

# A damage-recognition protein which binds to DNA containing interstrand cross-links is absent or defective in Fanconi anemia, complementation group A, cells

Bo Hang, Anthony T.Yeung<sup>1</sup> and Muriel W.Lambert\*

Department of Laboratory Medicine and Pathology, UMDNJ, New Jersey Medical School and the Graduate School of Biomedical Sciences, 185 South Orange Avenue, Newark, NJ 07103 and <sup>1</sup>Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA

Received June 29, 1993; Revised and Accepted August 4, 1993

## ABSTRACT

**A DNA binding protein with specificity for DNA containing interstrand cross-links induced by 4,5',8-trimethylpsoralen (TMP) plus long wavelength ultraviolet (UVA) light has been identified in normal human chromatin. Protein binding to DNA was determined using a gel mobility shift assay and an oligonucleotide containing a hot spot for formation of psoralen interstrand cross-links. Specificity of the damage-recognition protein for cross-links was demonstrated both by a positive correlation between level of cross-link formation in DNA and extent of protein binding and by effective competition by treated but not undamaged DNA for the binding protein. Chromatin protein extracts from cells from individuals with the genetic disorder, Fanconi anemia, complementation group A (FA-A), which have decreased ability to repair damage produced by TMP plus UVA light, failed to show any protein binding to TMP plus UVA treated DNA. We have previously shown that these chromatin protein extracts contain a DNA endonuclease complex, pI 4.6, which specifically recognizes and incises DNA containing interstrand cross-links and which in FA-A cells is defective in its ability to incise this damaged DNA (Lambert et al. (1992) *Mutation Res.*, 273, 57-71). Together, these findings suggest that the DNA binding protein identified is involved in recognition and repair of DNA interstrand cross-links.**

## INTRODUCTION

DNA interstrand crosslinks have been shown to be repaired in mammalian cells (1-4). However, unlike bacterial cells, the precise mechanisms and proteins involved in the repair of this type of lesion are largely unknown. Critical to this repair process is the initial step which involves protein recognition of the damage followed by endonucleolytic incision of the DNA at or near the site of the lesion. Proteins which specifically recognize and bind

to DNA containing interstrand cross-links and which have been shown to play a role in the repair process have not been identified in mammalian cells. DNA binding proteins which recognize other types of DNA damage such as base modifications produced by either chemical or physical agents (5-15), bulky lesions (16-18), abasic sites (19,20), and mismatches in DNA (21), have been described in mammalian cells but whether these proteins play a role in the DNA repair processes is not clear, though it has been suggested by the results of several of the studies.

A number of different agents can produce interstrand cross-links in DNA, however, one which serves as an excellent model for studying such cross-links is psoralen plus long wavelength UVA light. Psoralen, in conjunction with UVA light, covalently binds to a pyrimidine base in DNA to form first a monoadduct and then a diadduct (interstrand cross-link) and conditions can be adjusted so that the majority of the adducts formed are interstrand cross-links (22-24). We have isolated a chromatin-associated DNA endonuclease complex from the nuclei of normal human cells which specifically recognizes and incises DNA containing interstrand cross-links induced by 8-methoxypsoralen (8-MOP) or 4,5',8-trimethylpsoralen (TMP) plus UVA light (25,26). Kinetic analysis indicates that this complex has increased affinity for (or a higher rate of) association with DNA damaged by psoralen plus UVA light compared with undamaged DNA (27). Its role in the initial damage recognition/incision step of the repair process has been confirmed by demonstration of its ability to correct the defect in repair of damage from psoralen plus UVA light in a repair deficient cell line xeroderma pigmentosum, complementation group A (XPA), when introduced into these cells in culture via electroporation (28).

We have recently examined this endonuclease complex in cells from individuals with another repair disorder, Fanconi anemia, complementation group A (FA-A). Cells from individuals with FA-A are extremely sensitive to DNA interstrand cross-linking agents and show decreased cell survival, increased chromosomal aberrations (29-32) and a reduced ability to repair DNA damage produced by these agents (33-36). We have shown that the DNA endonuclease complex, pI 4.6, is present in FA-A cells but that

\* To whom correspondence should be addressed

it has reduced levels of activity on DNA treated with 8-MOP plus UVA light compared to the normal complex (36). The nature of the repair defect is unclear and could lie in a defect in ability to recognize the damage and/or in an inability to incise DNA at sites of interstrand cross-links. Further evidence that a defect in this complex is related to the repair defect in FA-A cells is provided by our finding that the normal complex, when introduced into FA-A cells in culture via electroporation, is able to correct the defect in the ability of these cells to repair 8-MOP plus UVA damage to DNA (in preparation).

In the present study we describe the presence of a damage-recognition protein, associated with chromatin in the nuclei of normal human cells, which binds to DNA containing interstrand cross-links produced by TMP plus UVA light. Specificity of protein binding to DNA was determined using an electrophoretic gel mobility shift assay and a custom designed oligonucleotide which contained a hot spot for formation of interstrand cross-links induced by TMP plus UVA light. We also demonstrate that FA-A cells are defective in this damage recognition protein. The present data, combined with our previous findings, suggest that this DNA damage recognition protein is involved in the repair of DNA interstrand cross-links produced by TMP plus UVA light.

## MATERIALS AND METHODS

### Chromatin-associated protein extracts

Normal human lymphoblastoid cell lines (GM 1989 and GM 3299) (transformed with Epstein-Barr virus) were obtained from the Coriel Institute for Medical Research, Camden, NJ. The lymphoblastoid cell line (transformed with Epstein-Barr virus) obtained from a FA patient, complementation group A (HSC 72), was a gift from Dr Manuel Buchwald. The cells were grown in suspension culture in RPMI 1640 medium, supplemented with 12.5% fetal calf serum, and harvested under conditions of maximal proliferation as previously described (37). Cell cultures were routinely tested for mycoplasma (37).

Cell nuclei were isolated and the chromatin-associated proteins separated from the nucleoplasmic proteins in a series of steps as previously described (25,38). The chromatin-associated proteins were dialyzed into 50 mM potassium phosphate, pH 7.1, 1 mM B-mercaptoethanol, 1 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride (PMSF) and 40% ethylene glycol and passed through a CM sephadex column (25,38). Protein concentrations were determined by the BioRad protein assay (BioRad Laboratories).

### Oligonucleotide probe

We designed a DNA sequence which facilitates the study of damage-specific DNA binding and repair (Figure 1). This sequence contains hot spots for the formation of pyrimidine dimers, cisplatin alkylation, and psoralen cross-linking. The two 82-mer oligonucleotides used to assemble this duplex were synthesized by an Applied Biosystems DNA synthesizer, using the FC3 program (39), and purified from denaturing polyacrylamide gels. The oligonucleotides were hybridized and cloned into the plasmid pUC19 to replace the EcoRI-HindIII multicloning site to produce the plasmid pATY2701. When pATY2701 was restricted with BamHI (Boehringer Mannheim Inc.) and Asp718 (Asp718, Boehringer Mannheim Inc., cuts at the KpnI site to produce a 4 nucleotide 5' overhang), a 76-mer duplex is obtained in which the hot spots for DNA damage are located near the

middle of the fragment. The original EcoRI and HindIII sites of pUC19 are destroyed by the site fusions. Instead, they are placed internally in the fragment so that the ends of this fragment can be trimmed efficiently as needed to allow specific labelling at any of the four termini. By preparing the substrate from plasmid DNA instead of directly using the product of oligonucleotide synthesis, our substrate is free of the lesions known to be produced by chemical DNA synthesis (40,41). The pATY2701 is propagated in *Escherichia coli* HB 101 cells.

DNA was extracted from the *E. coli* via lysis by alkali (42) and purified by precipitation with polyethylene glycol (42). The 76-mer probe was cut from the plasmid by restriction with BamHI and Asp718. The probe was end-labeled with [ $\alpha$ - $^{32}$ P]dGTP (800 Ci/mmol, New England Nuclear) using DNA polymerase I large fragment (Bethesda Research Laboratories). Unincorporated label was removed by a Nucletrap push column (Stratagene) and the DNA was purified by passage through a Millipore Ultrafree-probind filter unit. The DNA was then ethanol precipitated, resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA and separated by polyacrylamide gel electrophoresis. The 76-mer probe was excised from the gel and eluted in a buffer containing 0.5 M ammonium acetate, pH 8.0, 1 mM EDTA.

### Reaction of psoralen with DNA

The radiolabeled probe and unlabeled competitor plasmid DNA pATY2701 were reacted in the dark with 4,5',8-trimethylpsoralen (TMP) (5  $\mu$ g/ml) (Sigma Chemical Co.) in 10 mM Tris-HCl, pH 7.6, 0.4 mM EDTA, 50 mM NaCl for 20 min at 20°C (43), and then irradiated with UVA light (principally 366 nm) using a General Electric F15T8/BLB lamp at 5–30 KJ/m<sup>2</sup> (10 W/m<sup>2</sup> for various periods of time). The DNA was extracted with chloroform twice to remove unbound TMP, precipitated with ethanol, washed twice with 80% ethanol, and dissolved in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Formation of DNA interstrand cross-links in the TMP plus UVA treated radiolabeled probe was determined by denaturing 8% polyacrylamide gel electrophoresis (44). Gels were exposed to X-ray film and the fraction of DNA containing interstrand cross-links was determined by scanning the films with an Imager (Appligene) and analyzing the data using the QuantiScan densitometry program (Biosoft).

### Treatment of DNA with UVC light

The radiolabeled probe was irradiated with 1200 J/m<sup>2</sup>, 3600 J/m<sup>2</sup>, and 7200 J/m<sup>2</sup> UVC (254 nm) light from a germicidal lamp (G15T8, American Ultraviolet Co.) at 20° Celsius.

### Gel mobility shift assay

The gel mobility shift assay utilized for testing for proteins binding to DNA containing interstrand cross-links was a modification of the method of Carey (45). [ $\alpha$ - $^{32}$ P]-probe (0.1–0.2 ng), either undamaged or treated with TMP plus UVA light, was incubated with the extract of chromatin-associated proteins (2–3  $\mu$ g) in a buffer containing 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT and 0.6  $\mu$ g of poly(dI-dC)·poly(dI-dC) (Boehringer Mannheim Inc), in a total volume of 30  $\mu$ l, for 30 min at 20°C. In separate experiments, prior to incubation with the DNA, extracts were additionally treated with 500  $\mu$ g/ml proteinase K (Sigma Chemical Co.) at 37°C for 15 minutes. The reaction mixture was loaded onto 5% polyacrylamide gels (acrylamide/bisacrylamide weight ratio, 80:1) prepared in a low ionic-strength buffer containing 10 mM sodium phosphate, pH

5.5, 2.5% glycerol, 0.14% ammonium persulfate and 0.028% TEMED. Gels were preelectrophoresed for at least 1.5 hr at 100 V and then electrophoresed at 200 V for approximately 3 hr in 10 mM sodium phosphate, pH 4.8, at room temperature with recirculation of the buffer. The gels were exposed to Kodak XAR-5 X-ray film with an intensifying screen at  $-80^{\circ}\text{C}$ .

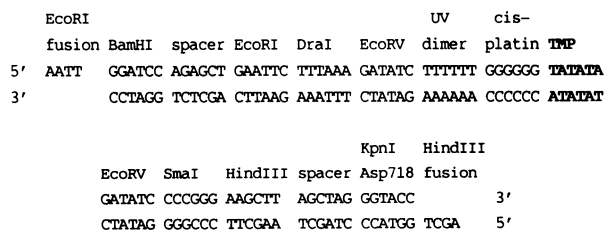
The gel mobility shift assay utilized to test for proteins binding to UVC irradiated DNA was carried out using a modification of the method of Carthew et al. (46). The UVC-irradiated or unirradiated [ $^{32}\text{P}$ ]-probe (0.1–0.2 ng) was incubated with the protein extract (3  $\mu\text{g}$ ) and 0.8  $\mu\text{g}$  poly(dI-dC)·poly(dI-dC) in 50 mM Tris-HCl, pH 7.6, 10 mM  $\text{MgCl}_2$ , 2 mM ATP for 30 minutes at  $20^{\circ}\text{C}$  in a total volume of 30  $\mu\text{l}$ . The binding reactions were resolved on a 4% polyacrylamide gel prepared and run in a low ionic strength buffer containing 7.6 mM Tris-HCl, pH 7.9, 3.3 mM sodium acetate, and 1 mM EDTA. Electrophoresis was carried out at 200 V at room temperature with recirculation of the buffer.

### Competition assays

Competition assays were carried out by incubating unlabeled competitor DNA with the chromatin protein extracts for 10 minutes at room temperature prior to addition of labeled probe. After addition of the probe, incubation was continued for an additional 20 minutes. The competitor was pATY2701 DNA (200 and 2000  $\mu\text{g}$ ) that was either undamaged or damaged with TMP (5  $\mu\text{g}/\text{ml}$ ) plus UVA light (20  $\text{KJ}/\text{m}^2$ ).

## RESULTS

A DNA substrate containing a hotspot for formation of DNA interstrand cross-links induced by psoralen plus UVA light was developed and utilized as a probe for identification of nuclear, chromatin-associated proteins, in human cells, which bind to DNA containing interstrand cross-links (Figure 1). Reaction conditions with TMP plus UVA light were used which have been shown to produce DNA interstrand cross-links (43). The extent of interstrand cross-linking in the treated oligonucleotide substrate was determined by denaturing gel electrophoresis. Figure 2 shows that, at a constant TMP concentration (5  $\mu\text{g}/\text{ml}$ ), as the dosage of UVA light increases from 0 to 20  $\text{KJ}/\text{m}^2$  the degree of interstrand cross-linking of the DNA increases. An increase is observed in both the number and intensity of the slower migrating DNA bands containing interstrand cross-links. The

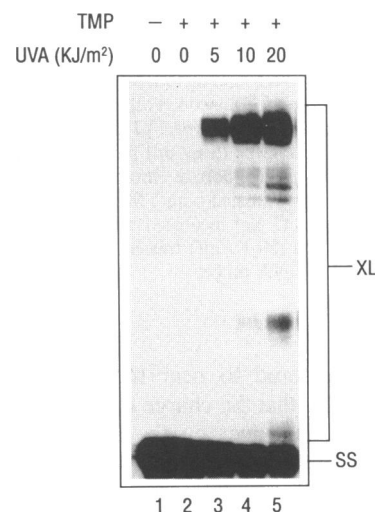


**Figure 1.** Sequence of the oligonucleotide duplex used to replace the EcoRI-HindIII multicloning site fragment of pUC19 to produce plasmid pATY2701. Restriction cutting of pATY2701 with BamHI and Asp718 generates a 76 base pair duplex which was used as a probe for detection of damage specific DNA binding proteins. A hot spot for psoralen cross-link formation is located in the site indicated by TMP. Other sites for the preferential formation of pyrimidine dimers and cis-platin adducts are indicated.

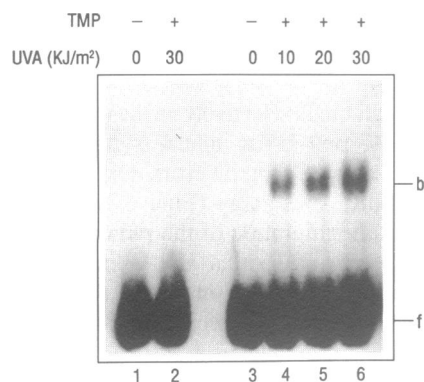
fraction of the DNA containing interstrand cross-links at UVA dosages of 0, 5, 10, and 20  $\text{KJ}/\text{m}^2$  was 0%, 50%, 61%, and 72%, respectively.

A protein which binds selectively to the oligonucleotide duplex modified by TMP plus UVA light was detected in the extracts of chromatin-associated proteins from normal human cell nuclei by the gel mobility shift assay (Figure 3). The DNA binding protein retarded the migration of the radiolabeled DNA, which was modified with TMP plus UVA light, through the gel (Figure 3, lanes 4–6). No mobility shift was seen with undamaged DNA probe (Figure 3, lane 3) or in the absence of protein extract (Figure 3, lanes 1 and 2). Non-specific protein binding was eliminated by addition of an excess of poly(dI-dC)·poly(dI-dC). As the degree of interstrand cross-linking of the DNA substrate increased with increasing dose of UVA light (from 10 to 30  $\text{KJ}/\text{m}^2$ ), the extent of protein binding to the DNA increased. This was demonstrated by a greater intensity of the retarded band (Figure 3, lanes 4–6). These results were obtained using a low ionic strength PAGE system. Protein binding to TMP cross-linked DNA was not observed when a high ionic strength gel system was used. Pretreatment of the extract with proteinase K before incubation with TMP cross-linked DNA eliminated the retarded band in the gel, showing that this band was due to protein bound to DNA (data not shown).

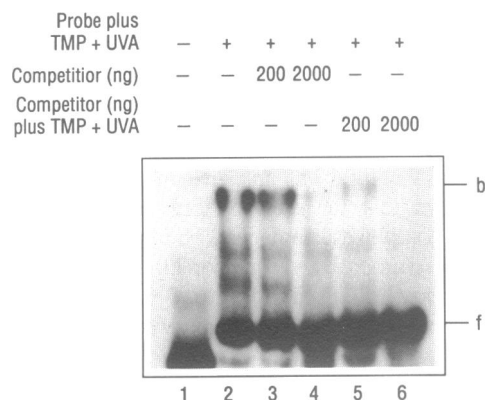
This retarded DNA band was not observed when the pH of the gel and the running buffer was between 6.5 and 8.5. Under these conditions the protein/DNA complex failed to resolve from free DNA in the gels (data not shown). We have previously shown that a human endonuclease complex which is specific for DNA containing interstrand cross-links induced by TMP or 8-MOP plus UVA light has an isoelectric point of 4.6. Carey (45) has shown that the electrophoretic mobility of specific protein/DNA complexes can depend on the pH of the gel system. For the *Escherichia coli trp* repressor/DNA complex, above the pI of the protein, little retardation of DNA was observed (45). These studies showed that retardation was enhanced as the pH



**Figure 2.** Electrophoretic resolution of the 76 base pair DNA oligonucleotide containing interstrand cross-links produced by TMP plus UVA light. DNA was treated with TMP (5  $\mu\text{g}/\text{ml}$ ) and varying dosages of UVA light and the extent of cross-linking was determined by denaturing 8% polyacrylamide gel electrophoresis. XL, cross-linked DNA; SS, single-stranded DNA containing monoadducts.



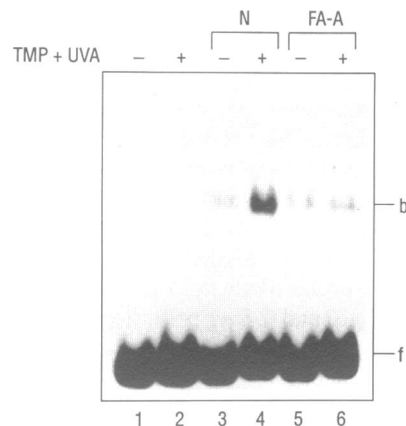
**Figure 3.** Identification of a damage specific DNA binding protein in normal human cells which binds to DNA containing TMP plus UVA light induced interstrand cross-links. Chromatin protein extracts (2  $\mu$ g) from normal human lymphoblastoid cells were incubated with [<sup>32</sup>P]-probe either untreated (lane 3) or treated with TMP (5  $\mu$ g/ml) plus increasing dosages of UVA light (lanes 4–6). Extent of protein binding correlates with the level of TMP + UVA induced DNA interstrand cross-link formation which increased with increasing UVA dose. The control lanes contained untreated probe (lane 1) and treated probe (lane 2) incubated with the extract buffer only. f, unbound free DNA probe; b, protein-bound DNA probe.



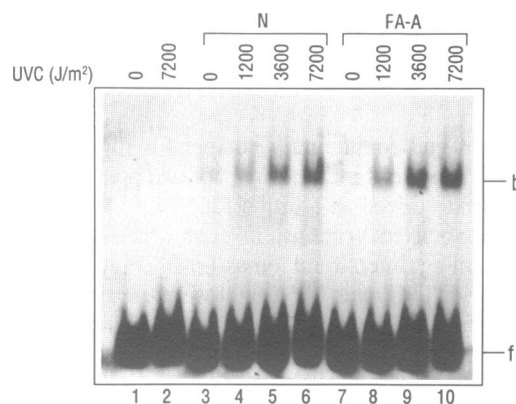
**Figure 4.** Specificity of protein binding to DNA containing TMP plus UVA light induced interstrand cross-links. Competition experiments were carried out in which increasing amounts of cold competitor (pATY2701 DNA) either undamaged (lanes 3 and 4) or damaged with TMP (5  $\mu$ g/ml) plus UVA light (20 KJ/m<sup>2</sup>) (lanes 5 and 6) were incubated with the normal chromatin protein extracts (2  $\mu$ g) prior to addition of labeled probe treated with TMP plus UVA light. Control lanes contained protein extracts (2  $\mu$ g) incubated with undamaged (lane 1) or TMP (5  $\mu$ g/ml) plus UVA light (20 KJ/m<sup>2</sup>) treated (lane 2) probe. f, unbound free DNA; b, protein-bound DNA probe.

of the gel was lowered to near the isoelectric point of trp repressor, indicating that the charge on a protein is an important factor that exerts an effect on the mobility of protein/DNA complexes (45). We likewise found that by lowering the pH of the gel system to 5.5, which is near the isoelectric point of the endonuclease complex specific for DNA interstrand cross-links, the separation of the free DNA and the protein bound DNA could be observed.

Competitive binding experiments were performed to assess the specificity of the binding protein for the TMP plus UVA light damaged DNA. As shown in Figure 4, protein binding to the radiolabeled damaged DNA was competed by addition of unlabeled pATY2701 DNA, modified by TMP plus UVA light,



**Figure 5.** Lack of protein binding in FA-A chromatin protein extracts to TMP plus UVA light damaged probe. FA-A chromatin protein extracts did not show any specific binding to DNA containing TMP (5  $\mu$ g/ml) plus UVA light (20 KJ/m<sup>2</sup>) induced interstrand cross-links (lane 6) compared to undamaged DNA (lane 5). This is in contrast to the damage recognition protein in normal cells which binds to the damaged probe (lane 4) and has little affinity for undamaged DNA (lane 3). Control lanes contained undamaged probe (lane 1) and TMP plus UVA light treated probe (lane 2) incubated with the extract buffer only. Symbols are as in Figure 4.



**Figure 6.** Damage-recognition proteins in normal human and FA-A chromatin protein extracts which bind to UVC irradiated DNA. Labeled probe was irradiated with UVC light (1200, 3600, 7200 J/m<sup>2</sup>) and incubated with either normal human or FA-A extracts. Both normal (lanes 3–6) and FA-A (lanes 7–10) extracts contained a protein which bound to the UVC irradiated probe. Control lanes contained undamaged (lane 1) and UVC irradiated (7200 J/m<sup>2</sup>) (lane 2) probe incubated with the extract buffer only. Symbols are as in Figure 4.

in a 50 and 500 fold molar excess (Figure 4, lanes 5 and 6). By contrast, undamaged pATY2701 DNA did not effectively compete with labeled modified DNA for binding of the protein (Figure 4, lanes 3 and 4). This indicates that the protein has binding specificity for the cross-linked DNA. The intermediate bands observed migrating in front of the protein bound DNA band could be due to local variations in the pH of the gel, resulting in some of the protein/DNA complex migrating faster than the rest.

When extracts of chromatin-associated proteins from FA-A cell nuclei were examined with the gel shift assay for protein binding to DNA cross-linked with TMP plus UVA light, no increase in protein binding to the damaged DNA compared to undamaged

DNA was observed (Figure 5, lanes 5 and 6). Varying the range of NaCl concentrations in the gel shift assay reaction mixture, from 0 mM to 100 mM, had no effect on these results (data not shown). The protein, which from normal human cell chromatin binds to this cross-linked DNA, thus appears to be absent or defective in FA-A cells. These same results were obtained when several different FA-A extract preparations were tested. The FA-A extracts were tested for ability to bind to UVC irradiated DNA. Incubation of the FA-A extracts with UVC irradiated DNA produced a band of reduced electrophoretic mobility (Figure 6, lanes 8–10) just as did the normal human extracts (Figure 6, lanes 4–6). These results demonstrate that the FA-A extracts were active. For both the normal and the FA-A extracts, the extent of protein binding increased as the UVC dose increased. The presence of a protein in FA-A extracts which binds to UVC irradiated DNA correlates with the ability of FA-A cells to repair UVC light damage to DNA (33,34,47).

## DISCUSSION

We have identified a protein in a chromatin non-histone protein extract, obtained from the nuclei of normal human lymphoblastoid cells, which has high affinity for DNA containing interstrand cross-links produced by TMP plus UVA light. Specificity of binding to cross-linked DNA was demonstrated both by a positive correlation between level of cross-link formation in DNA and extent of binding of the protein, and by competition experiments in which DNA treated with TMP plus UVA light, but not undamaged DNA, was an effective competitor for the binding protein. By contrast, this binding protein was not detectable in the same protein extract obtained from FA-A cells.

We have previously shown that this chromatin protein extract, obtained from normal human lymphoblastoid cells, contains a DNA endonuclease complex, pI 4.6, which specifically recognizes and incises DNA containing interstrand cross-links produced by TMP or 8-MOP plus UVA light (25–27). The damage recognition and DNA incision capabilities of this complex may be associated with the same protein or with different proteins. This complex also incises a shuttle vector containing a site-directed nitrogen mustard interstrand cross-link (obtained from Dr Edward Loechler) (unpublished observation). Associated with this endonuclease complex is a protein which interacts with DNA treated with psoralen plus UVA light, but not undamaged DNA, so as to increase activity of the endonuclease several fold when the DNA is in the form of nucleosomes (26,27). This protein is normal in the corresponding endonuclease complex derived from FA-A cells (36). In FA-A cells, however, the endonuclease associated with this complex shows reduced ability to incise DNA containing interstrand cross-links produced by TMP or 8-MOP plus UVA, with only about 25% of normal activity (36). This reduced activity could be due to reduced damage recognition capabilities or to reduced endonuclease activity. That the protein complex, pI 4.6, is involved in the repair process is further shown by our finding that introduction, by electroporation, of the normal, but not the FA-A endonuclease complex, into FA-A cells in culture restores their levels of UDS to normal levels following exposure to 8-MOP plus UVA light (manuscript in preparation).

Kinetic analysis of the normal endonuclease complex on psoralen plus UVA light treated DNA suggests that its activity on this DNA, compared to undamaged DNA, depends on selectively increased binding (27). The  $K_m$  of the normal complex on 8-MOP plus UVA light treated DNA is significantly lower than that on undamaged DNA, whereas the  $K_{cat}$  remains

similar, indicating that the association constant and/or the rate of association between the enzyme complex and the psoralen plus UVA light treated DNA is increased on damaged compared to undamaged DNA (27). Kinetic analysis of the activity of the FA-A endonuclease complex reveals the  $K_m$  of the FA-A complex on damaged DNA is also decreased compared to that on undamaged DNA (36). However, the decrease in  $K_m$  of the FA-A complex on damaged versus undamaged DNA is much less than that of the normal complex (i.e., the  $K_m$  of the FA-A complex on damaged DNA is much greater than that of the normal complex), while the  $K_{cats}$  remain similar, again indicating that the FA-A complex either associates more slowly with this damaged DNA substrate, or has a lower binding affinity, than the normal complex (36). This also is in agreement with the present study, since we were unable to detect a binding protein specific for TMP plus UVA light treated DNA in the FA-A extract analogous to the one we found in the normal human cell extract and this may be related to a deficiency in a 8-MOP plus UVA damage recognition protein in FA-A cells.

Damage recognition is an important component of the initial steps in the DNA repair process. A number of damage recognition proteins have been reported which bind to types of damaged DNA other than those containing psoralen plus UVA light induced interstrand cross-links (5–20). None of these proteins has been found to have DNA endonucleolytic activity but many have been hypothesized to play a role in the repair process. The endonuclease complex, pI 4.6, which we have isolated, and which has specificity for DNA interstrand cross-links, has both damage recognition and DNA incision capabilities (25–27). Whether the damage recognition component of this complex is associated with a specific DNA binding protein or with the endonuclease, or with both, is under investigation. The normal human DNA binding protein identified in the present study may be a component of this complex. The finding that this binding protein could not be detected in the FA-A chromatin protein extracts, yet the demonstration of significantly reduced but not totally absent UDS in FA-A cells in response to 8-MOP or TMP plus UVA light and to reduced incisibility, by the endonuclease complex, pI 4.6, of lesions produced by these agents (36) could be due to several different causes. The binding protein could be present but binding to DNA not detected due to modifications of this protein. It is also possible that this damage-recognition protein plays a role in recruiting or in aiding the physical delivery of the endonuclease complex to the site of damage in the DNA, thus increasing the efficiency of excision repair as has been suggested in other systems (6,48). A defect in this protein would thus not be likely to totally abolish the repair process but would be expected to reduce it, consistent with our findings. The defect which we have found in this DNA binding protein in FA-A cells, however, may or may not be the only deficiency responsible for defective DNA repair in FA-A. It is possible that more than one alteration is involved which could possibly be caused by the simultaneous unopposed expression of defective alleles at more than one locus. Such involvement of more than one locus in certain DNA repair-deficient inherited diseases is, in fact, a specific prediction of the co-recessive inheritance hypothesis which we have proposed for the etiopathogenesis of these disorders (49,50).

Whatever the mechanism, it would appear that the lack of binding of a protein in the FA-A chromatin non-histone protein extracts to DNA damaged by 8-MOP plus UVA light is related to the defective repair of DNA interstrand cross-links observed in FA-A cells in culture. Thus the damage-recognition protein

identified in the present study, in normal human cells, may be involved in mediating the efficient repair of psoralen plus UVA light induced interstrand cross-links in DNA.

## ACKNOWLEDGEMENTS

We thank Dr W.Clark Lambert for critical reading of the manuscript and Mr Robert Lockwood for culturing of the human cell lines. These studies were supported, in part, by grants from the N.I.H. to M.W.L. (RO1 ES05940) and to the Fox Chase Cancer Center (CA06927 and RR05539), from the N.S.F. to A.Y. (DMB88-02091 and DIR-8812108), an appropriation from the Commonwealth of Pennsylvania, and a grant from the Glennede Trust to the Fox Chase Cancer Center.

## REFERENCES

1. Baden, H.P., Parrington, J.M., Delhanty, J.M. and Pathak, M.A. (1972), *Biochem. Biophys. Acta.*, 262, 247-255.
2. Kaye, J., Smith, C.A. and Hanawalt, P.C. (1980) *Cancer Res.*, 40, 696-702.
3. Bredberg, A., Lambert, B. and Soderhall, S. (1982) *Mutation Res.*, 93, 221-234.
4. Gruenert, D.C. and Cleaver, J.E. (1983) *Cancer Res.*, 45, 5399-5404.
5. Rydberg, B., Dosanjh, M.K. and Singer, B. (1991) *Proc. Natl. Acad. Sci. U.S.A.*, 88, 6839-6842.
6. Treiber, D.K., Chen, Z. and Essigmann, J.M. (1992) *Nucleic Acids Res.*, 20, 5805-5810.
7. Hirschfeld, S., Levine, A.S., Ozato, K. and Protic, M. (1990) *Mol. Cell. Biol.*, 10, 2041-2048.
8. Chu, G. and Chang, E. (1988) *Science*, 242, 564-567.
9. Chao, C.C.-K. (1992) *Mutation Res.*, 281, 105-113.
10. Feldberg, R.S. (1980) *Nucleic Acids Res.*, 8, 1133-1143.
11. Teale, B., Singh, S., Khanna, K.K., Findik, D. and Lavin, M.F. (1992) *J. Biol. Chem.*, 267, 10295-10301.
12. Donahue, B.A., Augot, M., Bellon, S.F., Treiber, D.K., Toney, J.H., Lippard, S.J. and Essigmann, J.M. (1990) *Biochemistry*, 29, 5872-5880.
13. Chao, C.C.-K., Huang, S.-L., Lee, L.-Y., and Lin-Chao, S. (1991) *Biochem. J.*, 277, 875-878.
14. Pil, P.M. and Lippard, S.J. (1992) *Science*, 256, 234-237.
15. Hughes, E.N., Engelsberg, B.N. and Billings, P.C. (1992) *J. Biol. Chem.*, 267, 13520-13527.
16. Moranelli, F. and Lieberman, M.W. (1980) *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3201-3205.
17. Tsang, S.S. and Kuhnlein, U. (1982) *Biochim. Biophys. Acta.*, 697, 202-212.
18. Zytkovicz, T.H. (1982) *Cancer Res.*, 42, 4387-4393.
19. Lenz, J., Okenquist, S.A., LoSardo, J.E., Hamilton, K.K. and Doetsch, P.W. (1990) *Proc. Natl. Acad. Sci., U.S.A.*, 87, 3396-3400.
20. Hasegawa, S.L., Doetsch, P.W., Hamilton, K.K., Martin, A.M., Okenquist, S.A., Lenz, J. and Boss, J.M. (1991) *Nucleic Acids Res.*, 19, 4915-4920.
21. Jiricny, J., Hughes, M., Corman, N. and Rudkin, B.B. (1988) *Proc. Natl. Acad. Sci. U.S.A.*, 85, 8860-8864.
22. Ben-Hur, E. and Song, P.-S. (1984) *Adv. Radiat. Biol.*, 11, 131-177.
23. Cimino, G.D., Gamper, H.B., Isaacs, S.T. and Hearst, J.E. (1985) *Ann. Rev. Biochem.*, 54, 1151-1193.
24. Vigny, P., Gaboriau, F., Voituriez, L. and Cadets, J. (1985) *Biochimie*, 67, 317-325.
25. Lambert, M.W., Fenkart, D. and Clark, M. (1988) *Mutation Res.*, 193, 65-73.
26. Parrish, D.D., and Lambert, M.W. (1990) *Mutation Res.*, 235, 65-80.
27. Parrish, D.D., Lambert, W.C. and Lambert, M.W. (1992) *Mutation Res.*, 273, 157-170.
28. Tsongalis, G.J., Lambert, W.C. and Lambert, M.W. (1990) *Carcinogenesis*, 11, 499-503.
29. Wunder, E.E. and Fleischer-Reischman B. (1983) *Human Genetics*, 64, 167-172.
30. Moustacchi, E., Papadopoulo, D., Diatloff-Zito, C., and Buchwald, M. (1987) *Human Genet.*, 75, 45-47.
31. Digweed, M., Zakrzewski-Ludcke, S., and Sperling, K. (1988) *Human Genetics*, 78, 51-54.
32. Auerbach, A.D., Rogatko, A., and Schroeder-Kurth, T.M. (1989) *Blood*, 73, 391-396.
33. Gruenert, D.C. and Cleaver, J.E. (1985) *Cancer Res.*, 45, 5399-5404.
34. Plooy, A.C.M., van Dijk, M., Berends, F., and Lohman, P.H.M. (1985) *Cancer Res.*, 45, 4178-4184.
35. Averbeck, D., Papadopoulo, D., and Moustacchi, E. (1988) *Cancer Res.*, 48, 2015-2020.
36. Lambert, M.W., Tsongalis, G.J., Lambert, W.C., Hang, B. and Parrish, D.D. (1992) *Mutation Res.*, 273, 57-71.
37. Okorodudu, A.O., Lambert, W.C. and Lambert, M.W. (1982) *Biochem. Biophys. Res. Commun.*, 108, 576-584.
38. Lambert, M.W., Lee, D.E., Okorodudu, A.O., and Lambert, W.C. (1982) *Biochim. Biophys. Acta*, 69, 192-203.
39. Yeung, A.T. and Miller, C.G. (1990) *Anal. Biochem.*, 187, 66-75. 4539-4554.
41. Van Houten, B., Gamper, H., Sancar, A. and Hearst, J.E. (1987) *J. Biol. Chem.*, 262, 13180-13187.
42. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor, pp. 1.33-1.41.
43. Jones, B.K. and Yeung, A.T. (1990) *J. Biol. Chem.*, 265, 3489-3496.
44. Maxam, A.M. and Gilbert, W. (1980) *Methods Enzymol.*, 65, 499-560.
45. Arey, J. (1988) *Proc. Natl. Acad. Sci., U.S.A.*, 85, 975-979.
46. Carthew, R.W., Chodosh, L. and Sharp, P. (1985) *Cell*, 43, 439-448.
47. Klocker, H., Burtscher, H.J., Auer, B., Hirsch-Kauffmann, M. and Schweiger, M. (1985) *Europ. J. Cell Biol.*, 37, 240-242.
48. Sancar, A., Franklin, K.A. and Sancar, G.B. (1984) *Proc. Natl. Acad. Sci. U.S.A.*, 81, 7397-7401.
49. Lambert, W.C. and Lambert, M.W. (1985) *Mutation Res.*, 145, 227-234.
50. Lambert, W.C. and Lambert, M.W. (1992) *Mutation Res.*, 273, 179-192.