Ultraviolet radiation-induced DNA damage in promoter elements inhibits gene expression

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Abstract **Repair of DNA damage in gene promoters is slower than in actively transcribed genes. Persistent damage in gene promoters though transient can have significant biological effects on regulated gene expression. In this study we investigated the effect of ultraviolet radiation on gene promoter-associated functions when DNA damage is located within and outside transcription factor binding sites. Our results show that both cyclobutane pyrimidine dimers and (6-4) photoproducts inhibit DNA–protein interaction, in vitro transcript production and transactivation of reporter genes. The biological significance of transient DNA damage as a mechanism in carcinogenesis is discussed.**

Key words: Ultraviolet radiation; DNA damage; Gene expression

1. Introduction

Resolution of the unstable electronic configuration by excited adjacent pyrimidines following absorption of ultraviolet (UV) photons produces cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone dimers [(6-4) PD] as shown in Fig. 1 [1]. An important consequence of damage to the DNA template is that the vital process of transcription becomes hampered. Therefore mammalian cells take special precautions and prioritize elimination of DNA injury from active genes. However, DNA repair is not uniform throughout the genome. The faster, strand-directed sub-pathway of nucleotide excision repair designated transcription-coupled repair preferentially repairs the active genome compartment. The genome overall including gene promoters is repaired more slowly [2–5].

Eukaryotic gene regulation is governed by multiple DNA sequences that are recognized by specific transcription factors that function to activate or repress transcription by binding to regulatory elements in the promoter. Numerous kinds of DNA damage have been tested for DNA–protein interactions and found to have differential effects. For example, using a single site-specific 8-hydroxyguanine modification in the binding sites of AP-1 and Sp1, we have shown that transcription factor binding is inhibited in vitro. UV photoproducts such as CPD and (6-4) PD also inhibit binding of the sequence-specific transcription factors E2F, NF-Y, AP-1, and p53. O^6-Methylguanine, nitrogen mustard and quinacrine mustard modifications have all been found to inhibit the binding of transcription factors to DNA [6–10]. On the other hand, the presence of bulky benzo[*a*]pyrene diol epoxide (BPDE) adducts in a TATA promoter sequence has been shown to enhance binding of the TATA binding protein [11]. Further, DNA-damaging agents such as cisplatin and BPDE adducts have been shown to titrate transcription factors from their binding site [12]. However, the effect of changes in DNA–protein interaction in response to DNA damage on gene expression remains largely unexplored.

In the present study we tested the hypothesis that UV-induced DNA damage not only in the transcription factor binding sites, but anywhere in the promoter region has the capacity to inhibit gene expression. Our results underscore the importance of DNA damage in gene promoters to inhibit normal functions associated with the promoter for effective gene regulation. Since deregulation of gene expression plays an important role in neoplastic transformation our observations have great implications for the persistence of DNA damage in gene promoters in the carcinogenic process.

2. Materials and methods

2.1. Materials

Schneider's *Drosophila* line 2 (SL2) was purchased from ATCC (CRL-1963) and the SL2 cell culture medium was from Gibco BRL (Invitrogen, Rockville, MD, USA). Radioisotopes, [α-^{32}P]rGTP and [γ-^{32}P]ATP were purchased from NEN (Perkin Elmer Life Sciences, Boston, MA, USA). The luciferase assay kit and HeLa in vitro transcription kit were from Promega (Madison, WI, USA). Restriction and modifying enzymes were purchased from New England Biolabs (Beverly, MA, USA). *Escherichia coli* photolyase was from Trevigen (Gaithersburg, MD, USA) and T4 endonuclease V was from Pharmingen (San Diego, CA, USA). All other chemicals were molecular biology grade from Sigma Aldrich (St. Louis, MO, USA). pGL3 promoter vector was purchased from Promega and pNF-κB-luc reporter was from Clontech (Palo Alto, CA, USA). The p50 and p65 expression plasmids were kind gifts from Dr. Nancy Rice, at NIH. The source of UV light was six narrow-band germicidal lamps (Philips Sterilamp G8T5) that predominantly emit 254-nm light. The fluence rate was measured with an IL-1400A radiometer/photometer coupled to a 254-nm UV detector (International Light, Newburyport, MA, USA).

2.2. Cell culture

Drosophila SL2 cells were maintained in Schneider's *Drosophila*

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media supplemented with 10% heat-inactivated serum at 30°C as described [13].

2.3. Substrates for electrophoretic mobility shift assay (EMSA)

Consensus double-stranded oligonucleotide (5′-TGT GCA **GGG GAC TTT CCC** ACG C-3′) containing the binding site for transcription factor NF-κB (shown in bold with dipyrimidines underlined), was synthesized at Sigma-Genosys Biotechnology (The Woodlands, TX, USA). The immunoprecipitation method used to produce oligonucleotide substrates enriched for CPD and (6-4) PD was as described previously [14]. Briefly, consensus NF-κB oligonucleotides were irradiated either with 1 and 2 kJ/m^2 or 10 and 20 kJ/m^2 254-nm UV light and incubated with an excess of antiserum specific for CPD or (6-4) PD respectively for 3 h at 37°C and overnight at 4°C. The immune complex was precipitated with goat anti-rabbit IgG and carrier normal rabbit serum at 4°C, collected by centrifugation (3800 rpm), digested with 0.3 mg/ml proteinase K to release the damage-containing oligonucleotide. After organic extractions and precipitation complementary strands were annealed and duplex oligonucleotides containing specific types of DNA damage were obtained. The substrates were gel-purified prior to use in gel shift assays.

2.4. Preparation of pGL3 promoter vector containing UV-induced DNA damage

The pGL3 promoter vector construct has an SV40 promoter, which was digested with *Hin*dIII and *Sma*I to release a 217-bp fragment. The sequence of the promoter with the dipyrimidine sites underlined was as follows: 5′-TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG-TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAGAGGCCGAGGC CGCCTCGGCC TCTGAGCTA-T TCCAGAAGTA GTGAGGAGGC TTTTTTGGAGGCCTAGG-CTT TTGCAAAAAG CTT-3′. After gel purification the DNA was eluted from the gel with a Millipore gel elution kit. The precipitated promoter fragment was either left unirradiated or irradiated with 2.5, 5, 7.5 and 10 kJ/m^2 254-nm UV. After irradiation each of the DNA fragments was ligated to the pGL3 vector backbone using T4 DNA ligase. DNA damage induced by UV irradiation was verified by T4 endonuclease V digestion. Further radioimmunoassay was performed with antibodies specific for CPD and (6-4) PD to determine the number of CPD and (6-4) PD in each of the fragment as described elsewhere [15].

2.5. Preparation of UV-damaged NF-κB-luciferase reporter vector

The NF-κB-luc reporter vector contains four tandem copies of the NF-κB binding site, GGG AAT TTC C (dipyrimidines underlined) fused to a TATA-like promoter. The plasmid was digested with *Kpn*I and *Bgl*II to release a 62-bp fragment. The fragment was gel-purified and UV-irradiated as described for the pGL3 promoter vector. The irradiated fragments were ligated to the pNF-κB-luc vector backbone with T4 DNA ligase. The presence of UV damage and number of CPD and (6-4) PD induced was confirmed by T4 endonuclease V digestion and radioimmunoassay respectively.

2.6. Electrophoretic mobility shift assay

Gel mobility shift assays were performed as described elsewhere [16]. Briefly, double-stranded NF-κB oligonucleotide (unirradiated or irradiated as described above) was end-labelled with [γ-^{32}P]ATP (specific activity 3000 Ci/mol). Binding reactions consisted of 1 gel shift unit of recombinant p50 and 0.2 ng radiolabeled oligonucleotide in a reaction buffer containing 20 mM Tris (pH 8.0), 50 mM HEPES pH 7.9, 0.2 mM KCl, 2.5 mM EDTA, 25 mM $MgCl_2$, 5 mM dithiothreitol (DTT) and 60% glycerol for 20 min at room temperature. The bound products were resolved on a 5% native polyacrylamide gel. The gel was dried, subjected to autoradiography and quantified with the ScionImage software (Scion, Frederick, MD, USA). The gel shift assay was repeated three times.

2.7. In vitro transcription assay

UV-irradiated pGL3 promoter vector was linearized with *Nar*I and used as substrate in the in vitro transcription assay. The assay was performed in accordance with the instructions of Promega. Each 25 μl reaction contained 150 ng UV-irradiated or unirradiated DNA in 1×HeLa nuclear buffer (20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 5 mM DTT and 20% glycerol), 8 mM $MgCl_2$, 1 mM each of the ribonucleotides (rATP, rCTP and rUTP), 40 μM rGTP and 10 μCi [α-^{32}P]rGTP (3000 Ci/mol, 10 mCi/ml). The reaction was initiated with 8 units of HeLa nuclear extract, incubated at 30°C for 60 min, and terminated with 0.3 M Tris–HCl (pH 7.4), 0.3 M sodium acetate, 0.5% sodium dodecyl sulfate, 2 mM EDTA, 3 mg/ml tRNA. Products were extracted with organic solvents and precipitated. The pellet was resuspended in loading dye, heated for 10 min at 90°C and resolved by electrophoresis on a 10% denaturing polyacrylamide gel. A 100-bp marker labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase was used as molecular weight marker. The gel was dried after electrophoresis and subjected to autoradiography. Quantification of scanned gels was carried out with the ScionImage software. The experiment was repeated at least four times with two different plasmid preparations.

2.8. Photoreactivation with E. coli photolyase

Approximately 250 ng of each of the irradiated plasmids was incubated with 5 U of *E. coli* photolyase (Trevigen) in the reaction buffer containing 20 mM Tris–HCl (pH 7.8), 1 mM EDTA, 1 mM DTT, 50 mM NaCl and photoreactivated under 365-nm light for 1 h at 37°C. Supercoiled and relaxed forms of the plasmid were resolved by 1% agarose gel electrophoresis and detected by ethidium bromide staining. Photoreactivated plasmid was used in the in vitro transcription assay.

2.9. Transfection and luciferase assay

We validated the results of the in vitro transcription assay by performing co-transfection assays in *Drosophila* cells using NF-κB as a model transcription factor. NF-κB is an important transcription factor that controls transcription of a large number of genes involved in various cell functions. In unstimulated cells NF-κB is bound to inhibitory IκB proteins that sequester NF-κB in its inactive form in the cytoplasm. Stimulation by agents including UV, cytokines, and growth factors leads to phosphorylation, subsequent ubiquitination and degradation of IκB. This results in the translocation of NF-κB to the nucleus and transcriptional activation of NF-κB-dependent genes. Mammalian cells contain different subunits of NF-κB but the p50/p65 heterodimer is considered to be the most important [17].

The transfection assay was a modification of the method of Kumar et al. [13]. *Drosophila* Schneider cells were plated at 5×10^5 per well in six-well plates 24 h before transfection. Cells were set up in triplicate for every dose and each experiment was repeated five times with two different plasmid preparations. 4 μg of each of the damaged or undamaged reporter plasmids was co-precipitated overnight with 1 μg each of the expression plasmids p65 and p50, separately and in combination. DNA was dissolved in 2.5 M $CaCl_2$ and HEBS buffer (pH 7). After 30 min incubation at room temperature, the mixture was added to each well so that each well received 1 μg of reporter and 250 ng of expression plasmid. Forty-eight hours after transfection, cells were harvested and extracts were made in a buffer containing 25 mM Tris-phosphate (pH 7.8), 2 mM DTT, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10% glycerol, 1% Triton X-100, 1.25 mg/ml lysozyme and 2.5 mg/ml bovine serum albumin. After a couple of freeze–thaw cycles, debris was separated by centrifugation. Protein was estimated in the supernatant with the Coomassie Plus Protein assay reagent from Pierce according to the manufacturer's instructions. Equal amounts of protein (0.1 μg) were used to measure luciferase activity with the Luciferase Assay System (Promega). Triplicate measurements were obtained for each sample using a luminometer (Turner Designs, Sunnyvale, CA, USA).

3. Results

3.1. UV-induced DNA damage in the NF-κB consensus binding site inhibits DNA–protein interaction

We used EMSA to test the ability of recombinant p50 to bind the consensus NF-κB binding site with specific types of UV-induced DNA damage. As shown in Fig. 2, oligonucleotide substrates containing no damage produced a single bound complex (lane 1). However, binding to the substrate containing CPD (lanes 2 and 3) decreased by about 45% and 70% when irradiated with 1 and 2 kJ/m^2 UV respectively. Because

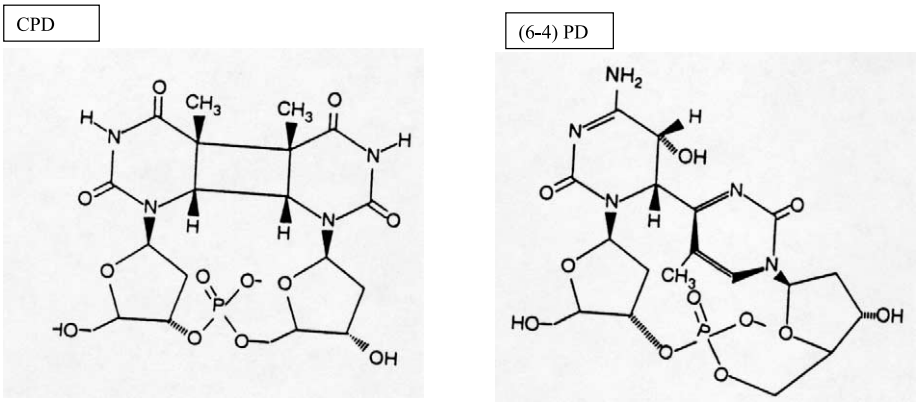

Fig. 1. Structure of UV-induced photoproducts: *cis-syn* cyclobutane thymine–thymine homodimer (CPD), and pyrimidine (6-4) pyrimidone 5′-cytosine–thymine-3′ heterodimer.

(6-4) PD are induced at a lower frequency than CPD we used a 10-fold higher UV dose to produce the (6-4) PD substrate for the EMSA. Fig. 2 (lanes 4 and 5) also shows that the (6-4) PD had a greater inhibitory influence on DNA–protein interaction with about 60% and 90% inhibition at 10 and 20 kJ/m^2 respectively over control binding. Substrates containing the Dewar photoproduct, which is a photoisomer of (6-4) PD, were found to exhibit greater than 95% inhibition in p50 binding at 10 kJ/m^2 (data not shown).

3.2. *DNA damage in the promoter inhibits in vitro transcription from the pGL3 promoter*

In order to test the ability of UV-damaged promoter fragments to produce transcripts in vitro, the excised SV40 promoter region from the pGL3 promoter vector was irradiated with increasing doses of UV (2.5, 5, 7.5 and 10 kJ/m^2). Irradiated and unirradiated control fragments were ligated back to the pGL3 vector prior to being used for in vitro transcription assay. As shown in Fig. 3A, the number of CPD increased in an UV dose-dependent manner. Although a dose-dependent increase in (6-4) PD was also observed, it was much lower compared to CPD. The number of CPD induced at 10 kJ/m^2 was approximately three-fold higher than (6-4) PD.

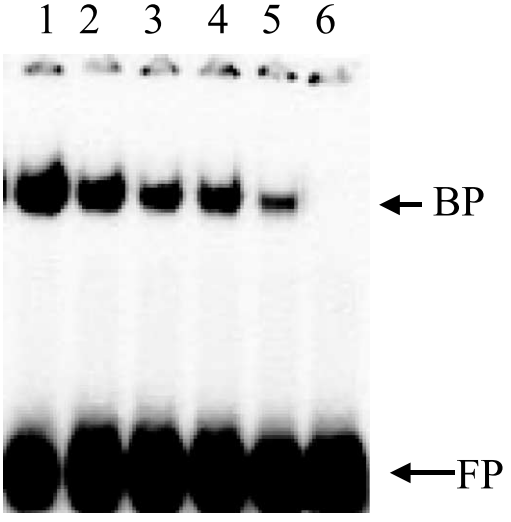

Fig. 2. Effect of UV-induced DNA damage on DNA–protein interaction. Oligonucleotides used as probes were either unirradiated or irradiated with UV and purified with CPD- and (6-4) PD-specific antibodies. EMSAs were performed as described in Section 2. Lane 1, binding to undamaged NF-κB probe. Lanes 2 and 3, NF-κB–CPD probe irradiated with 1 and 2 kJ/m^2 UV respectively. Lanes 4 and 5, NF-κB–(6-4) PD probe irradiated with 10 and 20 kJ/m^2 UV respectively. Lane 6, free probe with no protein. FP: free probe; BP: bound probe.

The pGL3 promoter vector containing increasing UV-induced DNA damage in the promoter fragment was used as substrate for in vitro transcription. Resolution of the in vitro transcripts shown in Fig. 3B indicates the production of an approximately 180-bp fragment from the undamaged substrate. At 2.5 kJ/m^2 the amount of full-length transcript produced decreased $>75\%$. At 5 kJ/m^2 we observed $>98\%$ inhibition of transcript production. We repeated this experiment four times and consistently found that increasing the UV dose beyond 5 kJ/m^2 did not change the inhibitory effect on transcription. To validate that the inhibition was due to the presence of CPD, we photoreactivated the promoter fragment with *E. coli* photolyase before ligation. We used T4 endonuclease V to check for remaining CPD. Resolution of the photoreactivated and unirradiated control by gel electrophoresis showed no changes in mobility (data not shown). This confirmed that the CPD were repaired. We used these photoreactivated substrates for in vitro transcription assays.

As shown in Fig. 3C, the photoreactivated substrates produced full-length transcripts in in vitro transcription assay at approximately 92% of the unirradiated control substrate at the highest dose of UV used. We attribute the low level of inhibition observed in Fig. 3C to the fact that (6-4) PD were still present in the substrates since photoreactivation with *E. coli* photolyase does not repair the (6-4) PD type of damage. These results taken together demonstrate that the presence of UV photoproducts in the pGL3 promoter region interferes with the production of transcripts in vitro and the observed inhibition is mainly attributed to CPD which were the major type of damage at the doses tested.

3.3. *Effect of DNA damage on transactivation of NF-κB-luciferase gene expression*

To test the biological significance of the in vitro observations we performed co-transfection assays in *Drosophila* SL2 cells using NF-κB as a model transcription factor. *Drosophila* cells were chosen to eliminate interference by the endogenous transcription factor in this assay [13,18]. We used both the p50 and p65 expression vectors separately and in combination

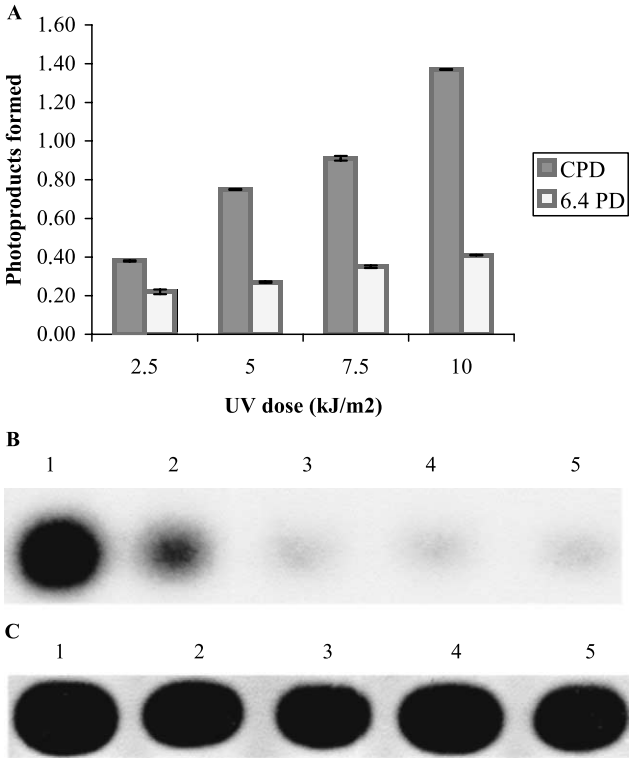

Fig. 3. Effect of DNA damage on in vitro transcription. A: Assessment of DNA damage in the pGL3 plasmid. *Hin*dIII-*Sma*I-digested pGL3 was isolated and irradiated with different doses of UV. The fragment was ligated to the vector backbone. Quantitative measurements of UV photoproducts in the pGL3 plasmid using radioimmunoassay for CPD and (6-4) PD are shown. Values are expressed as photoproducts/Mb DNA. B: In vitro transcription from substrates containing UV-induced DNA damage. After substrates were prepared in vitro transcription was carried out and products were resolved on a 10% polyacrylamide gel. Lane 1, product from undamaged pGL3 promoter vector. Lanes 2–5 were pGL3 promoter vectors irradiated with 2.5, 5, 7.5, and 10 kJ/m^2 UV, respectively. C: In vitro transcription after photoreactivation. The UV-irradiated substrates were photoreactivated under 365-nm light and in vitro transcription was carried out as described in Section 2. Lane 1, product from unirradiated substrate. Lanes 2–5, pGL3 promoter vector irradiated with 2.5, 5, 7.5 and 10 kJ/m^2 UV respectively and photoreactivated with 365-nm light.

in the co-transfection assays to test their ability to bind to and transactivate the NF-κB-luciferase reporter gene. Induction of luciferase expression was calculated as the ratio of basal NF-κB-luciferase expression in the absence of the expression vector to that in the presence of the expression vector using equal amounts of protein as described earlier [13,18]. We used equal amounts of protein to determine the luciferase activity since published literature demonstrates that β-galactosidase activity is too weak for accurate determination in these cells [13,18]. The number of CPD and (6-4) PD in the NF-κB-luc construct was quantified by radioimmunoassay. As shown in Fig. 4A there was more CPD than (6-4) PD at every dose with only about a third of the total damage at 10 kJ/m^2 being (6-4) PD. The effect of DNA damage on transactivation of gene expression can therefore be mostly attributed to the CPD.

Results of the co-transfection assay using the p50 expression plasmid are shown in Fig. 4B. The undamaged control reporter produced about nine-fold increase in NF-κB-dependent promoter activity (normalized to vector alone). DNA damage induced at the lowest dose (2.5 kJ) of UV produced ~10-fold inhibition in reporter activity. At the highest UV dose (10 kJ), reporter gene activity was almost undetectable. Fig. 4C shows that the transactivation by p65 was about three-fold greater than the basal activity with undamaged reporter. At 10 kJ/m^2 254-nm UV, we saw a six-fold inhibition in reporter gene activity. These results taken together indicate that while UV photoproducts inhibit both p50 and p65 from binding to the NF-κB binding sites in the NF-κB-luciferase reporter gene, p50 was inhibited to a greater extent than p65. We also used a combination of p50 and p65 expression plasmids (Fig. 4D) and found that there was approximately five-fold inhibition in the reporter gene activity at the highest dose of UV used. Although the inhibitory effect of the p50–p65 combination (1:1) was lower at the lowest dose of UV used, there was a good dose–response effect with the dimerized NF-κB proteins. It is possible that differences in the nucleotide(s) involved in the interaction with p50 alone and the dimerized protein binding and DNA bending may be involved in the differences observed between the different members of the NF-κB family. At this time we are not sure if changing the ratio of p50 to p65 proteins will change the observed inhibitory effect of the single protein. Experiments to examine the kinetics of inhibition are in progress.

4. Discussion

Studies on mutagenic and transformation processes have questioned the notion that DNA damage-directed mutagenesis is the only important process in the initiation of carcinogenesis. Further studies have shown that the frequencies of transformation induced by a carcinogen were at least 100-fold higher than mutation frequencies induced by the same treatment [19–21]. This has led to the proposition that epigenetic mechanisms including deregulated gene expression are involved in carcinogenesis. It is our overall hypothesis that UV radiation besides being mutagenic is also capable of changing the pattern of heritable changes in gene expression that leads to deregulation of cell growth and differentiation and ultimately to cancer. While DNA damage is induced in all parts of the genome including active and inactive genes, as well as in DNA elements necessary for regulation of gene expression, repair occurs preferentially in certain regions of active genes [22,23]. Since numerous cellular functions including gene expression and maintenance of chromatin structure are highly dependent upon precise recognition and binding of specific DNA elements by regulatory and structural proteins, it is very important to understand how the presence of DNA damage in the slowly repaired regulatory regions affect these functions.

Despite numerous published reports of the differential effects of DNA damage in transcription factor binding sites on transcription factor binding, it is unknown how DNA damage in promoter regions other than transcription factor binding sites affects gene expression. The biological significance of the observed inhibition of DNA–protein interaction and the downstream effects has been largely unexplored. Our observation that presence of both CPD and (6-4) PD in the consensus binding site of transcription factor NF-κB inhibits recombinant p50 binding is consistent with reports in the literature [6–10] that show that numerous types of DNA damage inhibit DNA–protein interactions. Although this is true of many transcription factors and types of damage a closer examina-

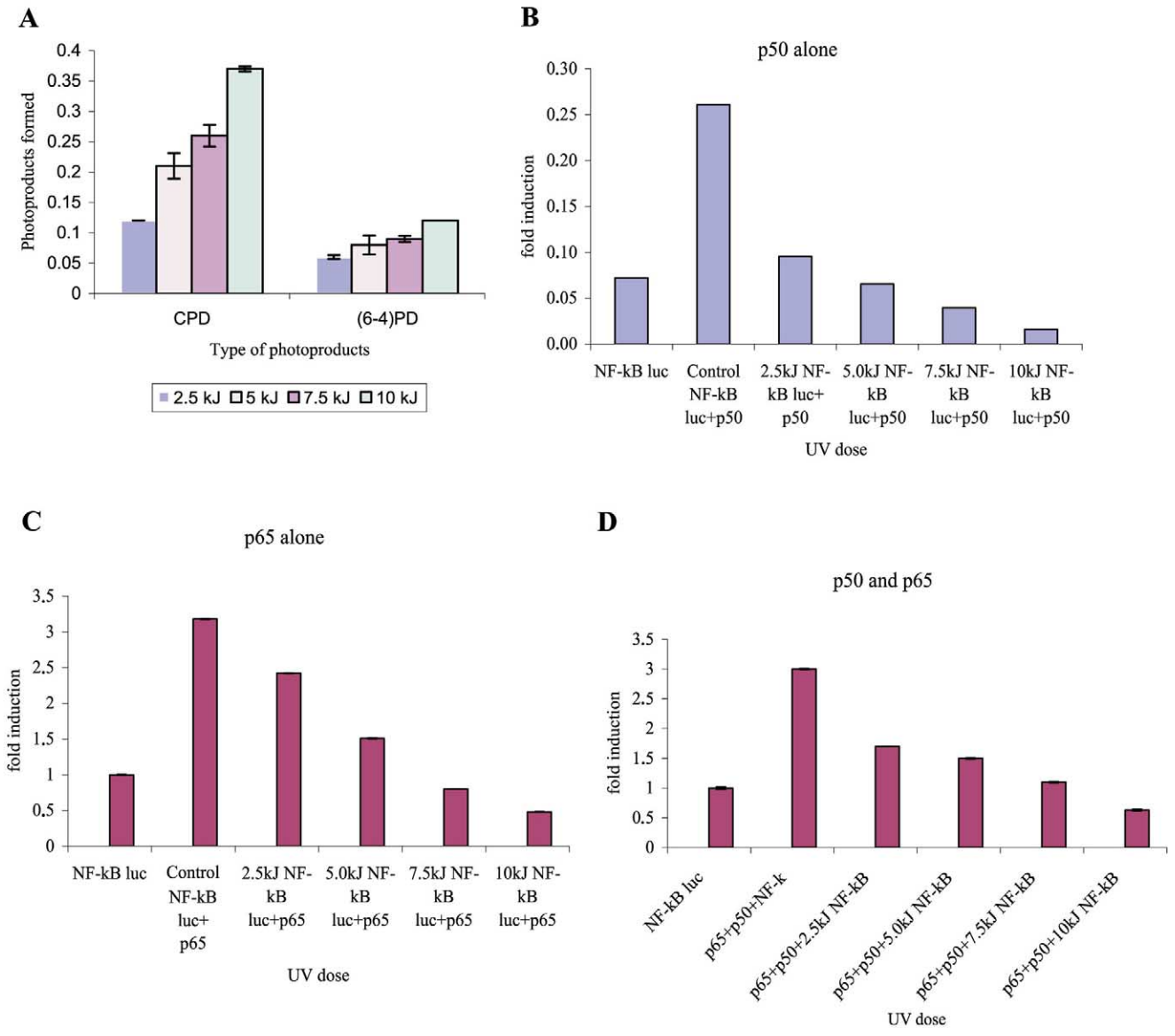

Fig. 4. Effect of DNA damage on reporter gene activity. A: Assessment of DNA damage in pNF-κB-luc. *Kpn*I-*Bgl*II-digested pNF-κB-luc was isolated and irradiated with different doses of UV. The fragment was ligated to the vector backbone. Radioimmunoassay was used for quantitative measurement of UV photoproducts in the pNF-κB-luc plasmid. Radioimmunoassays for CPD and (6-4) PD are shown. Values are expressed as photoproducts/Mb DNA. B: NF-κB-luciferase reporter activity from substrates containing UV-induced DNA damage. Co-transfection assays were carried out with NF-κB-luciferase substrates that were irradiated with different doses of UV and p50 expression plasmid as described in Section 2. The fold induction is ±S.D. of five independent experiments with two different plasmid preparations. C: Same as above except p65 was used in the co-transfection assay. D: Same as above except a combination of p50 and p65 (1:1) was used in the co-transfection assay.

tion shows that the relationship may be more complex. This was evident from our earlier studies that showed that NF-κB binding was not affected by a single 8-oxo-dG modification in its consensus binding site in contrast to the same modification in AP-1 and Sp1 [6]. Further BPDE modification created binding sites and therefore hijacked the transcription factor Sp1 from its binding site [11]. Therefore it is essential to examine other gene promoters and types of damage to begin to understand specific biological effects.

Our data presented in Fig. 2 (and data not shown) show a greater inhibition with (6-4) PD and the Dewar photoproduct compared to CPD. (6-4) PD produces a 44° bend in DNA compared to the 9° bend created by CPD [24], suggesting that the distortion in DNA caused by DNA damage may be an important determinant in the inhibition of transcription factor binding. DNA damage can occur both within and outside the transcription factor binding sites. Our observations (Fig. 3B) show that an UV-irradiated promoter produced less than 90% or no transcript in vitro from a pGL3 promoter vector whose entire SV40 promoter sequence was irradiated with UV. Normally the binding of transcription factors to promoter elements regulates gene expression. We tested the biological significance of the observed in vitro inhibition using transient expression assay in *Drosophila* cells. We used NF-κB proteins, p50 and p65 produced from expression plasmids to bind to and activate a reporter construct containing four NF-κB binding sites in tandem. Our results (Fig. 4) show that the presence of UV-induced DNA damage in the binding site of the NF-

κB-luc reporter construct inhibited or decreased transactivation of the reporter gene.

Although we did not examine the effect of specific types of UV photoproducts we showed that most of the lesions were CPD. Persistent CPD or (6-4) PD can result in base misincorporation and production of C:T transition mutations [25]. Our results taken together with published data suggest that such damage in the promoter region can deregulate gene expression either by persistence of the damage (which can lead to a mutation) or by preventing transcription factor binding. Nearly all transcription factor binding sites contain a dipyrimidine sequence and are thus potential targets for photoproduct formation. This vulnerability, coupled with the lack of repair of lesions in the binding site, could have direct consequences for transcription regulation and cause a significant perturbation of gene expression in cells exposed to UV. In this context it is interesting to note that predisposition in certain DNA repair-deficient diseases for skin cancer correlates with deficiencies in the global genome repair pathway and, presumably, repair of promoter DNA.

Although DNA damage in regulatory regions is considered to be transient, it can have immense biological significance since promoters have been shown to contain UV damage hotspots that are repaired slowly [26–28]. Despite the small target size of the promoter, the large number of genes that are transcriptionally regulated and can be targeted makes this immensely significant. In addition should DNA damage or mutation dislodge a transcription factor from its binding site it may leave the promoter open to methylation and subsequent inactivation. Biological effects of DNA damage in promoters may also arise from modified transcription rates of active genes, erroneous activation of non-active genes. A better understanding of the role of deregulated gene expression due to DNA damage in the gene regulatory regions will help sort out its contribution to the carcinogenic process.

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