JOURNAL OF VIROLOGY, Nov. 1989, p. 4540–4544
0022-538X/89/114540-05$02.00/0

Vol. 63, No. 11

UV-Induced Transcription from the Human Immunodeficiency Virus Type 1 (HIV-1) Long Terminal Repeat and UV-Induced Secretion of an Extracellular Factor That Induces HIV-1 Transcription in Nonirradiated Cells

BERND STEIN, MARCUS KRÄMER, HANS J. RAHMSDORF, HELMUT PONTA, AND PETER HERRLICH*

Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Postfach 3640, D-7500 Karlsruhe 1, Federal Republic of Germany

Received 24 April 1989/Accepted 19 July 1989

UV irradiation, but not visible sunlight, induces the transcription of human immunodeficiency virus type 1 (HIV-1). Chimeric constructs carrying all or parts of the HIV-1 long terminal repeat linked to an indicator gene were transfected into HeLa cells or murine and human T-cell lines, and their response to irradiation was tested. The *cis*-acting element conferring UV responsiveness is identical to the sequence binding transcription factor NFκB. UV irradiation enhances NFκB binding activity as assayed by gel retardation experiments. Interestingly, the requirement for UV irradiation can be replaced by cocultivation of transfected cells with UV-irradiated nontransfected (HIV-1-negative) cells. A UV-induced extracellular protein factor is detected in the culture medium conditioned by UV-treated cells. The factor is produced upon UV irradiation by several murine and human cell lines, including HeLa, Molt-4, and Jurkat, and acts on several cells. These data suggest that the UV response of keratinocytes in human skin can be magnified and spread to deeper layers that are more shielded, including the Langerhans cells, and that this indirect UV response may contribute to the activation of HIV-1 in humans.

The activation of latent human immunodeficiency virus type 1 (HIV-1) provirus is considered to be the limiting step in the development of acquired immunodeficiency syndrome (11, 22). Latent virus is found predominantly in cells carrying the CD4 surface marker, including T-helper cells (8, 16, 30a); macrophages and their multinucleated derivatives, e.g., the Langerhans cells of the epidermis (16, 30a); and microglia (20). In experimental systems the activation of HIV-1 transcription is achieved by antigenic stimulation (19, 27, 29), by treatment with chemicals (9, 14, 19), and by UV irradiation (12, 28, 31; P. Herrlich, H. Ponta, B. Stein, S. Gebel, H. König, A. Schönthal, M. Büscher, and H. J. Rahmsdorf, *in* C. E. Sekeris, ed., *Molecular Mechanisms and Consequences of Activation of Hormone and Growth Factor Receptors*, in press), suggesting that such external agents can influence the rate of HIV-1 multiplication in the organism. Exposure to UV irradiation is in fact a major environmental factor for the human population. However, most HIV-1-bearing cells are not normally exposed to UV irradiation. We now describe a mechanism by which these cells can be indirectly affected. UV irradiation induces the release of an extracellular factor which, in turn, activates HIV-1 transcription in nonirradiated cells. This factor, which is produced by and acts upon a variety of cell types, including epitheloid, fibroblast, and lymphoid cell lines, can be detected by cocultivation or by the transfer of conditioned medium. We show that both direct UV irradiation and factor-dependent HIV-1 transcription require the same major enhancer element (9, 19) and that the transcription factor binding to this element (NFκB) is activated upon UV irradiation. We suggest that the indirect mechanism of proviral activation could play an important role in the manifestation of the disease.

* Corresponding author.

MATERIALS AND METHODS

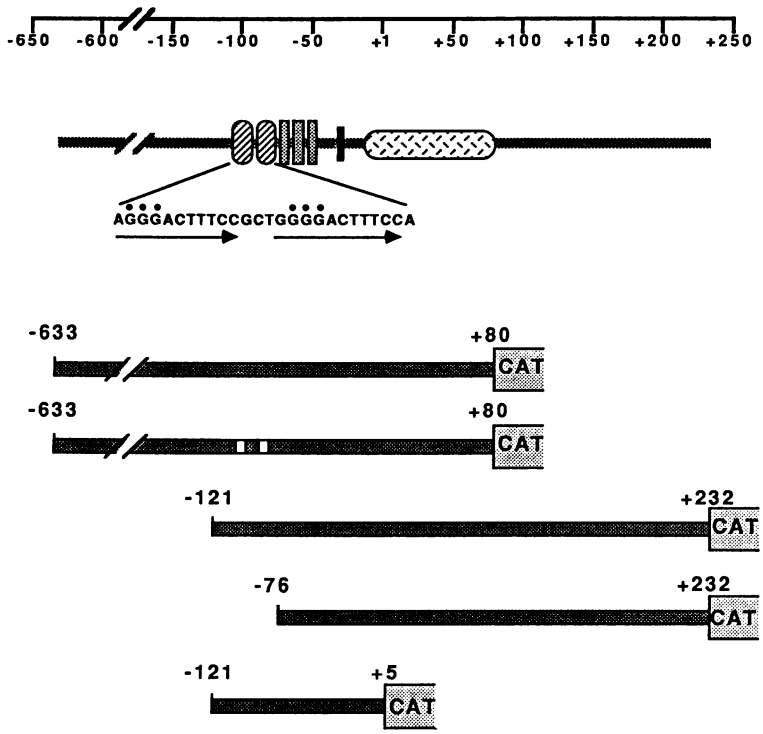
Cell and culture conditions. HeLa TK$^-$ cells (1) were grown in Earle modified Eagle medium supplemented with 10% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Molt-4 cells (17) were grown in RPMI supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Plasmids. The HIV-1 promoter mutations (18, 19) were recloned in pBLCAT5, a derivative of pBLCAT3 (15) from which a UV- and 12-*O*-tetradecanoyl-phorbol-13-acetate-responsive vector sequence has been deleted (C. Jonat, unpublished data). pBLCAT4 served as the control promoter, carrying the sequences from positions −105 to +51 of the herpes simplex virus (HSV) thymidine kinase promoter. It is derived from pBLCAT2 (15) from which a UV- and 12-*O*-tetradecanoyl-phorbol-13-acetate-responsive vector sequence has been deleted (C. J. Jonat, unpublished data).

Transient transfections of HeLa cells were performed as described previously (1). For Molt-4 cells, chloroquine treatment was replaced by a dimethyl sulfoxide shock.

Mobility shift experiments. Whole-cell extracts were prepared from control HeLa TK$^-$ cells or from cells 60 min or 8 h past UV irradiation (20 J/m^2) by a method described for chloramphenicol acetyltransferase (CAT) assay extracts (1). The content in active HIV-1 enhancer-binding protein was determined by gel retardation analysis (4), using the double-stranded synthetic oligonucleotide 5'TGGGGACTTTCCAG CCG3' as a probe.

Preparation of the extracellular factor. Cells were treated with 30 J of UV per m^2 and permitted to produce factor for 48 h (26). The concentration and action of the factor were measured by its inducing ability of the human exoenzyme collagenase.



	cat activity in pmol $mg^{-1}min^{-1}$		
	con	UV	fold induction
	42.1	304.9	7.2
	1.9	3.1	1.6
	68.7	626.5	9.1
	1.6	1.9	1.2
	37.4	252.5	6.8

FIG. 1. UV induction of HIV-1 promoter activity. Exponentially growing HeLa TK^- cells (1) were transiently transfected with one of the HIV-1 CAT constructs shown (18, 19). At 8 h after transfection, the cells were washed with phosphate-buffered saline and irradiated (20 J/m^2) at a wavelength of 254 nm (or mock irradiated [con]). Fresh medium was added, and CAT activity was determined 42 h later (10). Symbols: ▨, HIV-1 enhancer repeats; ■, SP1-binding site; ▮, TATA box; ▩, *tar* region. The dots in the enhancer DNA sequence indicate the point mutations in the mutant construct (CTC instead of GGG).

RESULTS AND DISCUSSION

In an attempt to define the molecular steps involved in UV-induced HIV-1 transcription, we transiently transfected HIV-1 promoter-reporter gene constructs (CAT) into several types of cells and determined promoter activity in response to UV irradiation. A construct carrying all of the HIV-1 long terminal repeat (LTR) and some *env* sequences (−633/+80) reacted to UV irradiation by a 7.2-fold increase in CAT activity in HeLa cells (Fig. 1) and a similar increase in lymphoid cell lines (not shown). The use of various mutants of the LTR sequence (a selection of which is shown in Fig. 1; the mutants were kindly provided by G. Nabel and D. J. Capon) allowed the delimiting of the *cis*-acting sequence elements required for the UV response. (i) The *tar* region, which is the target of the virus-coded *trans*-activating factor TAT (23), is not required. Mutant −121/+5 is fully UV responsive. (ii) The major enhancer element between positions −121 and −76 is necessary for conferring UV responsiveness to the promoter. A 5′ deletion to position −76 and point mutations in both repeats of the enhancer (Fig. 1) destroy reactivity. UV induction of the HIV-1 LTR is specific since promoter constructs with other elements are not responsive to UV irradiation, e.g., the HSV thymidine kinase promoter, the Rous sarcoma virus LTR, the mouse mammary tumor virus LTR, and various synthetic promoters (data not shown; see also reference 28 and B. Stein, H. J. Rahmsdorf, A. Steffan, M. Litfin, and P. Herrlich, Mol. Cell. Biol., in press).

The major enhancer element serves as the recognition region for transcription factor NFκB (19). We examined by gel retardation experiments whether the binding of this factor to its cognate sequence is influenced by irradiating cells with UV. In whole-cell extracts from nonirradiated HeLa cells grown in 10% fetal calf serum, a low level of binding activity is detectable (Fig. 2). This binding is specific for the HIV-1 enhancer since only competition with the authentic sequence obliterates formation of the complex.

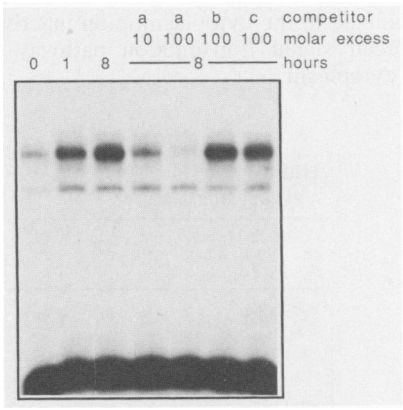


FIG. 2. UV-induced activation of HIV-1 enhancer binding factor. Whole-cell extracts were prepared from control HeLa TK^- cells or from cells 60 min or 8 h past UV irradiation (20 J/m^2) and incubated with the synthetic oligonucleotide 5′TGGGGACTTT CCAGCCG3′. The upper band is sequence specific, as shown by competition with the homologous oligonucleotide (a) and absence of competition with the mutant sequence 5′TGCTCACTTTCCAG CCG3′ (b) and the AP-1 recognition sequence of the collagenase gene 5′TGATGAGTCAGCCG3′ (2; c).

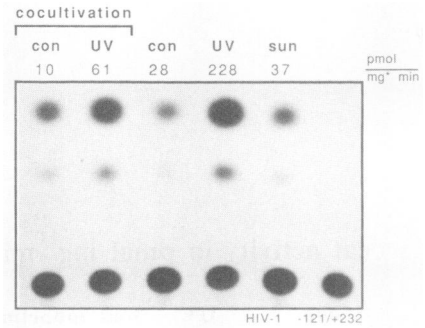


FIG. 3. Cocultivation transmits the inducing signal from irradiated to HIV-1-transfected nonirradiated cells. HeLa cells transiently transfected with HIV-1 (−121/+232) CAT were starved for 24 h in Earle modified Eagle medium containing 0.5% fetal calf serum, trypsinized, and mixed 1:1 with nontransfected starved cells. At 20 min before mixing, the nontransfected cells had been irradiated with 45 J of UV per m^2 (or mock irradiated). CAT assays were performed 40 h later (first two lanes). Lanes 3 to 5 show the CAT activity in nontreated cells, in cells irradiated with 45 J of UV per m^2, and in cells irradiated (600,000 J/m^2) with a lamp emitting a sunlightlike spectrum but missing shortwave UV. *, The protein is calculated for the cells transfected with HIV-1.

The nonfunctional point mutation of the enhancer shown in Fig. 1 and a synthetic oligonucleotide comprising the collagenase AP-1-binding site (2) cannot compete for binding. Extracts from UV-treated HeLa cells contain more binding activity. DNA binding activity is increased about 5-fold by 60 min and continues to rise to 10-fold by 8 h (Fig. 2). A complex of identical migration behavior is formed using extracts of phorbol ester-treated cells (data not shown; B. Kaina, B. Stein, A. Schönthal, H. J. Rahmsdorf, H. Ponta, and P. Herrlich, *in* Lambert et al., ed., *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells*, in press), suggesting that the complex contains NFκB whose binding ability is modulated upon phorbol ester treatment (3). Thus, UV-induced expression of HIV-1 is regulated at the transcriptional level and is based on an activation of NFκB (or of a closely related protein) and its binding to the major enhancer element. Since NFκB is normally localized in the cytoplasm in an inactive form (3), the UV-induced signal transduction pathway must pass through the cytoplasm.

Other DNA-damaging agents, such as 4-nitroquinoline-oxide and mitomycin C, can activate HIV-1 transcription through the same enhancer sequence (Stein et al., in press). Sunlight also activates the HIV-1 promoter (31) but only with its UV content since light of wavelengths longer than 300 nm is absolutely ineffective (Stein et al., in press), as is a mixed spectrum of sunlight without UV (Fig. 3).

Studies of UV-induced gene expression led earlier to the discovery of a UV-induced extracellular factor which influences the transcription of several UV-inducible genes in nonirradiated cells (24, 26). This extracellular factor enhances HIV-1 promoter activity (Fig. 3 and 4). One way to show this is by cocultivating UV-irradiated HIV-1-negative cells with nonirradiated cells that have been transfected with HIV-1 CAT constructs. CAT activity is elevated depending on the dose applied to the HIV-1-negative cells. In Fig. 3, 45 J/m^2 caused a 3- to 6-fold increase, which is about 30% of the increase reached by direct UV irradiation. Conditioned medium from UV-irradiated cells also transfers the induction to nonirradiated cells (Fig. 4). Conditioned medium from control cells (Fig. 4, con CM; compare lane 3 or 5 with lane 1) did not change CAT activity levels, while the medium harvested at 48 h after irradiation (Fig. 4, UV CM; compare lanes 4 and 6 with lanes 3 and 5, respectively) consistently increased CAT activity by three- to fourfold. This increase was not due to enhanced proliferation. Control cells and UV CM-treated cells grow at an equal rate (data not shown). UV CM acts in a concentration-dependent manner (data not shown). The extracellular factor addresses the same *cis*-acting element as does UV irradiation. The *tar* region can be deleted without effect (Fig. 4, lanes 7 to 10), while removal of the major enhancer obliterates factor-induced expression (Fig. 4, lanes 11 to 14). The action of the factor is, however, specific: HSV thymidine kinase promoter CAT constructs were nonresponsive (data not shown), and only a minority of the [^{35}S]methionine-labeled gene products separated by one- or two-dimensional gel electrophoresis were induced by the factor (unpublished data; 26). The major HIV-1 enhancer mediates induction by several pathways: those elicited by phorbol esters, UV, and the UV-induced factor. Phorbol esters are known to initiate a signal transduction chain initiating from the attachment of protein kinase C to the plasma membrane. UV-induced signal transfer is initiated at damaged DNA (28; Stein et al., in press). Since the UV-induced secreted factor is presumably a protein (see below),

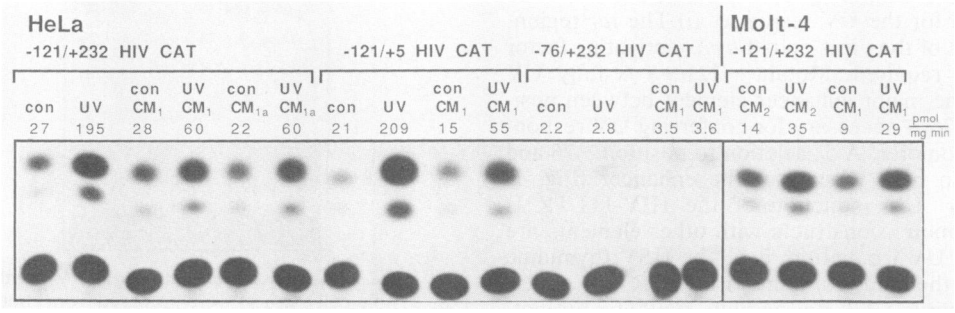


FIG. 4. Transfer of the UV response through conditioned medium to HeLa cells and the lymphoid cell line Molt-4. Logarithmically growing HeLa cells or Molt-4 cells were transfected with the HIV-1 CAT constructs indicated. At 8 h after transfection, the cells were either mock treated (con), irradiated with 20 J/m^2 (UV), or treated with the culture medium of nonirradiated cells (con CM) or of cells irradiated with 30 J/m^2 48 h past irradiation (UV CM; for methodology see reference 26). Cells were harvested for CAT determination 42 h after treatment. As sources of extracellular factor, two different cell culture medium preparations from HeLa cells (CM_1 and CM_{1a}) and one from Molt-4 cells (CM_2) were used. Before the culture medium from HeLa cells was applied to Molt-4 cells, it was dialyzed extensively against RPMI containing 10% fetal calf serum and 2 mM glutamine.

its likely interaction with a target cell will be at the cell surface, initiating a signal transduction pathway. This is confirmed by experiments using suramin (5, 7). The polyanion suramin, known to block the interaction of growth factors with their receptors, inhibits the activity of the UV-induced extracellular factor. That nonphysiological agents also reach physiological components and elicit signalling pathways that merge into physiological pathways is totally expected as the cell answers with a genetic response. The observation that several pathways converge onto the same transcription factor has been reported previously for factor AP-1 (serum, phorbol esters, and UV; 2, 25) and for the protein(s) binding to the serum response element of *fos* (erythrocyte growth factor, platelet-derived growth factor, serum, phorbol esters, and UV; 6, 21, 30).

Since HIV-1 resides largely in CD4-positive cells, we examined the induction of HIV-1 promoter activity in CD4-positive cells by the extracellular factor. A response to conditioned medium from UV-treated HeLa cells was observed in two different cell lines, Molt-4 (17) and Jurkat (13). For Molt-4 cells this is shown in Fig. 4 (compare lanes 17 and 18). Conditioned medium from UV-treated Molt-4 cells also contained activity (Fig. 4, lanes 15 and 16). In addition, UV-induced factor expression is detected in several other cell types, e.g., human primary skin fibroblasts from normal individuals and from patients with xeroderma pigmentosum (26), in the human hepatoma cell line HepG2, and in various murine cell lines (data not shown).

The identity of the factor is unknown. It is presumably a protein because it can be precipitated with ammonium sulfate, is not dialyzable, and can be enriched by chromatography on heparin-Sepharose and Q-Sepharose (Krämer et al., unpublished data). We have evidence for an elevated concentration of several factors in the UV CM, including Il-1α, Il-1β, and TNFα. These factors are released after UV irradiation in the presence or absence of normal fetal calf serum (Krämer et al., unpublished data). The complexity of UV-induced factors is being explored.

The observation of a UV-induced factor which activates HIV-1 promoter activity in nonirradiated cells may gain significance for the natural course of acquired immunodeficiency syndrome. CD4-positive cells are, by and large, not exposed to UV irradiation. We envisage, however, that keratinocytes and skin fibroblasts exposed to UV irradiation will trigger or magnify the activation of HIV-1 in lymphoid and Langerhans cells.

ACKNOWLEDGMENTS

We gratefully acknowledge technical assistance by Anja Steffen and gifts of HIV-1 LTR mutants by G. Nabel and D. J. Capon. C. Jonat constructed vectors pBLCAT4 and pBLCAT5.

Work was supported in part by Fonds der Chemischen Industrie.

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