dosimeter. Introduction of sites sensitive to pyrimidine dimer-DNA glycosylase from M. luteus (700 enzyme U per μg protein obtained from Applied Genetics Inc., Freeport, NY) was monitored on agarose gels by quantitating the conversion of closed circular molecules to nicked forms. The introduction of sites sensitive to E. coli Nth protein was measured in the same way. An average of one cyclobutane pyrimidine dimer per molecule was produced by a fluence of 37 J/m^2, while an average of one stable site sensitive to Nth protein was produced by a fluence of 450–500 J/m^2. Plasmid irradiated with 450 J/m^2 was treated with an excess of E. coli Nth protein (0.04 mg protein per mg DNA) for 60 min at 37°C to nick stable UV-induced Nth protein-sensitive sites. Closed circular form I irradiated DNA was purified from the reaction mixture by two sequential 5%–20% neutral sucrose gradient centrifugations. The closed circular DNA was insensitive to nicking by Nth protein, or by sequential incubation with uracil-DNA glycosylase and endonuclease IV from E. coli (Lindahl et al., 1977), or to nicking by mammalian cell extracts (standard reaction mixture) in the absence of Mg^{2+} (data not shown). pBR322 plasmid DNA was prepared from strain DH5 and used as an unirradiated control.

Repair Reactions

Standard 50 μl reaction mixtures contained (final concentration) 0.3 μg unirradiated closed circular form I pBR322, 0.3 μg pAT153 UV irradiated substrate, 45 mM HEPES-KOH (pH 7.8), 70 mM KCl, 7.4 mM $MgCl_2$, 0.9 mM dithiothreitol, 0.4 mM EDTA, 2 mM ATP, 20 μM each of dGTP, dCTP, and TTP, 8 μM dATP, 2 μCi [α-^{32}P]dATP (3000 Ci/mmol), 40 mM phosphocreatine, 2.5 μg creatine phosphokinase (Type I, Sigma), 3.4% glycerol, 18 μg bovine serum albumin, and (typically) 80 μg of extract protein. Reactions were incubated for 6 hr at 30°C.

Visualization of Repair by Autoradiography

Reactions were stopped by the addition of EDTA to 20 mM. After a 10 min incubation at 37°C with 80 μg/ml RNAase A, SDS was added to 0.5%, and proteinase K to 190 μg/ml. Tubes were incubated for 30 min at 37°C, and the mixture was extracted with phenol/chloroform. DNA was precipitated in the presence of 2.5 M ammonium acetate with 2 vol of ethanol at −70°C. Plasmids were digested with EcoRI in 30 μl buffer and loaded onto a 1% agarose gel cast and run in buffer containing 0.5 μg/ml ethidium bromide. After overnight electrophoresis, the gel was photographed under near-UV transillumination with Polaroid Type 55 positive/negative film. An autoradiograph of the dried gel was obtained using Kodak XAR-5 film and intensifying screens for 2 hr at −80°C. Band intensities on the autoradiograph and the photographic negative were quantified using an LKB UltroScan XL scanning laser densitometer. In a number of experiments, bands were excised from the gel and analyzed by scintillation counting in order to calibrate the densitometry results with reference to incorporation of radioactive material.

Preparation of Substrate Containing Psoralen Adducts

Plasmid pAT153 at 3 μg/ml in TE buffer was mixed with 8-methoxypsoralen (Sigma) at 30 μg/ml. Samples in a thin layer were irradiated at room temperature with near UV light (360 nm peak) 22 cm below two FL-15BLB fluorescent lamps, filtered through 6 mm glass and 1 mm plastic to remove wavelengths below 300 nm. The incident fluence rate was 11 W/m^2, measured with a Model J221 Blak-Ray ultraviolet meter (Ultraviolet Products, San Gabriel, CA). The solution was extracted with chloroform-isoamyl alcohol (24:1) and concentrated with Centricon-30 centrifugal microconcentrators. After ethanol precipitation, the DNA was resuspended in TE buffer. Cross-links were visualized by cutting the plasmids with EcoRI and observing the linearized forms on an alkaline agarose gel; cross-linked forms show retarded migration.

Introduction of DNAase I Nicks, Apurinic Sites, and Gaps

Closed circular pAT153 was nicked with DNAase I. An average of one nick per circle was obtained by incubation with 1.3 ng/ml DNAase I for 30 min at 26°C. Apurinic sites were introduced into plasmid by treatment at pH 5.0, and 70°C. The number of sites per molecule was determined by measuring the susceptibility to E. coli endonuclease IV (Lindahl et al., 1977) and alkaline hydrolysis. Randomly placed gaps were generated in pAT153 labeled with ^{3}H-thymidine by first introducing an average of one DNAase I nick per circle, followed by digestion with a precalibrated saturating amount (500 enzyme U per μg DNA) of E. coli

exonuclease III (obtained from Gibco BRL) for 15 sec at 14°C. The gap size was determined by monitoring the release of cold acid-soluble ^3H (West et al., 1982). Solubilization of 0.31% of the total radioactive material corresponded to a gap size of about 25 nucleotides.

Acknowledgments

We thank Sally Tomlinson and the staff of the ICRF Clare Hall Cell Production facility for providing human cell cultures, Tim Oates for preparing Nth protein and plasmids, and our colleagues for comments on the manuscript. R. W. was supported through much of this work by U.S. PHS grant number 5-F32-CA-07937, awarded by the National Cancer Institute, DHHS.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 5, 1988; revised January 25, 1988.

References

Amacher, D. E., and Lieberman, M. W. (1977). Removal of acetylaminofluorene from the DNA of control and repair-deficient human fibroblasts. Biochem. Biophys. Res. Commun. *74*, 285–290.

Andrews, A. (1983). Xeroderma pigmentosum. In Chromosome Mutation and Neoplasia, J. G. German, ed. (New York: A. R. Liss), pp. 63–83.

Andrews, A. D., Robbins, J. H., Kraemer, K. H., and Buell, D. N. (1974). Xeroderma pigmentosum long-term lymphoid lines with increased ultraviolet sensitivity. J. Natl. Cancer Inst. *53*, 691–693.

Arrand, J. E., Squires, S., Bone, N. M., and Johnson, R. T. (1987). Restoration of UV-induced excision repair in xeroderma D cells transfected with the *denV* gene of bacteriophage T4. EMBO J. *6*, 3125–3131.

Bachetti, S., and Benne, R. (1975). Purification and characterization of an endonuclease from calf thymus acting on irradiated DNA. Biochim. Biophys. Acta *390*, 285–297.

Bailly, V., and Verly, W. G. (1987). Escherichia coli endonuclease III is not an endonuclease but a β-elimination catalyst. Biochem. J. *242*, 565–572.

Boyce, R., and Howard-Flanders, P. (1964). Release of ultraviolet light-induced thymine dimers from DNA in E. coli K-12. Proc. Natl. Acad. Sci. USA *51*, 293–300.

Breimer, L. H. (1983). Urea-DNA glycosylase in mammalian cells. Biochemistry *22*, 4192–4197.

Breimer, L. H., and Lindahl, T. (1984). DNA glycosylase activities for thymine residues damaged by ring saturation, fragmentation, or ring contraction are functions of endonuclease III in Escherichia coli. J. Biol. Chem. *259*, 5543–5548.

Cleaver, J. E. (1968). Defective repair replication of DNA in xeroderma pigmentosum. Nature *218*, 652–656.

Cleaver, J. E. (1983). Xeroderma pigmentosum. In The Metabolic Basis of Inherited Disease, 5th ed., J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, J. L. Goldstein, and M. S. Brown, eds. (New York: McGraw-Hill), pp. 1227–1248.

Cunningham, R. P., and Weiss, B. (1985). Endonuclease III (*nth*) mutants of Escherichia coli. Proc. Natl. Acad. Sci. USA *82*, 474–478.

de Jonge, A. J. R., Vermeulen, W., Klein, B., and Hoeijmakers, J. H. J. (1983). Microinjection of human cell extracts corrects xeroderma pigmentosum defect. EMBO J. *2*, 637–641.

de Jonge, A. J. R., Vermeulen, W., Keijzer, W., Hoeijmakers, J. H. J., and Bootsma, D. (1985). Microinjection of Micrococcus luteus UV-endonuclease restores UV-induced unscheduled DNA synthesis in cells of 9 xeroderma pigmentosum complementation groups. Mutation Res. *150*, 99–105.

de Weerd-Kastelein, E. A., Keijzer, W., and Bootsma, D. (1972). Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. Nature New Biol. *238*, 80–83.

Doetsch, P. W., Helland, D. E., and Haseltine, W. A. (1986). Mechanism of action of a mammalian DNA repair endonuclease. Biochemistry *25*, 2212–2220.

Dresler, S. L., and Lieberman, M. W. (1983). Requirement of ATP for specific incision of ultraviolet-damaged DNA during excision repair in permeable human fibroblasts. J. Biol. Chem. *258*, 12269–12273.

Erixon, K., and Ahnström, G. (1979). Single-strand breaks in DNA during repair of UV-induced damage in normal human and xeroderma pigmentosum cells as determined by alkaline DNA unwinding and hydroxylapatite chromatography. Mutation Res. *59*, 257–271.

Fornace, A. J., Kohn, K. W., and Kann, H. E. (1976). DNA single-strand breaks during repair of UV damage in human fibroblasts and abnormalities of repair in xeroderma pigmentosum. Proc. Natl. Acad. Sci. USA *73*, 39–43.

Friedberg, E. C., Backendorf, C., Burke, J., Collins, A., Grossman, L., Hoeijmakers, J. H. J., Lehmann, A. R., Seeberg, E., van der Schans, G. P., and van Zeeland, A. A. (1987). Molecular aspects of DNA repair. Mutation Res. *184*, 67–86.

Giannelli, F., Pawsey, S. A., and Avery, J. A. (1982). Differences in patterns of complementation of the more common groups of xeroderma pigmentosum: possible implications. Cell *29*, 451–458.

Grafstrom, R. H., Park, L., and Grossman, L. (1982). Enzymatic repair of pyrimidine dimer-containing DNA: a 5′-dimer DNA glycosylase-3′-apyrimidinic endonuclease mechanism from Micrococcus luteus. J. Biol. Chem. *257*, 13465–13473.

Hearst, J. E., Isaacs, S. T., Kanne, D., Rapoport, H., and Straub, K. (1984). The reaction of the psoralens with deoxyribonucleic acid. Quart. Rev. Biophys. *17*, 1–44.

Hoeijmakers, J. H. J., Odijk, H., and Westerveld, A. (1987). Differences between rodent and human cell lines in the amount of integrated DNA after transfection. Exp. Cell Res. *169*, 111–119.

Holden, J. A., and Low, R. L. (1985). Characterization of a potent catenation activity of HeLa cell nuclei. J. Biol. Chem. *260*, 14491–14497.

Hollstein, M. C., Brooks, P., Linn, S., and Ames, B. N. (1984). Hydroxymethyluracil DNA glycosylase in mammalian cells. Proc. Natl. Acad. Sci. USA *81*, 4003–3007.

Husain, I., van Houten, B., Thomas, D. C., Abdel-Monem, M., and Sancar, A. (1985). Effect of DNA polymerase I and DNA helicase II on the turnover rate of Uvr ABC excision nuclease. Proc. Natl. Acad. Sci. USA, *82*, 6774–6778.

Kano, Y., and Fujiwara, Y. (1983). Defective thymine dimer excision from xeroderma pigmentosum chromatin and its characteristic catalysis by cell-free extracts. Carcinogenesis *4*, 1419–1424.

Kaposi, M. (1882). Xeroderma pigmentosum. Wiener mediz. Jahrbücher (Oct.), pp. 619–632, (French translation by P. Doumic, Annu. Derm. Venereol. *4*, 29–38 [1883]).

Kaufmann, W. K., and Briley, L. P. (1987). Reparative strand incision in saponin-permeabilized human fibroblasts. Mutation Res. *184*, 237–243.

Kaye, J., Smith, C. A., and Hanawalt, P. C. (1980). DNA repair in human cells containing photoadducts of 8-methoxy-psoralen or angelicin. Cancer Res. *40*, 696–702.

Kondo, S., Satoh, Y., and Kuroki, T. (1987). Defect in UV-induced unscheduled DNA synthesis in cultured epidermal keratinocytes from xeroderma pigmentosum. Mutation Res. *183*, 95–101.

Kraemer, K. H., Lee, M. M., and Scotto, J. (1987). Xeroderma pigmentosum: cutaneous, ocular, and neurologic abnormalities in 830 published cases. Arch. Dermatol. *123*, 241–250.

LaBelle, M., and Linn, S. (1982). In vivo excision of pyrimidine dimers is mediated by a DNA N-glycosylase in Micrococcus luteus but not in human fibroblasts. Photochem. Photobiol. *36*, 319–324.

Lee, K., McCray, W., Jr., and Doetsch, P. W. (1987). Thymine glycol-DNA glycosylase/AP endonuclease of CEM-C1 lymphoblasts: a human analog of E. coli endonuclease III. Biochem. Biophys. Res. Commun. *149*, 93–101.

Lehmann A., Kirk-Bell, S., Arlett, C., Paterson, M. C., Lohman, P. H. M., de Weerd-Kastelein, E. A., and Bootsma, D. (1975). Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. Proc. Natl. Acad. Sci. USA *72*, 219–223.

Li, J. J., and Kelly, T. J. (1984). Simian virus 40 DNA replication in vitro. Proc. Natl. Acad. Sci. USA *81*, 6973–6977.

Lindahl, T. (1982). DNA repair enzymes. Annu. Rev. Biochem. *51*, 61–87.

Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B., and Sperens, B. (1977). Properties of uracil-DNA glycosidase from E. coli. J. Biol. Chem. *252*, 3286–3294.

Maher, V. M., Ouellette, L. M., Curren, R. D., and McCormick, J. J. (1976). Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal cells. Nature *261*, 593–595.

Manley, J. L., Fire, A., Cano, A., Sharp, P. A., and Gefter, M. L. (1980). DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. Proc. Natl. Acad. Sci. USA *77*, 3855–3859.

Mitchell, D. L., Haipek, C. A., and Clarkson, J. M. (1985). (6–4) photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. Mutation Res. *143*, 109–112.

Mortelmans, K., Friedberg, E. C., Slor, H., Thomas, G., and Cleaver, J. E. (1976). Defective thymine dimer excision by cell-free extracts of xeroderma pigmentosum cells. Proc. Natl. Acad. Sci. USA *73*, 2757–2761.

Nishida, C., Reinhard, P., and Linn, S. (1988). DNA repair synthesis in human fibroblasts requires DNA polymerase δ. J. Biol. Chem. *263*, 501–510.

Pearlman, D. A., Holbrook, S. R., Pirkle, D. H., and Kim, S.-H. (1985). Molecular models for DNA damaged by photoreaction. Science *227*, 1304–1308.

Rao, S. N., and Kollman, P. A. (1985). Conformations of deoxydodecanucleotides with pyrimidine (6–4)-pyrimidone photoadducts. Photochem. Photobiol. *42*, 465–475.

Royer-Pokora, B., and Haseltine, W. A. (1984). Isolation of UV-resistant revertants from a xeroderma pigmentosum complementation group A cell line. Nature *311*, 390–394.

Sancar, A., and Rupp, W. D. (1983). A novel repair enzyme: UVRABC excision nuclease of Escherichia coli cuts a DNA strand on both sides of the damaged region. Cell *33*, 249–260.

Schultz, R. A., Barbis, D. P., and Friedberg, E. C. (1985). Studies on gene transfer and reversion to UV resistance in xeroderma pigmentosum cells. Somatic Cell Genet. *11*, 617–624.

Seeberg, E., Nissen-Meyer, J., and Strike, P. (1976). Incision of ultraviolet-irradiated DNA by extracts of E. coli requires three different gene products. Nature *263*, 524–526.

Stillman, B. W., and Gluzman, Y. (1985). Replication and supercoiling of simian virus 40 DNA in cell extracts from human cells. Mol. Cell Biol. *5*, 2051–2060.

Tanaka, K., Hayakawa, H., Sekiguchi, M., and Okada Y. (1977). Specific action of T4 endonuclease V on damaged DNA in xeroderma pigmentosum cells in vivo. Proc. Natl. Acad. Sci. USA *74*, 2958–2962.

Thielmann, H. W., Edler, L., Popanda, O., and Friemel, S. (1985). Xeroderma pigmentosum patients from the Federal Republic of Germany: decrease in post-UV colony forming ability in 30 xeroderma pigmentosum fibroblast strains is quantitatively correlated with a decrease in DNA-incising capacity. J. Cancer Res. Clin. Oncol. *109*, 227–240.

van Duin, M., de Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M. H. M., Hoeijmakers, J. H. J., and Bootsma, D. (1986). Molecular characterization of the human excision repair gene *ERCC-1*: cDNA cloning and amino acid homology with the yeast DNA repair gene *RAD10*. Cell *44*, 913–923.

van Houten, B., and Sancar, A. (1987). Repair of N-methyl-N′-nitro-N-nitrosoguanidine-induced DNA damage by ABC excinuclease. J. Bacteriol. *169*, 540–545.

Waldstein, E. A., Peller, S., and Setlow, R. B. (1979). UV-endonuclease from calf thymus with specificity toward pyrimidine dimers in DNA. Proc. Natl. Acad. Sci. USA *76*, 3746–3750.

Weinfeld, M., Gentner, N. E., Johnson, L. D., and Paterson, M. C. (1986). Photoreversal-dependent release of thymidine and thymidine monophosphate from pyrimidine dimer-containing DNA excision fragments isolated from ultraviolet-damaged human fibroblasts. Biochemistry *25*, 2656–2664.

West, S. C., Cassuto, E., and Howard-Flanders, P. (1982). Postreplication repair in E. coli: strand exchange reactions of gapped DNA by RecA protein. Mol. Gen. Genet. *187*, 209–217.

Westerveld, A., Hoeijmakers, J. H. J., van Duin, M., de Wit, J., Odijk, H., Pastink, A., Wood, R., and Bootsma, D. (1984). Molecular cloning of a human DNA repair gene. Nature *310*, 425–429.

Wood, R., and Burki, H. J. (1982). Repair capability and the cellular age response for killing and mutation induction after UV. Mutation Res. *95*, 505–514.

Yamaizumi, M., Sugano, T., Asahina, H., Okada, Y., and Uchida, T. (1986). Microinjection of partially purified protein factor restores DNA damage specifically in group A of xeroderma pigmentosum cells. Proc. Natl. Acad. Sci. USA *83*, 1476–1479.

Yang, L. L., Maher, V. M., and McCormick, J. J. (1980). Error-free excision of the cytotoxic and mutagenic N2-deoxyguanosine DNA adduct formed in human fibroblasts by (±)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. Proc. Natl. Acad. Sci. USA *77*, 5933–5937.

Yeung, A. T, Mattes, W. B., Oh, E. Y., Yoakum, G. H., and Grossman, L. (1986). The purification of the Escherichia coli UvrABC incision system. Nucl. Acids Res. *14*, 8535–8556.

Zelle, B., and Lohman, P. H. M. (1979). Repair of UV-endonuclease-susceptible sites in the seven complementation groups of xeroderma pigmentosum A through G. Mutation Res. *62*, 363–368.