Inhibition of Transcription Factor Binding by Ultraviolet-Induced Pyrimidine Dimers[†]

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ABSTRACT: The formation of DNA photoproducts by ultraviolet (UV) light is responsible for the induction of mutations and the development of skin cancer. Cis−syn cyclobutane pyrimidine dimers (pyrimidine dimers) are the most frequent lesions produced in DNA by UV irradiation. Besides being mutagenic, pyrimidine dimers may interfere with other important DNA-dependent processes. To analyze the effects of pyrimidine dimers on the ability of DNA sequences to be recognized by trans-acting factors, we have incorporated site-specific T∧T dimers into oligonucleotides containing the recognition sequences of the sequence-specific transcription factors E2F, NF-Y, AP-1, NFκB, and p53. In each case, presence of the photodimer strongly inhibited binding of the respective transcription factor complex. Reduction of binding varied between 11- and 60-fold. The results indicate that the most common UV-induced DNA lesion can interfere severely with binding of several important cell cycle regulatory and DNA damage responsive transcription factors. We suggest that inhibition of transcription factor binding may be a major biological effect of UV radiation since promoter regions are known to be repaired inefficiently and since UV damage can deregulate the function of a large number of different factors.

More than 700 000 new cases of skin cancer are diagnosed each year in the United States. Ultraviolet (UV)[1] irradiation from the sun is causing skin cancer. The increased incidence of skin tumors (melanoma and non-melanoma) is probably related to altered patterns of recreational activities of large parts of the population. The destruction of the stratospheric ozone layer is an additional concern since it may eventually result in an increased exposure of the earth's surface to UVB radiation (Lubin & Jensen, 1995).

An important goal is to determine the molecular mechanisms that are responsible for UV-induced cell transformation and carcinogenesis. UV irradiation produces several well-characterized DNA photoproducts. The most frequent UV-induced DNA lesions are the cis−syn cyclobutane pyrimidine dimers and the (6−4) photoproducts (Brash, 1988; Mitchell & Nairn, 1989; Sage, 1993; Tornaletti & Pfeifer, 1996). These DNA photoproducts are mutagenic in bacterial and eukaryotic cells. Cyclobutane pyrimidine dimers can be bypassed during DNA replication which results in base misincorporation and produces most commonly C to T transition mutations. Pyrimidine dimers may also interfere with other vital cellular DNA-dependent processes. For example, they are a block to transcription elongation when present on the transcribed DNA strand (Donahue et al., 1994). The distribution of UV-induced DNA photoproducts as well as the speed and specificity at which these lesions are removed are clearly heterogeneous along the genome (Bohr et al., 1985; Mellon et al., 1987; Smerdon & Thoma, 1990; Venema et al., 1991; Hanawalt & Mellon, 1993; Aboussekhra & Wood, 1994; Sancar, 1995; Ma et al., 1995; Bohr, 1995; Friedberg et al., 1995; Tornaletti & Pfeifer, 1996). Although transcribed sequences, in particular the transcribed strand of active genes, are repaired rapidly, promoter sequences are repaired at a much slower rate (Gao et al., 1994; Tu et al., 1996). Hence, the biological effects of a pyrimidine dimer may be most pronounced in promoter sequences, owing to a lack of repair. In addition, several transcription factor binding sites are strong targets for photoproduct formation in vivo (Pfeifer et al., 1992; Tornaletti & Pfeifer, 1995).

Many DNA recognition sequences of transcription factors contain two adjacent pyrimidines which makes them potential targets for pyrimidine dimer formation. It is presently unknown whether transcription factors can bind to their target sites if the site contains a UV photoproduct. We have investigated protein−DNA interactions using site-specifically placed photoproducts and have analyzed the binding of five different transcription factor complexes to DNA sequences containing a cyclobutane pyrimidine dimer. We chose to investigate factors that are involved in regulation of cell cycle-dependent transcription and DNA damage responses: NF-Y, E2F, AP-1, NFκB, and p53. The recognition sequences of these transcription factors all contain a 5′-TT dipyrimidine sequence. These TT sequences were replaced by cis−syn T∧T cyclobutane pyrimidine dimers in synthetic oligonucleotides, and the modified and unmodified sequences were used in transcription factor binding assays.

MATERIALS AND METHODS

Synthesis of the Site-Specific Pyrimidine Dimer

General Methods and Materials. Plates used for TLC were Kieselgel 60, F254 plates from Merck. Silica gel used

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[1] Abbreviations: UV, ultraviolet; CPD, cyclobutane pyrimidine dimer; EMSA, electrophoretic mobility shift assay; NFκB, nuclear factor κ B.

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for HPLC was Kieselgel H from Fluka. Photolysis was performed in a 1 L capacity reactor (Ace) with a Pyrex filter, a 450 W Honovia medium-pressure mercury lamp, and water cooling.

Separation, purification, and analytical analyses of photoproducts were performed on a Gilson HPLC system using preparative C18-Vydac columns (7 mm × 250 mm) at a flow rate of 4−5 mL/min and an analytical Zorbax 300SB-C18 column (4.6 mm × 250 mm) at a flow rate 1.0−1.5 mL/min. Acetonitrile/water gradients were used for the separation of the four photoproducts. Purification of protected and phosphitylated photoproducts was performed on a Waters HPLC system using preparative columns filled with silica gel H (Fluka) at a flow rate 5−10 mL/min. Dichloromethane/methanol gradients were used for the purification of photoproduct derivatives.

Syntheses of oligodeoxyribonucleotides were performed on a model 394 DNA/RNA synthesizer (Applied Biosystems). Phosphoramidites T, dC, dG, and dA were from PerSeptive Biosystems. Purification of oligomers was performed on PRP-1 cartridges from Hamilton.

^{1}H-NMR spectra of protected nucleosides and nucleotides were measured at 300 MHz with a Varian spectrometer.

Dichloromethane, methanol, and acetonitrile were from Fisher. 2-Cyanoethyl (*N*,*N*-diisopropylamine)chlorophosphoramidite, *N*,*N*-diisopropylethylamine, and hydrazine monohydrate were from Aldrich. (Dimethoxytrityl)thymidine and monomethoxytrityl chloride were from ChemImpex. Pyridine, acetic acid, and acetone were from J. T. Baker. Levulinic acid was from Sigma. 2-Propanol was from Baxter. Petroleum ether and toluene were from Malinckrodt, and triethylamine was from Pierce.

*Photodimerization of Thymidinyl(3′−5′)-3′-O-levulinylthymidine Cyanoethyl Phosphotriester **1** (MW = 695.58).* The substrate, the thymidinyl(3′−5′)-3′-*O*-levulinylthymidine cyanoethyl phosphotriester **1**, was prepared according to the procedure of Murata et al. (1990). Thymidinyl(3′−5′)-3′-*O*-levulinylthymidine cyanoethyl phosphotriester (2 g, 2.87 mmol) was dissolved in a mixture of 300 mL of acetonitrile, 50 mL of acetone, and 700 mL of water (Murata et al., 1990). The solution was bubbled with argon (45 min) to remove oxygen and was UV irradiated for 14 h. Analytical reverse phase HPLC, at a detection wavelength of 230 nm on a Zorbax 300SB-C18 column, showed the presence of four products α, β, γ, and δ with retention times of 10.3, 11.5, 11.9, and 12.0 min, respectively, using a gradient of acetonitrile/water of 10 to 16% in 15 min (see Figure 1). The reaction mixture was then concentrated under vacuum to a dry residue.

Separation of Photoproducts. The reaction mixture was partially separated by HPLC on silica gel H (20 g) in a gradient of 0 to 12% methanol/dichloromethane to yield 210 mg of δ. Fractions from HPLC were checked by TLC in 15% methanol/ dichloromethane. TLC plates were visualized by exposure to UV 254 nm light, causing the photoreversion to TpT. The final separation was performed by HPLC at 240 nm using Vydac-C18 preparatory columns in a gradient from 5 to 30% of acetonitrile/water in 25 min. Combined yields were 13.4% of α, 26.8% of β, 18.7% of γ, 24.5% of δ, and 7.1% of TpT.

Structure Correlation. Thymidinyl(3′−5′)-3′-*O*-levulinylthymidine cyanoethyl phosphotriester **1** exists as a mixture of two diastereoisomers. Upon photolysis, each of those

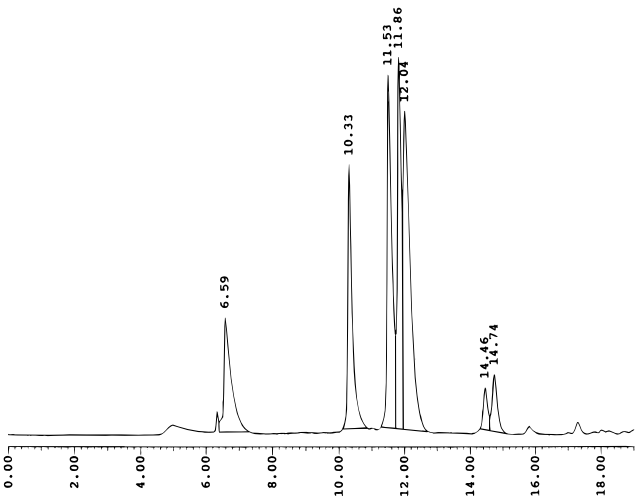

FIGURE 1: Separation of the photoreaction products by HPLC. Analytical reverse phase HPLC, at 230 nm, on a Zorbax 300SB-C18 analytical column using acetonitrile water (10 to 16%) for 15 min. The presence of four products α, β, γ, and δ with retention times of 10.33, 11.53, 11.86, and 12.04 min, respectively, is shown. Peaks α and β (10.33 and 11.53 min) represent the trans−syn dimer; peaks γ and δ (11.86 and 12.04 min) represent the cis−syn dimer. Peaks at 14.46 and 14.74 min show remains of unphotolysed TpT. The peak at 6.59 min shows the presence of acetone.

diastereoisomers creates a mixture of cis−syn and trans−syn isomers, thus yielding four photoproducts.

A mixture of cis−syn and trans−syn photodimer standards was prepared by photolysis of TpT (Liu & Yang, 1978). Aliquots of α, β, γ, and δ were each separately deprotected by treatment at 55 °C with concentrated ammonia for 16 h. After removal of the solvent under reduced pressure, samples were analyzed separately by HPLC on an analytical Zorbax 300SB-C18 column with 0 to 10% acetonitrile/water in 15 min at a detection wavelength of 230 nm. Comparison with the standards indicated that deprotected α and β are identical with the trans−syn dimer and γ and δ are identical with the cis−syn dimer (Liu & Yang, 1978).

NMR Data. Photodimer α: HO-T[t,s]PO(OCH$_2$CH$_2$CN)T-OLev; ^{1}H-NMR (300 MHz CD_3CN, ppm from TMS) 5.56 (dd, J = 4.75, 11.0 Hz, H1′), 5.20 (dd, H1′), 1.35, 1.33 (s, C5CH$_3$); UV (acetonitrile) no λ_{max} of >200 nm.

Photodimer β: HO-T[t,s]PO(OCH$_2$CH$_2$CN)T-OLev; ^{1}H-NMR (300 MHz CD_3CN, ppm from TMS) 5.83 (dd, J = 4.9, 10.7 Hz, H1′), 5.22 (dd, J = 4.7, 10.3 Hz, H1′), 1.35, 1.32 (s, C5CH$_3$); UV (acetonitrile) no λ_{max} of >200 nm.

Photodimer γ: HO-T[c,s]PO(OCH$_2$CH$_2$CN)T-OLev; ^{1}H-NMR (300 MHz CD_3CN, ppm from TMS) 6.18 (t, J = 7.4 Hz, H1′), 5.84 (t, J = 7.15, Hz, H1′), 1.36, 1.49 (s, C5CH$_3$); UV (acetonitrile) no λ_{max} of >200 nm.

Photodimer δ: HO-T[c,s]PO(OCH$_2$CH$_2$CN)T-OLev; ^{1}H-NMR (300 MHz CD_3CN, ppm from TMS) 5.92 (dd, J = 9.90, 5.55 Hz, H1′), 5.38 (dd, J = 4.8, 10.0 Hz, H1′), 1.35, 1.33 (s, C5CH$_3$); UV (acetonitrile) no λ_{max} of >200 nm.

*Synthesis and Purification of MMT-O-T[c,s]PO(OCH$_2$CH$_2$CN)T-OLev **2** (MW = 967.92).* The cis−syn photodimer δ (210 mg, 0.30 mmol) was coevaporated with dry pyridine (20 mL, three times), then dissolved in dry pyridine (10 mL), and treated with monomethoxytrityl chloride (108 mg, 0.35 mmol). The reaction mixture was then concentrated to a volume of 5 mL and kept at 35 °C for 16 h. Methanol (0.5 mL) was added, and the reaction mixture was concentrated to a syrupy residue and coevaporated with toluene and

2-propanol (20 mL, two times in turn). The residue was then dissolved in dichloromethane and purified by HPLC on silica gel H (20 g) using a gradient of 0 to 5% methanol/dichloromethane. The yield was 78% (226 mg, 0.23 mmol). Fractions from HPLC were checked by TLC in 10% methanol/dichloromethane. TLC plates were visualized by exposure to vapors of trifluoroacetic acid which showed the tritylated product as a yellow spot.

Synthesis and Purification of MMT-O-T[c,s]PO(OCH$_2$-CH$_2$CN)T-OH ***3*** *(MW = 869.82).* The 5′-*O*-monomethoxytritylated 3′-*O*-protected photodimer δ **2** (220 mg, 0.227 mmol) was dissolved in pyridine (4 mL), and 0.75 M hydrazine monohydrate in pyridine/acetic acid (3/2, v/v) (5 mL, 3.75 mmol) was added. After 30 min, acetone (0.5 mL) was added, and the reaction mixture was diluted with 150 mL of dichloromethane and washed with water, 5% aqueous $NaHCO_3$, and saturated brine. The organic layer was then dried with anhydrous $MgSO_4$ and concentrated under vacuum to a dry residue which was then dissolved in dichloromethane and purified by HPLC on silica gel H (20 g) using a gradient of 0 to 7% methanol/dichloromethane. Fractions from HPLC were checked by TLC in 15% methanol/dichloromethane. TLC plates were visualized by exposure to vapors of trifluoroacetic acid. Fractions containing pure product were concentrated, under reduced pressure, dissolved in dichloromethane (2 mL), and precipitated into petroleum ether (45 mL). The yield of **3** was 81% (160 mg, 0.184 mmol).

Synthesis and Purification of 5′-O-[(*Monomethoxytrityl)-thymidinyl](3′−5′)-3′-(O-2-Cyanoethyl N,N-diisopropylphosphoramidite) Cyanoethyl Phosphotriester* ***4*** *(MW = 1070.04).* The 5′-*O*-monomethoxytritylated 3′-*O*-deprotected dimer **3** (150 mg, 0.172 mmol) was dissolved in a solution of *N,N*-diisopropylethylamine (0.12 mL, 0.70 mmol) in dry acetonitrile (2 mL) and treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.08 mL, 0.35 mmol). The reaction mixture was stirred at room temperature for 120 min, rapidly concentrated to a syrupy residue under reduced pressure, dissolved in 2 mL of dichloromethane, centrifuged, and injected onto an HPLC column of silica gel H (5 g). The elution was accomplished using a gradient of 0 to 2% methanol/dichloromethane. Dichloromethane used for HPLC contained 0.1% triethylamine. Fractions from HPLC were checked by TLC in 5% methanol/dichloromethane. TLC plates were visualized by exposure to vapors of trifluoroacetic acid. Fractions containing pure product were concentrated under reduced pressure, dissolved in dichloromethane (2 mL), and precipitated into petroleum ether (45 mL). The yield of pure amidite was 50% (91 mg, 0.85 mmol). Petroleum ether used for precipitation contained 0.1% triethylamine.

Synthesis of the Oligodeoxyribonucleoside Containing the Cis−Syn Photodimer. Synthesis was performed on a DNA/RNA synthesizer (Applied Biosystems 394). Extended time (300 s) was used for the coupling of the phosphoramidite of the cis−syn photodimer δ (20 mg in 200 μL of acetonitrile). The oligomer was synthesized in a DMT-ON mode, in 0.2 μmol scale, and cleaved in concentrated ammonia at 55 °C for 16 h. Then the resin was separated and the filtrate, containing the oligomer, concentrated under reduced pressure. The crude oligomer was then dissolved in 1 M aqueous triethylamine acetate (2 mL) and loaded onto a prewashed PRP-1 cartridge. The cartridge was then washed with 3% aqueous ammonia, water, 3% trifluoroacetic acid, and water. The purified sample was eluted from the cartridge with 30% acetonitrile/water containing 1% ammonia. The sample was then purified by preparative polyacrylamide gel electrophoresis. The yield was approximately 2 ODU (260 nm) of pure oligomer.

T4 Endonuclease V Cleavage. The purified oligomer was phosphorylated with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP and annealed to an unlabeled oligonucleotide representing the opposite strand. After ethanol precipitation, the duplex DNA was incubated at 37 °C for 30 min with 0.075 μg of T4 endonuclease V in 50 mM Tris-HCl (pH 7.6), 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, and 100 μg/mL bovine serum albumin. The cleavage products were separated on 20% denaturing polyacrylamide gels.

Nuclear Extracts and Gel Mobility Shift Assays. HeLa nuclear extracts (gel shift grade) were obtained from Promega (Madison, WI). Nuclear extracts from normal diploid human fibroblasts were prepared as previously described (Tommasi & Pfeifer, 1995). Equal amounts of protein were used in each binding reaction. DNA binding assays were carried out according to Ausubel et al. (1993). Each binding reaction mixture contained 13 mM Hepes (pH 7.9), 13% glycerol, 64 mM KCl, 0.13 mM EDTA, 0.5 mM $MgCl_2$, and 0.3 mM phenylmethanesulfonyl fluoride. Nuclear extracts (6−8 μg) were preincubated in this mixture for 10 min on ice in the presence or absence of competitor DNA and 0.5 μg of salmon sperm DNA. The probe was then added, and incubation was continued for an additional 20 min on ice. DNA−protein complexes were resolved by electrophoresis through low-ionic strength 6% polyacrylamide gels at 4 °C in TAE buffer (Ausubel et al., 1993). The synthetic oligonucleotides used as probes were phosphorylated with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP to result in approximately equal specific activities and were annealed with a 2-fold excess of the unlabeled complementary strand. Oligonucleotides were annealed in molar equivalent quantities when used as nonradioactive competitors. The following oligonucleotides were used for gel retardation assays: *JUN*-CCAAT, 5′-GCCTTCCCATTGGCTCGCGTCGC; *JUN*-CCAAT−CPD, 5′-GCCTTCCCA-**T**∧**T**GGCTCGCGTCGC; AP-1, 5′-GCGGAGCATTACCTCATCCCGTG; AP-1−CPD, 5′-GCGGAGCA**T**∧**T**ACCTCATCCCGTG; E2F, 5′-ATTTAAGTTTCGCGCCCTTTCTCAA; E2F−CPD-5′-ATTTAAGT**T**∧**T**CGCGCCCTTTCTCAA; NFκB, 5′-TCGACAGAGGGGACTTTCCGAGAGGC; NFκB−CPD, TCGACAGAGGGGACT**T**∧**T**CCGAGAGGC; p53, 5′-TCGAGCAACATGTTGGGACATGTTCCTCGA; and p53−CPD, 5′-TCGAGCAACATG**T**∧**T**GGGACATGTTCCTCGA. In the modified oligonucleotides, the site-specific pyrimidine dimers are indicated by **T**∧**T**.

In antibody supershift experiments, 1−2 μg of specific antibody was added to the binding reaction mixtures. The following antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA): anti-Sp1 antibody PEP2, anti-E2F-4 antibody C-108, anti-p107 antibody SD9, anti-p130 antibody C-20, anti-Rb antibody C-15, anti-cyclin A antibody BF683, anti-DP-1 (K-20), anti-DP-2 (C-20), anti-NFκB p50 (NLS), anti-NFκB p65 (A), anti-ATF2 (C19), anti-c-Jun/AP-1 (N), and anti-NF-1 antibody N-20. The anti-E2F-1 (KH20) and the anti-E2F-2 (TFE25) antibodies were a gift from K. Helin. The anti-E2F-5 antibody was from R. Bernards. Anti-NF-YA Mab YA1a and anti-NF-YB rabbit serum were kindly provided by R. Mantovani and D. Mathis. Crude extracts of baculovirus-produced human wild-type p53

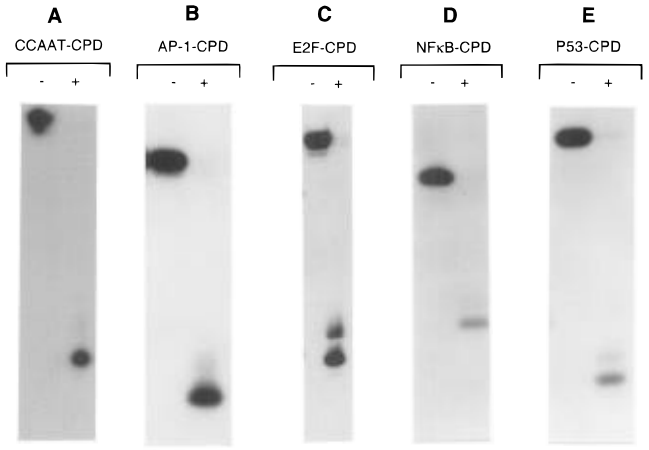

FIGURE 2: Cleavage of the pyrimidine dimer-containing oligonucleotides with T4 endonuclease V. The 5′-end-labeled oligonucleotides containing a pyrimidine dimer within the *JUN*-CCAAT (A), AP-1 (B), E2F (C), NFκB (D), and p53 (E) consensus binding sites were cleaved with the cyclobutane pyrimidine dimer-specific endonuclease T4 endonuclease V: lanes 1, uncut oligonucleotide; and lanes 2, the same oligonucleotide after T4 endonuclease V cleavage. The samples (A−E) were run on separate gels.

and the anti-p53 monoclonal antibody (PAb421) were a gift from J. Momand.

For quantitation, radioactivity in the specific complexes was counted with a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Synthesis of Oligonucleotides Containing Site-Specific Pyrimidine Dimers. A modification of the building block method (Taylor et al., 1987; Murata et al., 1990) was used to incorporate T∧T cis−syn cyclobutane pyrimidine dimers into synthetic oligonucleotides (see Materials and Methods). The initial photoreaction products that comprise cis−syn and trans−syn isomers were separated by HPLC (Figure 1). To verify the presence of the cis−syn pyrimidine dimers in the synthetic oligonucleotides, the dimer-containing strands were labeled at the 5′-end with T4 polynucleotide kinase and annealed with the opposite strand to form duplex DNA. Digestion with the cyclobutane dimer-specific enzyme T4 endonuclease V revealed the disappearence of the full length oligonucleotides and the appearance of the expected cleavage products (Figure 2). In some cases, the cleavage product migrates as two bands which differ only by the nature of the fragmented 3′-terminal deoxyribose residue (Smith & Taylor, 1993). The data confirm the identity and purity of the oligonucleotide substrates containing pyrimidine dimers within the transcription factor binding sites.

NF-Y Complexes. NF-Y (also called CP1 or CBF) is a ubiquitous sequence-specific transcription factor that recognizes CCAAT box elements in a wide variety of different promoters. It is a heteromeric protein composed of at least two subunits, NF-YA and NF-YB (Mantovani et al., 1992). The NF-YA subunit contains a glutamin-rich activation domain. A role of NF-Y proteins in the regulation of cell growth-associated genes has been suggested (Ladomery & Sommerville, 1995).

Nuclear extracts from human fibroblasts were used as the source of NF-Y complexes. The double-stranded oligonucleotide used for binding studies was a sequence containing the CCAAT box element located at position −91 within the human *JUN* promoter (Rozek & Pfeifer, 1993). We first characterized the identity and composition of the CCAAT box binding complexes by antibody supershift in gel retardation assays. Figure 3A shows that the specific complex is supershifted by antibodies directed against the A and B subunits of NF-Y. Control antibodies, including an antibody directed against NF-1, did not change the mobility of the complex. The results show that the CCAAT box binding complex is consistent with NF-Y.

The same nuclear extracts were then used for mobility shift assays with an oligonucleotide containing a site-specific T∧T pyrimidine dimer within the CCAAT box sequence (T∧T on the opposite strand). There was an approximately 60-fold reduction of complex formation when this oligonucleotide was used as a probe (Figure 3B; quantitated by phosphorimaging). When the intact CCAAT box sequence was used as a probe, the pyrimidine dimer-containing oligonucleotide did not function as a competitor (Figure 3B), confirming the lack of binding to the dimer-containing site. We conclude that binding of NF-Y complexes to the pyrimidine dimer-containing sequence is almost completely abolished.

AP-1 Complexes. AP-1 is an important transcription factor that is involved in a variety of cellular responses, including activation of DNA damage response pathways (Fornace, 1992; Devary et al., 1992; Herrlich & Rahmsdorf, 1994). The standard AP-1 recognition sequence is the palindromic element TGA(C/G)TCA. Unfortunately, this sequence does not contain a 5′-TT dipyrimidine, and TC dimers cannot be synthesized with the present technology. However, there are variant AP-1 sites, for example at position −190 within the human *JUN* promoter. This element is involved in induction of the *JUN* gene following UV irradiation (Devary et al., 1991; Stein et al., 1992) and contains the sequence 5′-TTACCTCA. The 5′-TT was replaced with a 5′-T∧T cyclobutane dimer.

It was reported that the AP-1 complexes that interact with this particular site are composed of heterodimers of JUN and ATF-2 proteins (Van Dam et al., 1993). However, the supershift assays shown in Figure 4A indicate that antibodies directed against JUN and ATF-2 do not perturb the mobility of the complexes. HeLa cells were used as the source of nuclear extracts. The exact identity of the complex that binds to this variant AP-1 site remains therefore unknown, but it is likely that the complex contains other members of the JUN, FOS, ATF, or CREB families of proteins.

Regardless of this, we have analyzed the effect of the site-specific T∧T pyrimidine dimer on complex formation. Figure 4B shows that binding to the modified sequence is significantly reduced (approximately 11-fold). This is supported by the competition assays in which the lesion-containing sequence was a less efficient competitor. The reduction of binding is not quite as dramatic as that seen with the CCAAT box sequence, perhaps because the lesion is located at the 5′-border of the recognition site within the AP-1 sequence.

NFκB Complexes. Nuclear factor κ B (NFκB) is a ubiquitous transcription factor, although its function is most notable in the immune system (Schmitz & Baeuerle, 1995). NFκB is a multiprotein complex that can activate a variety of genes. In nonstimulated cells, NFκB resides in the cytoplasm in an inactive complex with the inhibitor IκB. Pathogenic stimuli such as cytokines, tumor necrosis factor,

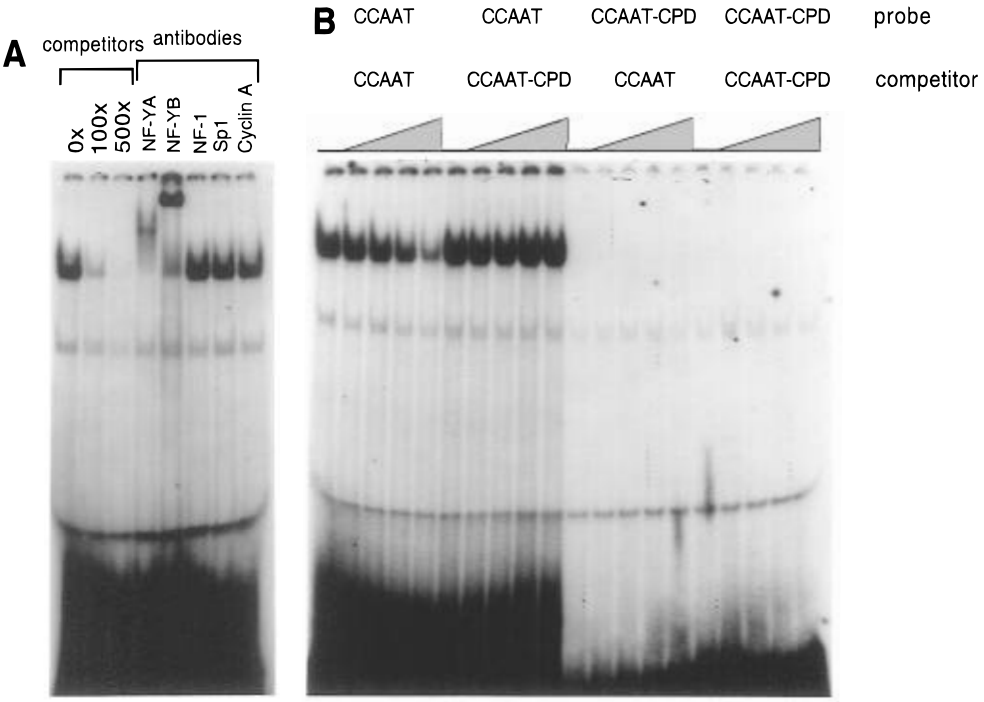

FIGURE 3: Electrophoretic mobility shift assays (EMSAs) of an oligonucleotide containing an intact CCAAT box sequence and the same sequence containing a site-specific T∧T pyrimidine dimer. Nuclear extracts were prepared from dividing human fibroblasts. ^{32}P-labeled, double-stranded oligonucleotides, having the same specific activity, were used for EMSAs. (A) Specificity of binding to the intact CCAAT box sequence. A 100- and 500-fold excess of unlabeled oligonucleotide was used to assess the specificity of the complexes. The identity of the specific complex was determined by supershift assays with anti-NF-YA and anti-NF-YB antibodies. (B) In the left 10 lanes, the intact CCAAT box sequence was used as a probe. In the right 10 lanes, the same sequence containing the site-specific pyrimidine dimer was used as a probe. Competition experiments were carried out with increasing amounts of intact oligonucleotide or pyrimidine dimer-containing oligonucleotide as a competitor (0-, 3-, 10-, 30-, and 100-fold excess).

viruses, double-stranded RNA, endotoxins, phorbol esters, UV light, or ionizing radiation can cause release of IκB and translocation of NFκB to the nucleus where the complex binds to its target sequences (Schreck et al., 1992). A consensus recognition sequence is 5′-GGGA(A/C)TTTCC (Faisst & Meyer, 1992). Because there is a stretch of several adjacent pyrimidines, this sequence may form a preferential target for UV damage formation in vivo.

We have incorporated a T∧T dimer into the sequence 5′-TCGACAGAGGGGACT**T**∧**T**CCGAGAGGC. Nuclear extracts were prepared from human fibroblasts at 12 h following serum stimulation of serum-starved cells, a time point at which specific NFκB complexes had reached a maximum level (data not shown). Competition experiments with an intact NFκB olgonucleotide and antibody supershift assays (Figure 5A) indicate that the upper one of the two most dominant complexes is a specific complex that contains the p50 and p65 subunits of nuclear factor κ B. Incubation with the pyrimidine dimer-containing oligonucleotide shows that binding of this complex is more than 95% (greater than 20-fold) inhibited by the presence of the lesion (Figure 5B). The lesion-containing site also functions as a less efficient competitor (Figure 5B).

E2F Complexes. E2F is a sequence-specific transcription factor that targets the promoters of many cell cycle-regulated genes [reviewed in Nevins (1992), La Thangue (1994), and Dyson (1994)]. E2F stands for a family of proteins consisting of heterodimers of E2F proteins (E2F1−5) and DP proteins (DP1−3). The consensus binding site for E2F complexes is 5′-TTT(C/G)(C/G)CGC. E2F sites can function as activating or repressing elements, depending on the particular promoter, the cell cycle phase, and association of the E2F proteins with inhibitory proteins such as the retinoblastoma protein (pRb), the p107 protein, or the p130 protein [for reviews, see Nevins (1992), La Thangue (1994), and Dyson (1994)].

Nuclear extracts from unsynchronized human fibroblasts were used as the source of E2F complexes (Tommasi & Pfeifer, 1995). With an oligonucleotide representing the E2F recognition site from the adenovirus E2 promoter, one (and occasionally two) major E2F complex can be observed (Figure 6). These complexes contain E2F-4 and the p130 protein, as was determined using specific antibodies in supershift assays (Figure 6A). There is also a significant supershift with antibodies directed against the DP-1 protein which can form heterodimers with E2F-4 (La Thangue, 1994).

The intact oligonucleotide and the pyrimidine dimer-containing oligonucleotide were used for electrophoretic mobility shift assays (Figure 6B). Binding of E2F complexes was almost completely abolished with the oligonucleotide that contains the pyrimidine dimer (Figure 6B). There was a 19-fold reduction of binding as quantitated by phosphorimaging. With the dimer-containing oligonucleotide, two weak bands are still visible. However, these complexes have a different mobility than the two major E2F complexes, and they appear to be nonspecific since they cannot be competed with an excess of unlabeled oligonucleotide (Figure 6B, right 10 lanes). When the intact E2F sequence was used as a probe, the pyrimidine dimer-containing oligonucleotide was a much weaker competitor than the undamaged oligonucleotide (Figure 6B). We conclude that binding of E2F complexes to the pyrimidine dimer-containing sequence is greatly reduced.

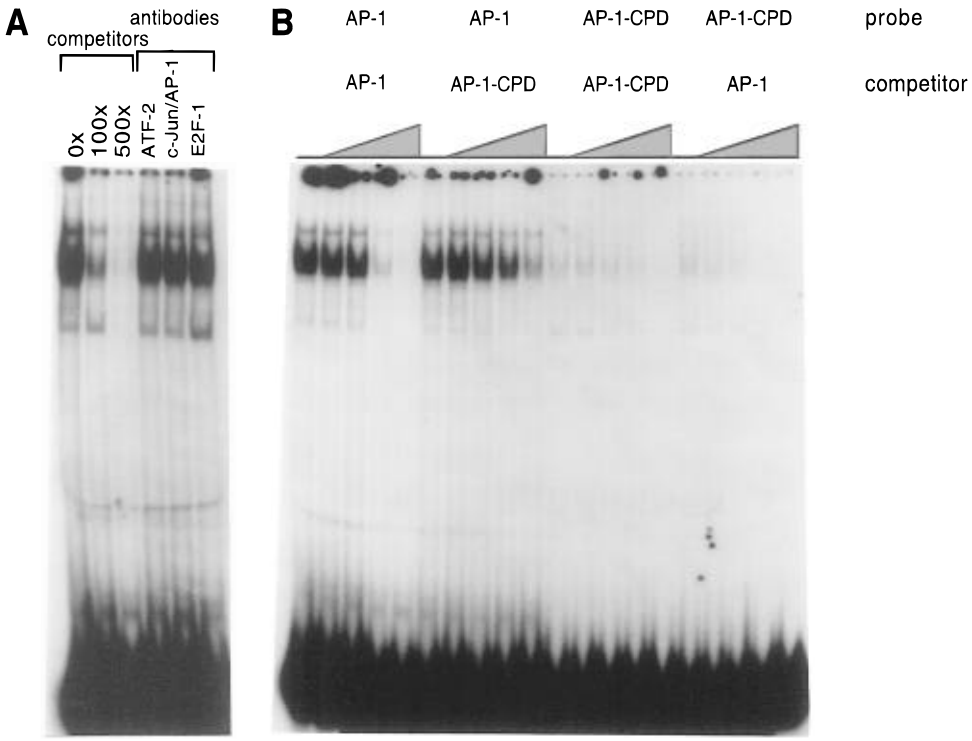

FIGURE 4: EMSAs of an oligonucleotide containing an intact AP-1 site and the same sequence containing a site-specific T∧T pyrimidine dimer. (A) The ^{32}P-labeled, double-stranded intact oligonucleotide was used as a probe with HeLa cell nuclear extracts. A 100- and 500-fold excess of unlabeled oligonucleotide was used to assess the specificity of the complexes. Incubation with anti-JUN and anti-ATF-2 antibodies did not result in a supershift. (B) In lanes 1−10, the intact AP-1 binding site from the *JUN* promoter was used as a probe. In lanes 11−20, the same sequence containing the site-specific pyrimidine dimer was used as a probe. Competition experiments were performed with increasing amounts of intact oligonucleotide or pyrimidine dimer-containing oligonucleotide as a competitor (0-, 3-, 10-, 100-, and 500-fold excess).

p53 Complexes. The p53 protein is part of a DNA damage response pathway and is induced by UV irradiation (Maltzman & Czyzyk, 1984; Hall et al., 1993). Apparently, p53 is involved in cell cycle checkpoint (and possibly apoptosis and DNA repair) pathways through activation of specific genes after DNA damage. One downstream target is the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 (Xiong et al., 1993; Dulic et al., 1994; Waldman et al., 1995). The induction of p21 leads to cell cycle arrest. A region upstream of the p21 gene contains two elements that mediate p53-dependent expression of the p21 gene, and these sequences bind p53 protein in vitro.

One of the p53 response elements upstream of the p21 gene contains the sequence 5′-TCGAGCAACATGTTGG-GACATGTTCCTCGA. A T∧T dimer was incorporated in place of the more 5′-located TT dipyrimidine sequence. The source of p53 protein was an extract from baculovirus-infected insect cells overproducing p53. The sequence-specific binding function of p53 could be strongly activated by coincubation with the monoclonal anti-p53 antibody PAb421 (Figure 7A). This antibody-induced activation has been described previously [e.g., Hupp et al. (1992), Zauberman et al. (1993), Hupp and Lane (1994), Hecker et al. (1996), and Hansen et al. (1996)]. The antibody p421 recognizes an epitope in the C-terminal domain of the p53 protein which causes a conformational change that activates the DNA binding activity of p53. It is thought that a mechanistically similar process might activate p53's DNA binding activity in vivo in reponse to stimuli such as certain types of DNA damage. The antibody-activated p53 was used for mobility shift assays with the intact oligonucleotide from the p21 promoter and the pyrimidine dimer-containing site (Figure 7B). As was the case with all other transcription factors tested, binding of p53 was severely inhibited by the UV lesion. Approximately 14-fold inhibition of binding was seen. The remaining binding activity may be explained by the fact that p53 binds to DNA as a tetramer and recognizes four repeats of a consensus DNA site 5′-PuPuPuC(A/T). There may be some residual affinity to DNA if only one of the repeat elements contains a lesion.

DISCUSSION

Effects of DNA Damage on Transcription Factors. UV damage can interfere with specific protein−DNA interactions as exemplified by the inhibition of restriction enzyme cleavage by UV damage (Cleaver, 1983). The influence of DNA damage on binding of sequence-specific transcription factors has been analyzed in only a few cases. It was shown that treatment of oligonucleotides with alkylating agents can inhibit binding of transcription factors NFκB, Sp1, OTF-1, and AP2 (Bonfanti et al., 1991; Fabbri et al., 1993; Sun & Hurley, 1994; Gray, 1995). The degree of inhibition depends on the sequence composition of the factor binding site, the type of alkylating agent, and the level of base modification. Adriamycin-induced DNA adducts inhibit the DNA interactions of octamer transcription factors and RNA polymerase (Cutts et al., 1996). Minor groove binding drugs can block the binding of the TATA box binding protein (Chiang et al., 1994). A different effect was observed for two other types of DNA adducts. The high-mobility group protein HMG1 and human upstream binding factor (hUBF) have a high affinity for any DNA substrate containing a cisplatinum adduct (Pil & Lippard, 1992; Treiber et al., 1994). Similarly, adducts of the polycyclic aromatic hydrocarbon benzo[*a*]-

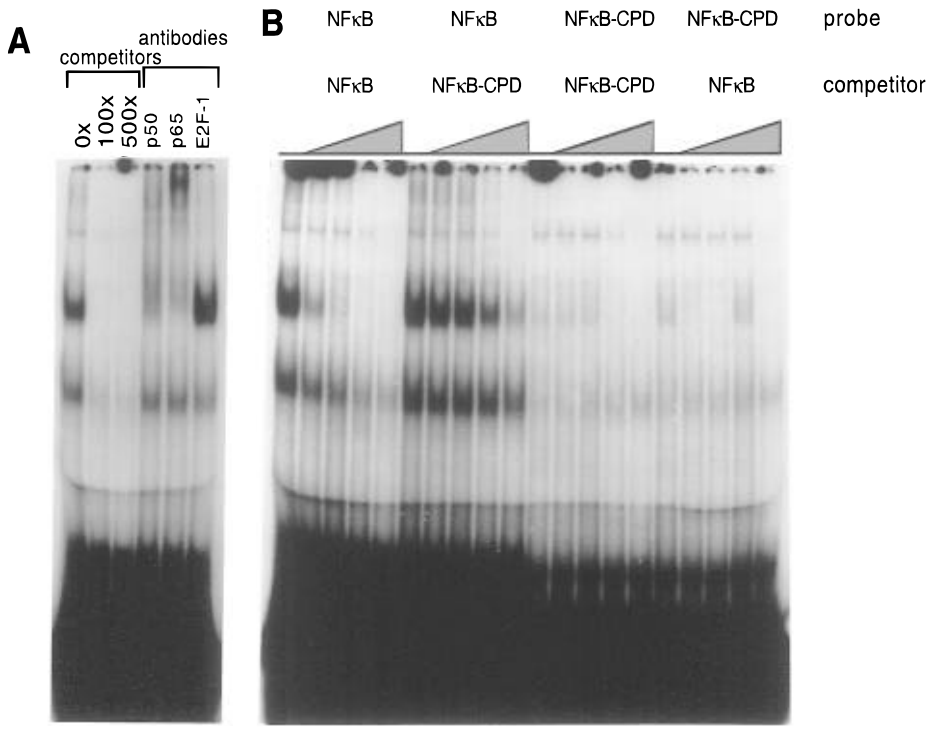

FIGURE 5: EMSAs of an oligonucleotide containing an intact NFκB binding site and the same sequence containing a site-specific pyrimidine dimer. (A) Nuclear extracts were prepared from serum-starved, synchronized human fibroblasts, at 12 h following serum stimulation. These extracts were incubated with an end-labeled, double-stranded intact NFκB oligonucleotide in the presence of 1.5 μg of poly(dI-dC) as a nonspecific competitor. A 100- and 500-fold excess of unlabeled oligonucleotide was used to assess the specificity of the complexes. NFκB p50 and NFκB p65 antibodies were used for supershift experiments. (B) In the left 10 lanes, the intact oligonucleotide spanning the NFκB consensus binding site was used as a probe. In the right 10 lanes, the same sequence containing the site-specific pyrimidine dimer was used as a probe. Competition experiments were performed with increasing amounts of intact oligonucleotide or pyrimidine dimer-containing oligonucleotide as a competitor (0-, 3-, 10-, 100-, and 500-fold excess).

pyrenediol epoxide (BPDE) promote increased binding of Sp1, even to nontarget sequences (MacLeod et al., 1995). There may be a selective effect of DNA adducts on the expression of specific genes, depending on the type of DNA-damaging agent and depending on which factor binding it can affect. The effects of UV irradiation on transcription factor binding has never been analyzed before, although UV is the DNA-damaging agent humans are exposed to most. Our data suggest that UV radiation damage may perturb regulation of a large number of genes since many different transcription factors can be inhibited. Almost every transcription factor binding site contains a dipyrimidine sequence (Faisst & Mayer, 1992) and is thus a potential target for formation of photolesions. Although not tested here, it is assumed that cyclobutane pyrimidine dimers at 5′-T$\wedge$C, 5′-C$\wedge$T, and 5′-C$\wedge$C would similarly inhibit protein−DNA interactions.

Mechanisms of Inhibition. The specific mechanistic basis of this inhibition is not understood at present. The formation of a pyrimidine dimer would be expected to change the basic shape and local electronic nature of the DNA substrate (Nielsen, 1991). Three types of interactions that TT would be involved with would be expected to change upon formation of the photodimer. These interactions are thymine−O4 (hydrogen acceptor), the C5 methyl group of thymine (a van der Waals attraction), and the $\pi-\pi$ interactions (stacking energy). The formation of a pyrimidine dimer is known to cause specific changes in the DNA structure. Near a pyrimidine dimer, DNA is bent by approximately 7−9° (Wang & Taylor, 1991; Kim et al., 1995) and is unwound by 15° (Wang & Taylor, 1993). These deformations may not be considered as being very dramatically different from those of B-DNA. In fact, a pyrimidine dimer can often be bypassed by DNA polymerases (Spivak & Hanawalt, 1992; Thomas & Kunkel, 1993; Carty et al., 1993; Gibbs et al., 1993; Smith et al., 1996). On the other hand, many transcription factors are known to deform and bend DNA upon binding to their recognition sequence (Harrington & Winicov, 1994). The transcription factors analyzed here, AP-1 (Kerppola & Curran, 1991), NF-Y (Ronchi et al., 1995), NFκB (Schreck et al., 1990), E2F (Huber et al., 1994), and p53 (Balagurumoorthy et al., 1995), are all known to bend or distort the DNA double helix. The pyrimidine dimer-induced bending and unwinding may not be compatible with the conformation of the DNA double helix that is present in transcription factor−DNA complexes. The situation may be more complex in vivo, however, where genomic DNA is under considerably more constraint. Clearly, the transcription factors "see" the pyrimidine dimer-containing DNA substrates as nonrecognition sites.

Slow Repair of Promoters. In previous work on the human *JUN* promoter, we found that promoter sequences that contain binding sites for transcription factors are repaired very slowly (Tu et al., 1996); repair rates were up to 10 times slower in the promoter region compared to those of sequences near the transcription initiation site and transcribed sequences. Slow repair of promoter regions appears to be a more general phenomenon since it was also observed along the promoter of the human *PGK1* gene (Gao et al., 1994). In addition, promoters can be target areas for strongly increased formation of photoproducts, in particular at binding sites of certain transcription factors where the UV damage

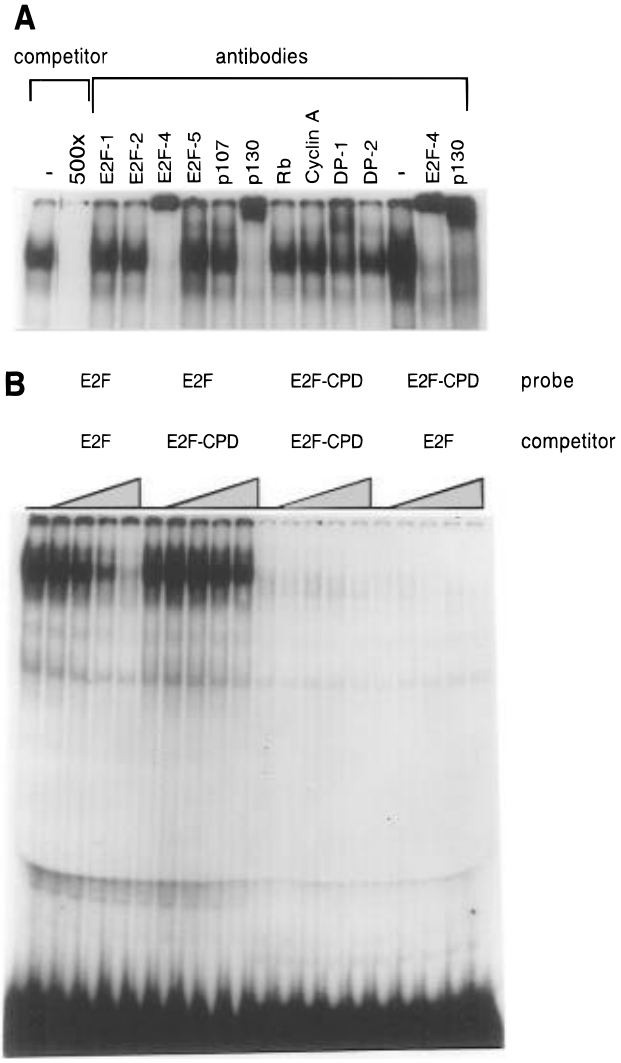

FIGURE 6: EMSAs of an oligonucleotide containing an intact E2F binding site and the same sequence containing a site-specific pyrimidine dimer. (A) Composition of the complexes analyzed with antibody supershift assays. Nuclear extracts from nonsynchronized fibroblasts were incubated with the antibodies indicated; − indicates no antibody. (B) In the lanes 1−10, the intact E2F binding site from the adenovirus E2 promoter was used as a probe. In lanes 11−20, the same sequence containing the site-specific pyrimidine dimer was used as a probe. Competition experiments were performed with increasing amounts of intact oligonucleotide or pyrimidine dimer-containing oligonucleotide as a competitor (0-, 3-, 10-, 30-, and 100-fold excess).

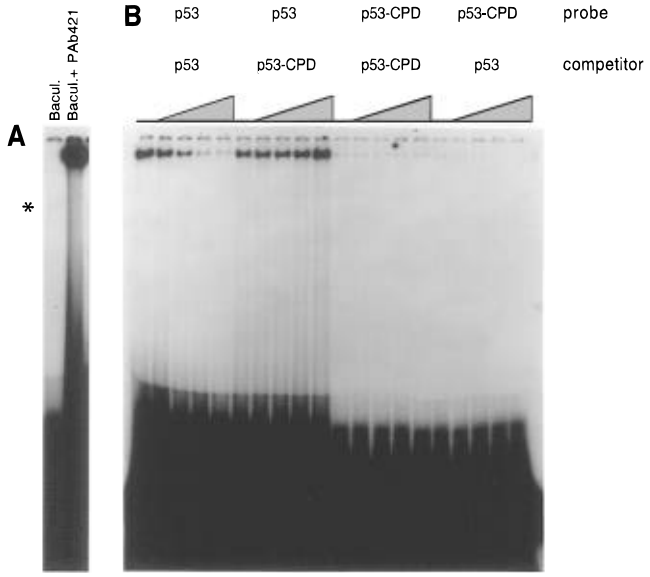

FIGURE 7: EMSAs with an intact p53 binding site and a pyrimidine dimer-containing site. (A) Nuclear extracts from a baculovirus-infected p53-enriched insect cell line were incubated with a ^{32}P-labeled, double-stranded oligonucleotide containing the p53 consensus binding site from the p21 gene. The asterisk marks a weak p53-specific complex in baculovirus extracts. A slower-migrating band of greatly increased intensity appears upon addition of a monoclonal antibody specific for p53 (PAb421, lane 2). (B) The same extracts containing the PAb421 antibody were assayed for the binding to an oligonucleotide containing the p53 consensus binding site and the respective dimer-containing sequence. In the left 10 lanes, the intact p53 oligonucleotide was used as a probe. In the right 10 lanes, the p53 oligonucleotide containing a site-specific pyrimidine dimer was used as a probe. Competition experiments were performed with increasing amounts of intact oligonucleotide or pyrimidine dimer-containing oligonucleotide as a competitor (0-, 3-, 10-, 30-, and 100-fold excess).

frequency can be increased by up to 30-fold relative to that of naked DNA or relative to those of sequences in the body of the gene (Pfeifer et al., 1992; Tornaletti & Pfeifer, 1995). In this context, it should be mentioned that it is unknown (and cannot easily be tested with existing methods) whether UV irradiation of a preformed protein−DNA complex leads to a detachment of the protein. However, protein−DNA complexes are always in an equilibrium with specific on and off rates for each factor. Thus, the factor is expected to dissociate from DNA either immediately during the occurrence of UV damage or some time thereafter, depending on the off rate. Thus, de novo binding and "rebinding" of a factor to its recognition site will be influenced by UV damage.

We hypothesize that this "vulnerability" and lack of repair of UV lesions in promoters together with a widespread inhibition of transcription factor binding could have direct consequences for transcriptional regulation and could cause a significant perturbation of gene expression in UV-irradiated cells. In this regard, it is also interesting that cancer predisposition in DNA repair-deficient diseases correlates with deficiencies in the genome-overall repair pathway but not with deficiencies in the transcription-coupled repair pathway (Bootsma et al., 1995). The genome-overall repair pathway is expected to be involved in repair of promoter DNA.

Biological Significance. The most important question to ask at this point is whether the effect of UV radiation on promoters can be biologically significant. It has long been known that UV irradiation causes a decrease in total RNA synthesis (Sauerbier & Hercules, 1978; Kantor & Hull, 1979; Mayne & Lehmann, 1982). This is generally believed to be a consequence of RNA polymerase arrest when encountering a transcription-blocking lesion on the template strand. Soon after UV irradiation, there is a recovery of RNA synthesis, and it was discovered later that this is related to the preferential repair of the transcribed strand of active genes (Bohr et al., 1985; Mellon et al., 1987). The rate of recovery of RNA synthesis may vary between genes and could be dependent on the initial lesion density on the transcribed DNA strand, the rate of repair, and the rate of transcription (Evans et al., 1996). For some genes, damage to the promoter may also be significant, but this has never been investigated. The first reason why this should be significant is that promoters can contain UV damage hot spots and are repaired much more slowly than the transcribed strand of

the gene (as discussed above), making the promoter-specific effect much longer-lasting. The second reason is that UV damage to the promoter not only may result in a decrease of gene expression (and would thus have a similar effect as damage in the transcribed strand) but also could result in an inappropriate *increase* in gene expression. The most plausible mechanism allowing this to occur is a UV photoproduct decreasing the binding affinity of a repressor protein, and increased transactivation would ensue due to a lack of repression. Our data on the E2F-4−p130 complex (Figure 6) show that this is a realistic possibility. Thus, the interference of UV-induced lesions with binding of E2F complexes could be of particular importance. Complexes of the E2F-4 protein and p130 are believed to function as transcriptional repressors of S-phase-specific genes during the G0 and G1 phases of the cell cycle (Tommasi & Pfeifer, 1995; Vairo et al., 1995). If a pyrimidine dimer interferes with binding of this complex, a repression specific for the G0 and G1 phases of the cell cycle may be released, leading to inappropriate activation of S-phase-specific genes. Some of the genes controlled by E2F complexes appear to function as key mediators of cell cycle progression. Among them are the *E2F-1* gene (Hsiao et al., 1994; Johnson et al., 1994; Neuman et al., 1994), the *cdc2* gene (Tommasi & Pfeifer, 1995), and several cyclin genes which contain E2F binding sites in their promoters (Schulze et al., 1995; Ohtani et al., 1995). Untimely activation of these genes due to DNA damage-induced lack of repression through E2F sites could lead to a loss of cell cycle control and, perhaps, initiation of DNA replication. Premature replication of a genome that was exposed to a DNA-damaging agent (in this case UV irradiation) could result in an increased level of genomic instability.

The changes in gene expression after UV irradiation may be only transient, if there is a re-establishment of the original expression pattern after the DNA damage has been repaired. However, what could be most detrimental are those changes that result in heritable changes in patterns of gene expression. Of particular importance could be autoregulatory networks of transcription factors in which, for example, a transcription factor regulates the expression of its own gene through the promoter of that gene. The *E2F1* gene may be an example for this (Hsiao et al., 1994; Johnson et al., 1994; Neuman et al., 1994). Once the genes were perturbed by DNA damage, such perturbations in expression levels of a particular gene could be maintained, perhaps even through cell divisions, due to the autoregulatory nature of such networks (MacLeod, 1996). In another scenario, it is conceivable that the loss of a bound transcription factor would cause methylation and subsequent complete inactivation of a promoter. The p16 gene (coding for an inhibitor of cyclin-dependent kinases and tumor suppressor) is often inactivated in tumors by promoter methylation through an unknown mechanism (Merlo et al., 1995). One possibility is that DNA damage is the event that triggers this inactivation. For example, it can be hypothesized that a UV-induced DNA adduct interferes with binding of a crucial transcription factor complex and, perhaps because transcription factors can keep CpG islands methylation-free (Pfeifer et al., 1990; Brandeis et al., 1994), there is a subsequent complete shutdown of the promoter by de novo DNA methylation. This scenario would require that the methylation enzymes not be inhibited by the UV lesion. There is also a strong possibility that, once one transcription factor is lost, the stability of other protein−DNA interactions is reduced because factors can stabilize each other in a higher-order complex at the promoter [e.g., Wright et al. (1994)].

It may be argued that these promoter-mediated effects are unlikely because of the limited target size for such events. However, the following considerations argue against this. Even if the target size of a promoter is only a few hundred base pairs, there are probably 100 000 human genes that could potentially be targeted. UV radiation damage produces significant amounts of DNA damage. After a moderate to severe sunburn, there can be approximately one cyclobutane dimer every 2−5 kb (Freeman et al., 1989; Rosenstein & Mitchell, 1991). The targeted cell population (sun-exposed skin) is also large, probably millions of cells. For a UV damage effect that decreases gene expression, the effects may be most severe for genes that are functionally hemizygous, for example X-linked genes and imprinted genes. For effects that result in an inappropriate increase in gene expression or activation of a silenced gene, the effects would become apparent even for autosomal genes (only one allele needs to be activated). However, sunlight appears to be such a powerful DNA-damaging agent and mutagen that the same gene is often mutated twice; for example, two mutations can be found on two separate p53 alleles (Ziegler et al., 1993).

In summary, the following arguments strongly suggest that the interference of UV damage with binding of transcription factors is biologically significant. (i) Promoters can contain UV damage hot spots. (ii) Promoters are repaired much more slowly than the transcribed sequences of a gene. (iii) Inappropriate increases in gene expression can result when a repressor cannot bind. (iv) There can be heritable changes in patterns of gene expression for autoregulated genes. (v) Loss of a bound transcription factor could cause methylation and subsequent complete inactivation of a promoter. (vi) There is a large number of human genes that can be targeted. (vii) UV radiation damage (sunburn) produces very significant amounts of DNA damage. (viii) A large number of different transcription factors are affected. The biological effects of ultraviolet (UV) radiation include premature skin aging and the development of skin cancer. Our hypothesis is that UV damage, besides being mutagenic, exerts much of its biological effect by perturbing gene expression through promoters. During photoaging, there could be a widespread perturbation of gene expression patterns caused by long-term UV exposure. During photocarcinogenesis, UV light could act by causing heritable changes in the gene expression patterns or by altering the expression of critical cell cycle control genes, leading to untimely replication of damaged DNA or loss of growth control.

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REFERENCES

Aboussekhra, A., & Wood, R. D. (1994) *Curr. Opin. Genet. Dev. 4*, 212−220.

Ausubel, F. M., Roger, B., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman, J. G., & Struhl, K. (1993) *Current Protocols in Molecular Biology Vol. 3*, J. Wiley, New York.

Balagurumoorthy, P., Sakamoto, H., Lewis, M. S., Zambrano, N., Clore, G. M., Gronenborn, A. M., Appella, E., & Harrington, R. E. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8591−8595.
Bohr, V. A. (1995) *Carcinogenesis 16*, 2885−2892.
Bohr, V. A., Smith, C. A., Okumoto, D. S., & Hanawalt, P. C. (1985) *Cell 40*, 359−369.
Bonfanti, M., Broggini, M., Prontera, C., & D'Incalci, M. (1991) *Nucleic Acids Res. 19*, 5739−5742.
Bootsma, D., Weeda, G., Vermeulen, W., van Vuuren, H., Troelstra, C., van der Spek, P., & Hoeijmakers, J. (1995) *Philos. Trans. R. Soc. London, Ser. B 347*, 75−81.
Brandeis, M., Frank, D., Keshet, I., Siegfried, Z., Mendelsohn, M., Nemes, A., Temper, V., Razin, A., & Cedar, H. (1994) *Nature 371*, 435−438.
Brash, D. E. (1988) *Photochem. Photobiol. 48*, 59−66.
Carty, M. P., Hauser, J., Levine, A. S., & Dixon, K. (1993) *Mol. Cell. Biol. 13*, 533−542.
Chiang, S.-Y., Welch, J., Rauscher, F. J., III, & Beerman, T. A. (1994) *Biochemistry 33*, 7033−7040.
Cleaver, J. E. (1983) *J. Mol. Biol. 170*, 305−317.
Cutts, S. M., Parsons, P. G., Sturm, R. A., & Phillips, D. R. (1996) *J. Biol. Chem. 271*, 5422−5429.
Devary, Y., Gottlieb, R. A., Lau, L. F., & Karin, M. (1991) *Mol. Cell. Biol. 11*, 2804−2811.
Devary, Y., Gottlieb, R. A., Smeal, T., & Karin, M. A. (1992) *Cell 71*, 1081−1091.
Donahue, B. A., Yin, S., Taylor, J.-S., Reines, D., & Hanawalt, P. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 8502−8506.
Dulic, V., Kaufmann, W. K., Wilson, S. J., Tlsty, T. D., Lees, E., Harper, J. W., Elledge, S. J., & Reed, S. I. (1994) *Cell 76*, 1013−1023.
Dyson, N. (1994) *J. Cell Sci. Suppl. 18*, 81−87.
Evans, M. K., Chin, K.-V., Gottesman, M. M., & Bohr, V. A. (1996) *Oncogene 12*, 651−658.
Fabbri, S., Prontera, C., Broggini, M., & D'Incalci, M. (1993) *Carcinogenesis 14*, 1963−1967.
Faisst, S., & Meyer, S. (1992) *Nucleic Acids Res. 20*, 3−26.
Fornace, A. J., Jr. (1992) *Annu. Rev. Genet. 26*, 507−526.
Freeman, S. E., Hacham, H., Gange, R. W., Maytum, D. J., Sutherland, J. C., & Sutherland, B. M. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5605−5609.
Friedberg, E. C., Walker, G. C., & Siede, W. (1995) *DNA repair and mutagenesis*, ASM Press, Washington, DC.
Gao, S., Drouin, R., & Holmquist, G. P. (1994) *Science 263*, 1438−1440.
Gibbs, P. E. M., Kilbey, B. J., Banerjee, S. K., & Lawrence, C. W. (1993) *J. Bacteriol. 175*, 2607−2612.
Gray, P. J. (1995) *Nucleic Acids Res. 23*, 4378−4382.
Hall, P. A., McKee, P. H., Menage, H. d. P., Dover, R., & Lane, D. P. (1993) *Oncogene 8*, 203−207.
Hanawalt, P. C., & Mellon, I. (1993) *Curr. Biol. 3*, 67−69.
Hansen, S., Hupp, T. R., & Lane, D. P. (1996) *J. Biol. Chem. 271*, 3917−3924.
Harrington, R. R., & Winicov, I. (1994) *Prog. Nucleic Acid Res. Mol. Biol. 47*, 195−270.
Hecker, D., Page, G., Lohrum, M., Weiland, S., & Scheidtmann, K. H. (1996) *Oncogene 12*, 953−961.
Herrlich, P., & Rahmsdorf, H. J. (1994) *Curr. Opin. Cell Biol. 6*, 425−431.
Hsiao, K.-M., McMahon, S. L., & Farnham, P. J. (1994) *Genes Dev. 8*, 1526−1537.
Huber, H. E., Goodhart, P. J., & Huang, P. S. (1994) *J. Biol. Chem. 269*, 6999−7005.
Hupp, T. R., & Lane, D. P. (1994) *Curr. Biol. 4*, 865−875.
Hupp, T. R., Meek, D. W., Midgley, C. A., & Lane, D. P. (1992) *Cell 71*, 875−886.
Johnson, D. G., Ohtani, K., & Nevins, J. R. (1994) *Genes Dev. 8*, 1514−1525.
Kantor, G. J., & Hull, D. R. (1979) *Biophys. J. 27*, 359−370.
Kerppola, T. K., & Curran, T. (1991) *Science 254*, 1210−1214.
Kim, J.-K., Patel, D., & Choi, B.-S. (1995) *Photochem. Photobiol. 62*, 44−50.
La Thangue, N. B. (1994) *Curr. Opin. Cell Biol. 6*, 443−450.
Ladomery, M., & Sommerville, J. (1995) *BioEssays 17*, 9−11.
Liu, F.-T., & Yang, N. C. (1978) *Biochemistry 17*, 4865−4876.
Lubin, D., & Jensen, E. H. (1995) *Nature 377*, 710−713.
Ma, L., Hoeijmakers, J. H. J., & van der Eb, A. J. (1995) *Biochim. Biophys. Acta 1242*, 137−164.
MacLeod, M. C. (1996) *Mol. Carcinog. 15*, 241−250.
MacLeod, M. C., Powell, K. L., & Tran, N. (1995) *Carcinogenesis 16*, 975−983.
Maltzman, W., & Czyzyk, L. (1984) *Mol. Cell. Biol. 4*, 1689−1694.
Mantovani, R., Pessara, U., Tronche, F., Li, X.-Y., Knapp, A.-M., Pasquali, J.-L., Benoist, C., & Mathis, D. (1992) *EMBO J. 11*, 3315−3322.
Mayne, L. V., & Lehmann, A. R. (1982) *Cancer Res. 42*, 1473−1478.
Mellon, I., Spivak, G., & Hanawalt, P. C. (1987) *Cell 51*, 241−249.
Merlo, A., Herman, J. G., Mao, L., Lee, D. J., Gabrielson, E., Burger, P. C., Baylin, S. B., & Sidransky, D. (1995) *Nat. Med. 1*, 686−692.
Mitchell, D. L., & Nairn, R. S. (1989) *Photochem. Photobiol. 49*, 805−819.
Murata, T., Iwai, S., & Ohtsuka, E. (1990) *Nucleic Acids Res. 18*, 7279−7286.
Neuman, E., Flemington, E. K., Sellers, W. R., & Kaelin, W. G., Jr. (1994) *Mol. Cell. Biol. 14*, 6607−6615.
Nevins, J. R. (1992) *Nature 358*, 375−376.
Nielsen, P. E. (1991) *Bioconjugate Chem. 2*, 1−12.
Ohtani, K., DeGregori, J., & Nevins, J. R. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 12146−12150.
Pfeifer, G. P., Steigerwald, S. D., Hansen, R. S., Gartler, S. M., & Riggs, A. D. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 8252−8256.
Pfeifer, G. P., Drouin, R., Riggs, A. D., & Holmquist, G. P. (1992) *Mol. Cell. Biol. 12*, 1798−1804.
Pil, P. M., & Lippard, S. J. (1992) *Science 256*, 234−237.
Ronchi, A., Bellorini, M., Mongelli, N., & Mantovani, R. (1995) *Nucleic Acids Res. 23*, 4565−4572.
Rosenstein, B. S., & Mitchell, D. M. (1991) *Radiat. Res. 126*, 338−342.
Rozek, D., & Pfeifer, G. P. (1993) *Mol. Cell. Biol. 13*, 5490−5499.
Sage, E. (1993) *Photochem. Photobiol. 57*, 163−174.
Sancar, A. (1995) *Annu. Rev. Genet. 29*, 69−105.
Sauerbier, W., & Hercules, K. (1978) *Annu. Rev. Genet. 12*, 329−363.
Schmitz, M. L., & Baeuerle, P. A. (1995) *Immunobiology 193*, 116−127.
Schreck, R., Zorbas, H., Winnacker, E.-L., & Baeuerle, P. A. (1990) *Nucleic Acids Res. 18*, 6497−6502.
Schreck, R., Albermann, K., & Baeuerle, P. A. (1992) *Free Radical Res. Commun. 17*, 221−237.
Schulze, A., Zerfass, K., Spitkovsky, D., Middendorp, S., Berges, J., Helin, K., Jansen-Dürr, P., & Henglein, B. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 11264−11268.
Smerdon, M. J., & Thoma, F. (1990) *Cell 61*, 675−684.
Smith, C. A., & Taylor, J.-S. (1993) *J. Biol. Chem. 268*, 11143−11151.
Smith, C. A., Wang, M., Jiang, N., Che, L., Zhao, X., & Taylor, J.-S. (1996) *Biochemistry 35*, 4146−4154.
Spivak, G., & Hanawalt, P. C. (1992) *Biochemistry 31*, 6794−6800.
Stein, B., Angel, P., van Dam, H., Ponta, H., Herrlich, P., van der Eb, A., & Rahmsdorf, H. J. (1992) *Photochem. Photobiol. 55*, 409−415.
Sun, D., & Hurley, L. H. (1994) *Gene 149*, 165−172.
Taylor, J.-S., Brockie, I. R., & O'Day, C. L. (1987) *J. Am. Chem. Soc. 109*, 6735−6742.
Thomas, D. C., & Kunkel, T. A. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 7744−7748.
Tommasi, S., & Pfeifer, G. P. (1995) *Mol. Cell. Biol. 15*, 6901−6913.
Tornaletti, S., & Pfeifer, G. P. (1995) *J. Mol. Biol. 249*, 714−728.
Tornaletti, S., & Pfeifer, G. P. (1996) *BioEssays 18*, 221−228.
Treiber, D. K., Zhai, X., Jantzen, H. M., & Essigmann, J. M. (1994) *Proc. Natl. Acad. Sci. U. S .A. 91*, 5672−5676.
Tu, Y., Tornaletti, S., & Pfeifer, G. P. (1996) *EMBO J. 15*, 675−683.

Vairo, G., Livingston, D. M., & Ginsberg, D. (1995) *Genes Dev. 9*, 869−881.

van Dam, H., Duyndam, M., Rottier, R., Bosch, A., Vries-Smits, L. d., Herrlich, P., Zantema, A., Angel, P., & van der Eb, A. J. (1993) *EMBO J. 12*, 479−487.

Venema, J., van Hoffen, A., Karcagi, V., Natarajan, A. T., van Zeeland, A. A., & Mullenders, L. H. F. (1991) *Mol. Cell. Biol. 11*, 4128−4134.

Waldman, T., Kinzler, K. W., & Vogelstein, B. (1995) *Cancer Res. 55*, 5187−5190.

Wang, C.-I., & Taylor, J.-S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 9072−9076.

Wang, C.-I., & Taylor, J.-S. (1993) *Chem. Res. Toxicol. 6*, 519−523.

Wright, K. L., Vilen, B. J., Itoh-Lindstrom, Y., Moore, T. L., Li, G., Criscitiello, M., Cogswell, P., Clarke, J. B., & Ting, J. P. (1994) *EMBO J. 13*, 4042−4053.

Xiong, Y., Hannon, G. H., Zhang, H., Casso, D., Kobayashi, R., & Beach, D. (1993) *Nature 366*, 701−704.

Zauberman, A., Barak, Y., Ragimov, N., Levy, N., & Oren, M. (1993) *EMBO J. 12*, 2799−2808.

Ziegler, A., Leffell, D. J., Kunala, S., Sharma, H. W., Gailani, M., Simon, J. A., Halperin, A. J., Baden, H. P., Shapiro, P. E., Bale, A. E., & Brash, D. E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 4216−4220.

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