UV-Induced DNA Damage Is an Intermediate Step in UV-Induced Expression of Human Immunodeficiency Virus Type 1, Collagenase, *c-fos*, and Metallothionein

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UV irradiation of human and murine cells enhances the transcription of several genes. Here we report on the primary target of relevant UV absorption, on pathways leading to gene activation, and on the elements receiving the UV-induced signal in the human immunodeficiency virus type 1 (HIV-1) long terminal repeat, in the gene coding for collagenase, and in the cellular oncogene *fos*. In order to induce the expression of genes, UV radiation needs to be absorbed by DNA and to cause DNA damage of the kind that cannot be repaired by cells from patients with xeroderma pigmentosum group A. UV-induced activation of the three genes is mediated by the major enhancer elements (located between nucleotide positions –105 and –79 of HIV-1, between positions –72 and –65 of the collagenase gene, and between positions –320 and –299 of *fos*). These elements share no apparent sequence motif and bind different *trans*-acting proteins; a member of the NF κ B family binds to the HIV-1 enhancer, the heterodimer of Jun and Fos (AP-1) binds to the collagenase enhancer, and the serum response factors p67 and p62 bind to *fos*. DNA-binding activities of the factors recognizing the HIV-1 and collagenase enhancers are augmented in extracts from UV-treated cells. The increase in activity is due to posttranslational modification. While AP-1 resides in the nucleus and must be modulated there, NF κ B is activated in the cytoplasm, indicating the existence of a cytoplasmic signal transduction pathway triggered by UV-induced DNA damage. In addition to activation, new synthesis of AP-1 is induced by UV radiation.

Under severe stress, e.g., replication arrest by UV-induced DNA damage, many bacteria mount a productive response which involves the new synthesis of a large number of specific gene products (39, 74). The response has been named SOS (51), and it is triggered by the activation of preexisting *recA* protein and its increased synthesis, probably due to the interaction of *recA* protein with single-stranded regions of DNA (for a review, see reference 74).

Mammalian cells in culture react to UV irradiation with a number of genetic changes resembling those in bacteria (for reviews, see references 29–31, 52, 60). These changes include the altered expression of specific genes (see B. Kaina, B. Stein, A. Schöenthal, H. J. Rahmsdorf, H. Ponta, and P. Herrlich, in M. W. Lambert et al., ed., *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells*, in press, for a recent list of references). Many genes are induced, e.g., plasminogen activator (46), collagenase and metallothionein genes (2, 4), the cellular oncogene *fos* (5), and a human retrovirus, the human immunodeficiency virus type 1 (HIV-1) (B. Stein, cited in reference 29; 30, 69, 73).

In an attempt to identify the elements of signal transduction to these genes, we show here that UV-induced gene expression is preceded by and requires absorption of UV radiation by DNA and UV-induced covalent DNA changes (photoproducts). Moreover, we define the DNA elements in three of the responsive genes (HIV-1, the collagenase gene, and *fos*) which receive the signals and show that the transcription factors binding to these elements are modulated as a result of UV irradiation.

MATERIALS AND METHODS

Plasmids and oligonucleotides. The HIV-1-CAT constructs have been described previously (47, 48). They were re-cloned, as were all other gene constructs used, into the vector pBLCAT5; this vector was derived from pBLCAT3 (44) by deletion of a UV- and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-responsive element (C. Jonat, unpublished results). –105/–79(HIV)-TATA-CAT was generated by cloning the two HIV enhancer repeats from position –105 to –79 (with *Hind*III-*Bam*HI linkers) into the *Hind*III-*Bgl*II site of PL-T, a plasmid which was obtained from PL-TG (63) by removing the *Xho*I fragment carrying the G-free cassette. The collagenase-CAT constructs have been described previously (2, 3). The construct –517/+63 mutTREColICAT, in which the TRE between –72 and –65 has been mutated, was derived from the collagenase constructs –517/–70 tkCAT and –60/+63 CAT. –517/–70 tkCAT was cut with *Bam*HI (which cuts behind position –70), blunted, and recut with *Nco*I (which cuts in the CAT gene); –60/+63 CAT was cut with *Hind*III (which cuts at –60), blunted, cut with *Nco*I, and ligated into the former plasmid. Sequencing showed that the collagenase sequence –73 ATGAGTCAGACAC –61 was replaced by the sequence –76 ATGACGGATCAGCTT –61. The *fos* construct –320/–299 tkCAT was obtained by cloning an oligonucleotide containing the *fos* sequences between positions –320 and –299 (with *Acc*I 5' overhangs) into the *Acc*I site of pBLCAT4. pBLCAT4 (tkCAT in Fig. 7) has been derived from pBLCAT2 (44) by removing a UV- and TPA-responsive DNA element (C. Jonat, unpublished results).

The oligonucleotides shown in Fig. 1 were synthesized by using a Pharmacia Gene Assembler.

Cell culture. HeLa tk[–] cells were grown in Earle modified

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-105 -79

^{3'}GATCCGGAGGGACTTTCCGCTGGGGACTTTCACGCC^{3'}

 GCCTCCCTGAAAGCGCACCCCTGAAAGGTGGCCTAG_{5'}

5' AGCTTGGGGACTTTCCAGCCG 3'
3' ACCCCTGAAAGGTCGGCCTAG 5'

5' AGCTTGCTCACTTTCCAGCCG 3'
3' ACGAGTGAAAGGTCGGCCTAG 5'

5' AGCTTG⁻⁷²GTGAGTCAG⁻⁶⁵CCG 3'
3' AACTCAGTCGGCCTAG 5'

5' AGCTTGACCTCTGGCTTCCG 3'
3' ACTGGAGACCGAAAGGCCTAG 5'

-323 -292
 5' GACACAGGATGTCCATATTAGGACATCTGCGGCGGTC 3'
 3' CTGTGTCCTACAGGTATAATCCTGTAGACGCCGCCAG 5'

Eagle medium supplemented with 10% fetal calf serum (FCS). Primary human skin fibroblasts from a healthy 29-year-old male and from a patient with xeroderma pigmentosum group A (a gift from J. Cleaver, University of California, San Francisco, Calif.) were grown in Dulbecco modified Eagle medium supplemented with 10% FCS. Both media were supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml).

For UV irradiation, cells were washed twice with phosphate-buffered saline and irradiated without phosphate-buffered saline with a dose of 20 J/m² unless indicated otherwise, and the original medium was added back to the cells. For control cultures the same procedure was followed, but the UV treatment was omitted. TPA (in dimethylsulfoxide) was added to a final concentration of 60 ng/ml; 4-nitroquinolineoxide (NQO) (in ethyl alcohol) was added to a concentration of 1.3 μ M, and mitomycin C (MMC) was

UV sources. For a continuous UV spectrum between 240 and 303 nm, we used a 1,000-W Hg arc lamp equipped with a prism monochromator as described elsewhere in detail (77). Monochromatic UV lamps (15-W Hg lamp) emitting three defined wavelengths (254 nm [half-maximum width, 2.3 nm], 345 nm [half-maximum width, 43 nm], 360 nm [half-maximum width, 35 nm]) were obtained from Vetter (Heidelberg, Federal Republic of Germany).

All procedures were done at 4°C; dithiothreitol and phenylmethylsulfonyl fluoride were added as fresh solutions. Protein concentrations of the extracts were determined by the method of Lowry et al. (41). Gel retardation assays were done as described previously (10), with slight modifications: 5 µg of nuclear extract was incubated with 0.5 µg of poly(dI-dC) · poly(dI-dC) (Pharmacia), and then 2 fmol of end-labeled oligonucleotide was added. After 30 min at room temperature, the samples were subjected to electrophoresis on a 4% polyacrylamide gel.

Action spectrum of UV-induced gene expression. The primary target of UV irradiation could be any one of a large number of molecules carrying chromophores that absorb UV, e.g., aromatic systems and conjugated double bonds (thus including as candidates nucleobase compounds, tyrosine-containing substances, steroids, and pyrrole rings). These chromophores are characterized by their UV absorption spectra. To determine which of these transmit the relevant stimulus to the UV-inducible genes, we measured an action spectrum of gene induction. The action spectrum should be equivalent to the absorption spectrum of the compound and should thus give us clues about its chemical nature. Primary human skin fibroblasts or HeLa cells were irradiated with UV sources that emit different defined spectra (see Fig. 2 legend) or with monochromatic UV radiation of wavelengths between 240 and 303 nm (Fig. 2). An attempt was made to determine optimum curves of gene induction for each wavelength. Transcripts of the endogenous collagenase and *c-fos* genes were determined, and some of the experiments have been repeated, with similar outcome, with

FIG. 2. Action spectrum of UV-induced gene expression. The efficiency of radiation of various wavelengths in the activation of genes was determined. The relative action per quantum obtained with a half-maximum dose was plotted versus wavelength. Gene activity was measured by S1 nuclease mapping of poly(A)⁺ RNA transcribed from the endogenous *fos* and collagenase genes (in primary human skin fibroblasts). RNA was prepared 45 min (for the determination of *fos* mRNA) and 36 h (for the determination of collagenase mRNA) after irradiation of the cells, time points at which the two RNA species are accumulated maximally. For control purposes the RNA was also hybridized to a cDNA clone derived from a gene which is not influenced by UV irradiation (K7 [4]). HIV-1 activity of a transiently transfected HIV CAT plasmid (−121/+232 HIV CAT in HeLa tk[−] cells) was determined by CAT assay. The action spectrum of cell killing (Chinese hamster cell line V79) was redrawn from reference 77. Cells were irradiated as described in Materials and Methods. For wavelengths from 240 to 303 nm, we used a prism monochromator, and for 345 and 360 nm, monochromatic UV lamps were used (the widths of the spectra at half-maximum intensity were 43 and 35 nm, respectively).

transiently transfected collagenase-CAT gene constructs. Examples of dose-response curves are shown in Fig. 3. We then plotted the relative action per quantum obtained with a half-maximum dose versus wavelength (Fig. 2). We did not always obtain a satisfactory dose-response curve. This may account for some of the variability. For instance, with 303 nm, 1,200 J/m² was the highest dose applied and the only point marking a presumed plateau level. However, the relative efficiency of radiation of 303 nm is so low that an error by a factor of 2 would not change the principal statement.

The action spectra for the activation of all UV-responsive genes examined peaked at 265 to 275 nm and fell off steeply at longer wavelengths. Half-maximum induction per quantum was seen with 280 to 290 nm. At 303 nm, the efficiency was down to below 1/60 of the peak value. No gene activation was found with irradiation of 345 and 360 nm at doses up to 2,000 J/m², indicating an induction efficiency of less than 1/3,000 of maximum efficiency at 270 nm. A lamp emitting a visible spectrum (but no UV) did not activate any of the genes discussed, even at doses up to 600,000 J/m² (data not shown). The spectra between 240 and 303 nm are superimposable on the cell-killing spectrum obtained with the same UV lamp (Fig. 2; redrawn from reference 77) and the spectrum of T-T dimer formation (57). Phage inactivation and absorption of isolated DNA in parallel experiments, however, peaked at 260 nm (not shown), a difference which has been attributed to preferential absorption of short-wavelength UV radiation in the cytoplasm shifting a nuclear

DNA absorption spectrum towards longer wavelengths (59). The most likely interpretation of the spectra is that UV radiation is absorbed by a nucleobase-containing compound such as NAD, RNA, or DNA. Other aromatic compounds could also be the targets. However, proteins and compounds with conjugated double bonds, such as those found in various membrane constituents, are ruled out (55).

Dose-response curves of gene expression in DNA repair-deficient cells. If DNA were the primary target leading to gene activation, pyrimidine-pyrimidone 6-4 photoproducts or T-T dimers would result and we could investigate whether the UV-induced DNA damage was an intermediate in the induction process. Towards this end, we determined the dose-response curves of gene activation in primary skin fibroblasts, either from a healthy individual or from a patient with xeroderma pigmentosum group A (XPA). These cell types differ in ability to remove UV-induced DNA lesions (16). Thus, after sufficient periods of time, the number of residual lesions should diverge, diminishing progressively in wild-type cells while remaining constant in XPA cells, or a larger dose of UV radiation should be required to obtain a level of DNA lesions in wild-type cells equivalent to that in XPA cells at a given time. If DNA damage were a necessary intermediate in gene induction, doses required would differ correspondingly. For all genes except *fos*, a striking difference in the dose-response curves was observed. In XPA cells, about 2 to 5 J/m² sufficed for optimal induction, while in wild-type cells, a more than 10-fold-larger dose was required (Fig. 3). Similar dose-response curves were ob-

FIG. 3. DNA damage as an intermediate in UV-induced gene expression. Gene activation by various doses of radiation at 254 nm was measured in primary human skin fibroblasts from a healthy individual (wt) and from a patient with xeroderma pigmentosum group A (XPA). HIV-1 transcription was determined by transfecting transiently -121/+232 HIV CAT and measuring CAT activity. Collagenase and metallothionein IIA mRNA accumulation was measured 36 h after irradiation by Northern RNA transfer hybridization of the endogenous RNA by using radioactive cDNA probes. Controls for the amount of RNA used were performed as described for Fig. 2.

tained in either cell line when the activation of transiently transfected (Fig. 3 [HIV-1]) or endogenous (Fig. 3 [collagenase and metallothionein II A]) genes was compared, indicating the same activation mechanism. Also, the activation of transiently transfected collagenase-CAT gene constructs showed the same difference in UV dose dependence between normal and XPA fibroblasts. With *fos*, this type of experiment is not informative, because UV-induced *fos* gene activation is already turned off at 30 min after induction (15), at which time the number of UV-induced lesions may not yet sufficiently differ between wild-type and XPA cells (1). In contrast, UV irradiation enhances the transcription from the genes shown in Fig. 3 for several hours and later time points

TABLE 1. MMC activation of HIV-1 and collagenase UREs^a

Construct	CAT activity (pmol/mg per min) with:		Fold induction
	No treatment	MMC	
-121/+232 HIVCAT	58.3	308.9	5.3
-76/+232 HIVCAT	2.5	1.1	0.5
5 $\times$ (-73/-65) ColltkCAT	19.0	81.4	4.3
tkCAT	3.3	0.9	0.3

^a HeLa tk⁻ cells were transfected with 10 μg of the gene constructs indicated. After 8 h the cells were treated with MMC (10 $\mu\text{g}/\text{ml}$) or left untreated. The CAT activity of the cell extracts was determined 42 h later.

can be compared (see below). These data clearly speak for an involvement of a DNA lesion (one of those that is not repaired in XPA cells) in UV-induced gene activation and for DNA as the primary target of UV absorption.

Similarly to UV, other DNA-damaging agents can trigger the expression of genes. Thus, as in bacteria, other DNA lesions can be intermediates in the induction process. Such lesions are introduced by MMC, gamma irradiation, and NQO. Transcriptional responses to these agents have been described elsewhere (4, 53, 73; B. Stein, unpublished results) and are shown in Table 1 (also see Fig. 6B). Subsequent to DNA damage, the DNA repair enzymes further change the DNA structure, e.g., by incision and excision. These secondary structural changes are probably not necessary for the UV response, since XPA cells cannot initiate these repair processes and still mount the UV response.

Since the agents mentioned above interfere with the cell cycle, we considered whether an arrest of replication at the UV-induced lesion is necessary. This is not the case, because UV induction of all genes examined follows similar kinetics and reaches a similar magnitude in G_0 cells as in dividing cells (shown for *fos* and the collagenase gene in Fig. 4). We conclude that the altered DNA structure itself forms the inducing signal.

Transcriptional regulation and UV-responsive DNA elements. UV irradiation leads to an increased abundance of mRNA for all UV-responsive genes examined in our laboratory: these include metallothionein (Fig. 3) (4), collagenase (Fig. 2-5), HIV-1 (Fig. 5), *jun* (not shown; results obtained in collaboration with Peter Angel and Michael Karin), and *fos* (Fig. 4) (5). The increased abundance reflects an increased rate of transcription. We have examined the

FIG. 4. UV irradiation-induced gene expression in noncycling cells. Primary skin fibroblasts (A) and cells from a patient with XPA (B) were grown in 10% FCS or starved for 48 h in 0.5% FCS. Labeling indices after a 4-h pulse with tritiated thymidine were 17 to 20% and 2%, respectively. Cells were irradiated with 30 J/m^2 (A) and 2 J/m^2 (B). RNA was prepared at 45 min (A) and 36 h (B) after irradiation and probed by Northern RNA transfer hybridization for *fos*- (A) and collagenase- (B) specific RNA with radioactive cDNA probes. Lanes 1, untreated; lanes 2, UV irradiated. Controls were performed as described for Fig. 2.

FIG. 5. Activation by UV and phorbol ester of HIV-1 and collagenase promoter-controlled mRNA accumulation. (A) HeLa tk⁻ cells were transiently transfected with 5 μ g of -121/+232 HIV CAT and 5 μ g of 5x(-73/-65) ColltkCAT and induced for 30 min with either 60 ng of phorbol ester (TPA) per ml or with 20 J/m² of UV radiation (254 nm) or were left untreated (con). Total cytoplasmic RNA (20 μ g) was analyzed by RNase protection. As SP6 probe, the 360-base-pair *SalI-XhoI* fragment isolated from the plasmid -76/+232 HIV CAT and recloned into SP65 (45) served to detect HIV-1 RNA. A corresponding SP6 probe for collagenase RNA has been described elsewhere (3). These probes generate RNase-protected fragments of 232 (HIV) and of around 210 (Coll.) nucleotides (see references 2 and 3). M, Marker lane. (B) HIV transcripts were determined in UV-treated cells that were permitted to accumulate RNA in the absence or presence of 10 μ g of cycloheximide (Cx) per ml for 9 h. Controls for the amount of RNA used were performed as described for Fig. 2.

UV inducibility of chimeric constructs consisting of 5'-flanking sequences of the UV-inducible genes, of their own or a heterologous promoter, and of the CAT reporter gene. These constructs were transfected transiently into various cells, and either CAT RNA or CAT enzyme activity was measured. In all cases these constructs were UV inducible, whereas the corresponding vectors were not. For instance, the following constructs retain UV inducibility: an HIV-1 gene construct with replacement of HIV-1 sequences by CAT at +5 (Fig. 6 and 7A); the collagenase gene (-139/-42)tk CAT (Fig. 7B), in which collagenase gene sequences drive the promoter of the herpes simplex virus thymidine kinase (tk) gene; and Fos (-711/+45) CAT (not shown) and Fos (-320/-299)tk CAT (Fig. 7C). This localizes the regulatory properties in the 5'-flanking DNA of these genes and indicates transcriptional regulation.

Fast kinetics of induction. *fos* RNA accumulation is induced maximally at 10 to 15 min after UV irradiation of cells (15) and does not depend on ongoing protein synthesis. Similar kinetics are seen after treatment of cells with phorbol ester tumor promoters. In contrast, the accumulation of collagenase RNA after UV (not shown) or phorbol ester treatment (2) is delayed and depends at least partly on ongoing protein synthesis. When we used the minimal collagenase 5x (-73/-65)tk CAT constructs which carry, as will be shown below, only the UV-responsive element as a pentameric repeat, UV-induced RNA accumulation was easily detectable at 30 min after UV treatment (Fig. 5A). Obviously the collagenase enhancer is an absolute prerequisite for UV (and phorbol ester) induction of the collagenase gene, since destruction of the enhancer abolishes UV- (Fig. 7B) and phorbol ester- (2, 3) induced gene activation. Other promoter elements, however, modulate the course of induc-

FIG. 6. The HIV-1 enhancer responds to UV radiation, phorbol ester, and NQO. (A) The HIV CAT constructs indicated (10 μ g each) were transiently transfected into HeLa tk⁻ cells. The cells were UV irradiated (20 J/m²) 8 h later (UV) or left untreated (con). Determination of CAT activity was done 42 h after treatment. (B) Experimental procedures were as described for panel A. -633/+80 mutHIVCAT contains point mutations in both enhancer repeats (48). TPA treatment was with 60 ng/ml, and NQO treatment was with 1.3 μ M. Ethanol (EtOH) is the solvent of NQO.

tion of the endogenous collagenase gene in a manner not understood at this time. Likewise, HIV transcription was detectable early (-121/+232 HIVCAT in Fig. 5A). HIV RNA started at the natural cap site. The rapid response resembled that seen after treatment of cells with phorbol ester. The presence of an inhibitor of protein synthesis (cycloheximide) could not prevent UV induction (shown for HIV-1 in Fig. 5B). Thus, at least for the minimal gene constructs carrying the UV-responsive elements, the response to UV is direct and rapid.

Different UV-responsive elements (UREs) in HIV-1, collagenase, and *fos* genes. The finding that transiently transfected chimeric gene constructs retained the ability to respond to UV irradiation permitted conventional deletion mapping of the *cis*-acting sequences required for the UV response.

The URE of HIV-1 coincides with the HIV enhancer region, which was shown to be necessary for basal-level expression (56) and phorbol ester induction (18, 34). The HIV enhancer contains two direct repeats which were also found in the enhancer regions of simian virus 40 (SV40) (28, 75), cytomegalovirus (13), the kappa immunoglobulin light chain (49, 65), class I genes of the major histocompatibility complex (9), and the interleukin-2 receptor α chain gene (12, 38).

A construct containing viral sequences from positions -633 to -453 and the HIV-1 3' LTR from positions -453 to +80 in front of the CAT gene (48) was induced by UV, as well as by phorbol ester and NQO, 10- to 15-fold (Fig. 6B). Removal of the target of the HIV-1-encoded transactivator protein (tar region -17/+80 [56]) did not affect UV responsiveness; the induction levels by UV of tar⁺ (-121/+232 and

–121/+80) and *tar*[–] (–121/+5) constructs were identical (Fig. 6A and 7A). Furthermore, we could not find any significant influence of the described negative regulatory elements located between positions –340 and –185 (56) and between positions –173 and –159 (22), nor of a Jun/Fos-binding site between positions –357 and –316 (21), on either UV or phorbol ester induction (compare –633/+80 with –121/+232 or –121/+80; Fig. 7A). We have no explanation at this time for the increased basal expression from the –121/+20 construct compared with that from the constructs –121/+232, –121/+80, and –121/+5.

Mutations of the enhancer, however, destroyed the response of the gene. For instance, point mutations in the repeats diminished both the basal level and the UV- or phorbol ester-induced level of expression (Fig. 6B and 7A). Deletion of one repeat (–91/+232) reduced the basal level of expression, and deletion of both repeats (–76/+232) further decreased the basal level of expression. Upon deletion, in addition, of all three Sp1-binding sites (–29/+232 [32]), basal expression became undetectable. Only constructs containing at least one enhancer repeat were fully inducible by UV (–121/+232 and –91/+232). The unresponsiveness of the enhancerless mutant –76/+232 was not due to undetectable low-level expression of this gene construct; in several experiments the expression from this construct was significantly higher compared with that from constructs lacking one or several of the Sp1-binding sites (–65/+232 and –29/+232 [Fig. 7A]), but the residual transcription was not inducible by UV radiation. Note that all constructs used here have been recloned into a UV unresponsive vector (pBLCAT5; C. Jonat, unpublished results). In a previous report (69), the enhancerless 5' deletion (–76/+232) retained residual UV responsiveness because of a coincidental AP-1 site in the vector pBLCAT3, from which pBLCAT5 has been derived by removal of this site. To prove that the HIV-1 enhancer suffices to confer UV responsiveness, a synthetic oligonucleotide containing both enhancer repeats from –105 to –79 was cloned in both orientations in front of the TATA-box of the *Xenopus* albumin gene. These constructs were fully inducible by UV radiation (Fig. 7A). Several control plasmids expressing CAT mRNA and enzyme either constitutively or under the influence of steroid hormone showed that UV radiation does not alter the fate of CAT RNA or CAT protein (examples are shown in Fig. 7).

The collagenase gene URE is identical with a *cis*-acting element that had been characterized previously as conferring the responses to growth factors, phorbol esters, and oncogenes (3, 62). UV responsiveness of the collagenase promoter was reduced upon deleting from position –517 to –73 and was lost upon deleting to position –60 or upon destruction of the URE (Fig. 7B). The collagenase 5'-flanking sequence –73/–42 sufficed to confer UV regulation to the heterologous tk promoter. The sequence –517/–70, however, had no such property on its own. Also, the sequence –66/–42 did not confer UV responsiveness. A synthetic oligonucleotide corresponding to the sequence –73/–65, on the other hand, totally sufficed to subject the tk promoter to

	-73	-65
human collagenase	ATGAGTCAG	
	-105	-93
HIV-1 repeat 2	AGGGACTTTCGC	
	-91	-79
HIV-1 repeat 1	GGGGACTTTCAG	
	-320	-299
human c-fos	GGATGTCCATATTAGGACATCT	
	+18	+38
human c-fos	AGCGAGCAACTGAGAAGCCAA	

FIG. 8. Sequences of UREs.

UV regulation. A pentameric repeat of this sequence restored the efficiency and inducibility of the complete promoter (Fig. 7B).

The UREs of HIV-1 and collagenase also conferred inducibility by MMC (Table 1). HeLa tk[–] cells transfected with the constructs –121/+232 HIV CAT or 5x(–73/–65) ColltkCAT were induced by MMC by five- and fourfold, respectively. The enhancerless constructs –76/+232 HIV CAT and the tk CAT vector did not respond.

As is the case for collagenase, the *cis*-acting elements of the *fos* promoter that are responsible for UV-, phorbol ester-, and growth factor-dependent activation are identical (15). The major URE is located between positions –320 and –299 (dyad symmetry element; serum response element [SRE] [70]). A 5' deletion mutant lacking the –320/–299 sequence (–96) showed only residual activation by phorbol ester, growth factors, or UV radiation. This residual responsiveness depended on the presence of a site within the transcribed untranslated region (+12 to +45 [15]) where we have also seen the binding in vitro of a protein (strongly protected DNase I footprint from +18 to +38 [not shown] and sequence shown in Fig. 8). The –320/–299 sequence, however, sufficed to confer UV responsiveness to either a –96 deletion mutant (15) or to the tk promoter (Fig. 7C). An SRE which is mutated at two positions and which does not bind the serum-responsive factor (25) could not mediate induction by UV (not shown).

The structure of the UREs is shown in Fig. 8. The UREs differ from one gene to the next. All three, however, mediate the response to either UV, other DNA-damaging agents, phorbol esters, or growth factors.

Different transcription factors are the terminal stations of UV-induced signal transfer. The UREs show no sequence similarity (Fig. 8). Thus, it is likely that each URE binds a different specific transcription factor whose activity is enhanced upon UV irradiation of cells. To directly show the binding of different factors and to determine possible changes in amount or DNA-binding activity of these transcription factors, the binding in vitro of proteins to synthetic URE oligonucleotides was quantitated by gel retardation (shown for the HIV-1 and collagenase URE in Fig. 9). To this end, each radioactive oligonucleotide was incubated with either cytoplasmic or nuclear extracts from HeLa cells.

The HIV enhancer oligonucleotide formed in vitro a specific major (arrowhead) and a minor DNA protein com-

FIG. 7. Deletion analysis of the HIV-1, collagenase, and *fos* promoters. ■, Untranslated regions with their 5' and 3' deletion endpoints; light interruptions in the dark bars indicate point mutations in the enhancers (see Materials and Methods). □, CAT-coding region. The herpes simplex virus thymidine kinase promoter from position –105 to +51 (tk) and the TATA box derived from the albumin promoter (TATA) are indicated. HIV-1 (A), collagenase (B), and *fos* (C) promoter constructs were transiently transfected into HeLa tk[–] cells (A and B) or primary human fibroblasts (C). At 42 h after UV irradiation, cells were harvested and CAT activity was determined. Shown are the results from one representative experiment. Most of the transfections were repeated eight times, with similar outcomes. All gene constructs contain the same vector background (the promoterless pBLCAT-5 and the tk promoter vector pBLCAT-4).

plex when incubated with a nuclear extract from UV-irradiated cells (Fig. 9A). These complexes did not comigrate with the protein complex formed by the collagenase URE and could not be eliminated by 100 to 500 molar excess of either a mutated nonfunctional HIV-1 URE (mHIV), the collagenase gene URE (coll1), or the *fos* URE. Also, another unrelated sequence (−62/−50, coll2) could not compete for binding, in contrast to the authentic HIV enhancer oligonucleotide, which competed efficiently in the formation of both complexes. We noted that a larger excess of coll1 did compete with HIV. This indicates competition for a shared factor (Fos), which will be the subject of a separate paper.

Corresponding results were obtained with the UREs of the collagenase and the *fos* genes. At the ³²P-labeled oligonucleotide comprising the collagenase URE sequence, a single DNA-protein complex was formed (Fig. 9A) which migrated to a position identical to that obtained with purified AP-1 (Jun/Fos) protein (not shown). The specific interaction was prevented by excess amounts of the homologous URE but not by excess amounts of the HIV or *fos* UREs (Fig. 9A), indicating that the factor binding to the collagenase URE cannot recognize the two other enhancers. The UV response element of the *fos* gene formed two specific protein complexes with nuclear extracts from HeLa cells, which were efficiently eliminated by competition with the homologous URE but not by the UREs of the HIV or the collagenase gene (not shown).

Thus, at least the DNA-binding domains of the transcription factors binding to the three UREs must be totally different. UV cross-linking of the labeled oligonucleotide to the binding proteins in vitro and precipitation of the cross-linked material with anti-Fos antibodies have shown that the collagenase URE is recognized by the AP-1 complex (heterodimer of Fos and Jun; B. Stein, unpublished results). Because the major complex at the HIV sequence was formed in an identical manner with cell extracts from the UV-induced human T-cell lines Jurkat and Molt-4 (which are known to contain the transcription factor NFκB; 26, 48, 65) and because it shared biochemical properties with NFκB (see below), we assume that the UV-induced complex is formed by NFκB. The proteins binding to the *fos* URE (not shown) are known to represent the serum response factor (72) and a 62-kilodalton protein (66).

The extracts used to determine the binding specificity to the three UREs were prepared from cells harvested at 30 min after UV treatment, a time point at which UV-induced RNA accumulation is clearly visible (Fig. 5). When we performed the same binding experiments with cell extracts from nonirradiated cells, we found the same DNA-protein complexes (Fig. 9B and C) and the same behavior in competition experiments (not shown), suggesting that the qualitative composition of the protein-DNA complexes was not changed by UV irradiation. The amount of binding activity for two of the UREs, collagenase and HIV, was, however, changed upon treatment of the cells with UV radiation or the

phorbol ester TPA (Fig. 9B). Binding activity was enhanced as early as 30 min after treatment. The course of further enhancement differed between the cells treated with UV radiation and those treated with phorbol ester. Phorbol ester induced binding activity somewhat earlier than UV radiation. Whereas this late increase of binding to the collagenase URE was inhibited by the protein synthesis inhibitors anisomycin and cycloheximide, the phorbol ester- and UV-induced binding to the HIV URE was not (not shown). This suggests that the increased binding to the collagenase URE is due to UV- (or phorbol ester-) induced synthesis of AP-1 (Fos and Jun), while the increased binding to the HIV enhancer (and the early increase in binding to the collagenase enhancer) is due to posttranslational modification. The in vitro binding of protein factors to the *fos* URE was not detectably altered by UV radiation or phorbol ester (not shown) as has been reported for most other conditions (50, 67, 71).

UV-induced signal transduction can pass through the cytoplasm. Since NFκB is known to be activated by release from an inhibitor in the cytoplasm (7, 8), the UV-induced activation of NFκB suggested that the signal pathway passed through the cytoplasm. To document UV-induced cytoplasmic activation, we examined cytosolic extracts for DNA-binding activity. Figure 9C shows a comparison of gel retardation experiments performed with nuclear and cytoplasmic extracts from treated and untreated cells. To allow for quantitative comparison, the amount of cytoplasmic and nuclear extract used for complex formation was derived from the same number of cells. HIV enhancer-binding activity was found in the nucleus and in the cytoplasm of growing HeLa cells. In untreated cells most of the binding activity was found in the cytoplasm. Treatment of the cytoplasmic extract with the ionic detergent deoxycholate enhanced the binding activity considerably, suggesting that the binding protein is indeed NFκB, which is liberated by detergent treatment from its inhibitor, IκB (7, 8). After UV treatment of cells, the amount of binding activity in the cytoplasm declined, whereas it was found to be increased in the nuclear fraction. UV-induced NFκB accumulation in the nucleus occurred also in the presence of protein synthesis inhibitors (not shown). This suggests that UV radiation, like phorbol ester, causes the modification of the inhibitor, releasing active NFκB into the nucleus. In contrast to the findings with the HIV-1 enhancer, cytosolic extracts contained no collagenase gene URE-binding activity, indicating that these proteins were taken up by the nucleus immediately upon synthesis and also indicating that the proteins did not leak from the nuclei during preparation (Fig. 9C). Modification of these proteins upon treatment of cells with UV radiation or phorbol ester thus probably occurs in the nucleus, although the signal transduction pathway may pass through the cytoplasm. Most of the SRE-binding activity was found in the nucleus of the cell (not shown). Signal transduction to the SRE-binding factors occurred in the presence of cyclohexi-

FIG. 9. Protein complexes formed in vitro with the HIV-1 and collagenase UV-responsive element. (A) The responsive elements bind different transcription factors. Band shifts were performed with nuclear extracts prepared from HeLa tk[−] cells 30 min after irradiation with 30 J/m² of UV radiation. At the bottom of the figure, the radiolabeled oligonucleotides used in the bandshift reactions are indicated (see Materials and Methods). The molar excess of cold oligonucleotides used for competition is indicated at the top. Arrows indicate the major specific retarded protein-DNA complexes. (B) UV and TPA modulation of binding activities to the UREs. Nuclear extracts from control cells and from cells treated with UV or TPA for the times indicated were incubated with the labeled oligonucleotides and subjected to gel electrophoresis. (C) Partition of binding activity between nucleus and cytoplasm. Nuclear and cytoplasmic extracts were prepared from control cells and from cells in which binding activity was maximally induced (see panel B; HIV, UV for 8 h and TPA for 1 h; collagenase, UV for 4 h and TPA for 4 h). Extracts were incubated with labeled oligonucleotides and subjected to gel electrophoresis. Where indicated, extracts were treated with 0.6% sodium deoxycholate–1.2% Nonidet P-40 during incubation with the oligonucleotide (DOC).

mide or anisomycin, indicating that posttranslational modification occurred and that this modification could be performed in the nucleus.

DISCUSSION

The absorption of UV radiation relevant for UV-induced gene expression occurs at DNA, and it must result in a structural alteration of the DNA helix. One of the changes that cannot be repaired in human mutant cells defective in the repair of UV-induced DNA damage is an intermediate in the UV-induced signal transduction pathway. Although absolute proof is still lacking, we suggest that the UV-responsive genes do not need to absorb UV radiation themselves. In XPA cells, doses as low as 2 J/m² activated the collagenase and HIV-1 genes maximally (Fig. 3). A dose of 2 J/m² introduces 1 thymidine dimer per 20,000 base pairs of DNA (76). At this frequency, *cis*-acting sequences, such as the small UREs, would not be hit in a sufficiently large number of cells to detectably alter transcription in the mass culture. Moreover, introduction of UV-irradiated DNA into cells elicits the UV response (S. Mai et al., National Institute of Child Health and Human Development workshop on genetic response to environmental adversity, Airlie, Va., April 1989).

The UV lesions in DNA do not need to be processed for eliciting the UV response, since neither DNA excision repair nor postreplication repair is required; the UV response occurs both in XPA cells known to lack excision repair and in the absence of DNA replication. Thus, the altered DNA structure itself seems to act as the inducing signal. The induction by NQO and MMC (and other DNA-damaging agents [S. Mai, unpublished results]) indicates that the type of DNA damage accepted is rather broad. Either arrested transcription at these lesions or the lesions themselves are apparently recognized by one or several unknown preexisting protein components. This component(s) may exert protein kinase activity or activate a protein kinase, since inhibitors of protein kinases block the UV response (15, 69). An even more complicated pathway of signal transfer would be compatible with the data available. UV radiation may trigger the release of a factor into the extracellular space which acts on a receptor that establishes the cascade of reactions leading to gene expression. The extracellular loop would need to be extremely rapid. UV radiation does indeed induce the release of factors (58, 64). Their role in the UV induction of gene expression is not established.

The structurally altered DNA stimulates a signal transduction pathway at the other end of which various genes receive this signal and get activated. The genes studied for this report (HIV-1, collagenase, and *fos*) are activated on the level of transcription. Particularly with promoter constructs that contain only the UV-responsive element and with *fos*, transcriptional activation by UV radiation is a matter of minutes. UV-induced transcription can therefore not result from an increase in gene copy number by UV-induced gene amplification, which would take several hours to increase transcription (36, 42, 61). We have also followed the fate of total transfected DNA in the nucleus and have not seen an increase after UV irradiation (done with a UV-inducible SV40 promoter construct [not shown]). There is also no evidence that any of the promoter constructs used carry an origin of replication. Nor is it plausible that the UREs function as origins of replication. Conversely, SV40 amplification has been shown to depend on a very defined origin structure. Without the origin, the UV-inducible SV40 early

promoter cannot support replication (17, 36). One of the UV-responsive elements in the SV40 enhancer in fact represents an NFκB binding site, as in the HIV enhancer (unpublished data, in collaboration with P. Chambon). A UV-induced cellular DNA-binding protein required for SV40 amplification (43) does not bind to any one of the promoter constructs discussed here (not shown). That UV radiation indeed induces gene transcription, and not mRNA or protein stabilization, is clearly shown by two types of experiments: (i) destruction of the UV-responsive elements destroys the UV response of the genes, and (ii) the same RNA and protein produced by transcription from a hormone-inducible or constitutive UV-unresponsive promoter are not affected by UV irradiation (as shown here for the herpes simplex virus thymidine kinase promoter [Fig. 7]).

UV-induced transcription of *fos*, HIV-1, and the collagenase gene is mediated through major enhancer elements. The same elements function as phorbol ester- and growth factor-dependent elements (2, 3, 15, 18, 19, 70, 71). As far as we can tell, the *trans*-acting factors that are required for the physiologic responses also mediate the activation of genes following UV irradiation. UV radiation augments the activity of two of the factors, AP-1 (3, 37) and NFκB (48, 65), as determined by gel retardation experiments. The DNA-protein complexes share the migration behavior with those formed with extracts from phorbol ester-treated cells. The material augmented by UV and binding to the collagenase URE reacts with Fos and Jun antibodies (not shown), proving its identity with the Fos/Jun complex originally termed AP-1 (3, 37). The cytoplasmic localization and behavior after stimulation of the HIV-1-binding factor speaks for an identity with NFκB.

For collagenase, the early UV-induced increase in enhancer-binding activity also occurs in the presence of protein synthesis inhibitors and may be due to posttranslational modification of the transcription factor complex (AP-1, Fos/Jun). A similar increase in binding activity to the collagenase enhancer has been shown to occur after phorbol ester treatment (3). The increase in binding activity which occurs at later times after UV irradiation (at 2 to 8 h) depends on ongoing protein synthesis and results from the coinduction of *fos* and *jun* by UV irradiation (Kaina et al., in press).

For HIV-1, the majority or all of the increase in binding activity is due to posttranslational modification. Our findings suggest that the activity may be generated in a reaction resembling that after phorbol ester treatment. The treatment inactivates an inhibitor which represses NFκB activity (7, 8). Upon UV-induced release, the factor is translocated to the nucleus. Our results thus suggest that UV radiation induces a modifying activity in the cytoplasm. The components of AP-1, Jun, and Fos are always located in the nucleus. Whether their activation is an exclusively nuclear event or also involves a cascade of modifying enzymes passing through the cytoplasm is an open question.

Most attempts to demonstrate an increase in *fos* enhancer-binding activity upon treatment of cells with agents that induce *fos* transcription have failed (50, 67, 71). However, in cells that overexpress the epidermal growth factor receptor, epidermal growth factor seems to increase the binding activity of protein recognizing the SRE (50). Also, UV radiation does not seem to modulate transcription factor-binding activity to the *fos* enhancer as measured in *in vitro* binding experiments, although *fos* transcription *in vivo* is induced efficiently (15). The UV-induced signal chain may posttranslationally modify a transcription factor which is always bound to the enhancer, thereby increasing its potential to

activate transcription without modifying its DNA-binding activity. In vivo genomic footprints have suggested that the SRE is indeed always protected and that the proteins binding there are not altered in their binding abilities during induction of *fos* transcription (28a).

The UV-response phenotype appears to be a property of several cell types. In our own experiments we detected the UV activation of genes in HeLa cells, NIH 3T3 cells (15), primary and SV40-transformed human skin fibroblasts, murine mammary epithelial cells (H. J. Rahmsdorf, unpublished results), and murine cell lines of the B-lymphocyte lineage (53). Keratinocytes (33), Chinese hamster V 79 lung fibroblasts (20), and T-cell lines (B. Stein, unpublished results) can also be induced to express responsive genes by UV radiation.

Particularly with respect to the activation of viruses such as HIV-1, it is relevant to ask whether the doses we applied in vitro occur at all in humans. For exposure to sunlight, only the UV portion of the spectrum is relevant for the induction of HIV-1 and other genes. A dose that induces genes in cultured cells to half-maximal transcription (10 J/m² [Fig. 3]) is equivalent to the amount of germicidal irradiation reached at the skin surface after 40 min of exposure to sun at noon in September in Dallas, Texas (27) or after 25 min of exposure to sun at noon in July at 2,300 m above sea level (35). These data should be taken with caution since different cells were used in these experiments. We can, however, conclude that the range is relevant for human sunbathing. Of the incident dose, less than 5% reaches the cellular layers of the epidermis (14). This is again variable with tanning due to sun exposure and the thickness of the keratin-containing dead-cell layer (for a review, see reference 40). Thus, a fully inducing dose would require on the order of 20 h of sun. The dose range obtained by sunbathing is likely to be relevant, since in cell culture the dose suffices to induce the UV response in the majority of cells, while in skin the induction of expression in the rare cell may suffice to produce consequences. In addition, a spread by the UV-induced extracellular protein synthesis-inducing factor (58, 64) will magnify the response (68). This induction may participate in disease progression. The physiologic meaning of the UV response is open to speculation. We favor the idea that it primarily serves a protective function. The induction of metallothionein expression is as yet the only plausible example for a protective function in that metallothionein causes resistance to killing by several mutagenic agents (Kaina et al., in press).

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