THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 34, Issue of August 23, pp. 20820–20827, 1996
© 1996 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

An NF-κB Site in the 5′-Untranslated Leader Region of the Human Immunodeficiency Virus Type 1 Enhances the Viral Expression in Response to NF-κB-activating Stimuli*

(Received for publication, February 22, 1996, and in revised form, May 31, 1996)

Massimo Mallardo, Emilia Dragonetti‡, Francesca Baldassarre‡, Concetta Ambrosino§, Giuseppe Scala§, and Ileana Quinto§¶

From the Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi Federico II di Napoli, 80131 Naples and the §Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Reggio Calabria, 88100 Catanzaro, Italy

The 5′-untranslated leader region of human immunodeficiency virus, type 1 (HIV-1), includes a complex array of putative regulatory elements whose role in the viral expression is not completely understood. Here we demonstrate the presence of an NF-κB-responsive element in the *trans*-activation response (TAR) region of HIV-1 that confers the full induction of HIV-1 long terminal repeat (LTR) in response to NF-κB-activating stimuli, such as DNA alkylating agents, phorbol 12-myristate 13-acetate, and tumor necrosis factor-α. The TAR NF-κB site GGGAGCTCTC spans from positions +31 to +40 and cooperates with the NF-κB enhancer upstream of the TATA box in the NF-κB-mediated induction of HIV-1 LTR. The conclusion stems from the following observations: (i) deletion of the two NF-κB sites upstream of the TATA box reduces, but does not abolish, the HIV-1 LTR activation by NF-κB inducers; (ii) deletion or base pair substitutions of the TAR NF-κB site significantly reduce the HIV-1 LTR activation by NF-κB inducers; (iii) deletions of both the NF-κB sites upstream of the TATA box and the TAR NF-κB site abolish the activation of HIV-1 LTR in response to NF-κB inducers. Moreover, the p50·p65 NF-κB complex binds to the TAR NF-κB sequence and *trans*-activates the TAR NF-κB-directed expression. The identification of an additional NF-κB site in the HIV-1 LTR points to the relevance of NF-κB factors in the HIV-1 life cycle.

The human immunodeficiency virus, type 1 (HIV-1),[1] is a lentivirus infecting $CD4^+$ cells and causing AIDS, a progressive degenerative disease of the immune and central nervous system (1). The variable latency period of this disease is possibly related to cellular and environmental factors determining the levels of HIV-1 expression and replication. The expression of HIV-1 is directed by the LTR that contains the sequences for DNA- and RNA-binding cellular and viral proteins (reviewed in Ref. 2). Upstream of the transcription start site, the LTR contains three functional regions, the minimal promoter, the enhancer, and the so-called negative regulatory region. The minimal promoter encompasses the TATA box (3, 4), an LBP-1 site (5–8), and three Sp1 sites (9). The enhancer contains the binding sites for the cellular transacting factors NF-κB (10), TCF-1α/LEF-1 (11, 12), and Ets-1 (13), which provide a signaling-specific activation of HIV-1 LTR, as well as a cell type-specific regulation of HIV-1 expression. The so-called negative regulatory region contains the binding sites for USF (14, 15), C/EBP (16), NFAT-1 (14), AP-1 (14, 17), and nuclear receptors (18), and it is still questionable whether it negatively affects the HIV-1 expression and replication (3, 14, 19, 20). The 5′-untranslated leader region of HIV-1 includes the initiator (Inr) sequences (21, 22), the inducer of short transcripts element (23, 24), and the binding sites for cellular proteins, such as LBP-1/UBP-1 (5–8), TFII I/USF (21, 25), UBP-2 (26), LBP-2 (27), TDP-43 (28), and CTF/NF-1 (5). In addition, this region overlaps the *trans*-activation response (TAR) element (nucleotides +19 to +42) that, as RNA hairpin, interacts with the viral *trans*-activator Tat and cellular RNA-binding proteins, increasing the elongation and/or initiation of HIV-1 transcription (2). Several stimuli such as cytokines (29–32), DNA-damaging agents (33, 34), and viral proteins (35–39) induce the HIV-1 expression. These different inducers mainly act through the activation of NF-κB complexes that bind to the NF-κB sites located upstream of the TATA box.

We previously showed that DNA alkylating agents induce the HIV-1 expression in human B lymphocytes (34). In the absence of Tat, the full induction of HIV-1 LTR required the integrity of both the NF-κB sites upstream of the TATA box and the +34/+37 sequence of TAR. Moreover, DNA alkylating agents rapidly induced a DNA-binding activity to the two NF-κB sites in the HIV-1 enhancer, as well to the +24/+47 sequence of TAR DNA. These results suggested that a mutagen-responsive element is located within the TAR DNA region of HIV-1 (34). In the present study, we have analyzed the Tat-independent enhancer activity of TAR. For this purpose, a set of TAR mutants were used in transient expression and DNA band shift assays to identify the sequence of TAR required for the enhancement of HIV-1 expression and binding to cellular proteins in response to activating stimuli, such as DNA alkylating agents, PMA and TNF-α. Results demonstrate the presence of a NF-κB consensus in the TAR region, which is required for the full induction of HIV-1 expression by NF-κB activating stimuli. The NF-κB site encompasses nucleotides +31 to +40 of the 5′-untranslated leader region of HIV-1; it binds to p50·p65

* This study was supported in part by grants from Istituto Superiore della Sanità, Rome-VIII Progetto Ricerche sull'AIDS, and Associazione Italiana per la Ricerca sul Cancro. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a fellowship from Istituto Superiore di Sanità-Progetto di Ricerche sull'AIDS.

¶ To whom correspondence should be addressed: Dipartimento di Biochimica e Biotecnologie Mediche, Via S. Pansini 5, I-80131 Napoli, Italy. Tel.: 39-81-7463157; Fax: 39-81-7463150.

[1] The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; AIDS, acquired immunodeficiency syndrome; LTR, long terminal repeat; PMA, phorbol 12-myristate 13-acetate; TNF-α, tumor necrosis factor-α; CAT, chloramphenicol acetyltransferase; *tk*, thimidine kinase; EMSA, electrophoretic mobility shift assay; DTT, dithiothreitol; TAR, *trans*-activation response; Inr, initiator element.

A

Plasmid	Sequence	*trans*-activation Mit C	Tat
pHIVCAT0	$^{+24}$ T G A G C C T G G G A G C T C T C T G G C T A A C T A G $^{+51}$	1.00	1.00
pHIVCAT7	* * * * G * * * * * * * * * * * * * * * * *	0.95	0.02
pHIVCAT1	* * * * * A * * * * * * * * * * * * * * * *	1.25	0.26
pHIVCAT2	* * * * * A * * * * * * * * * * * * * * * *	0.98	0.10
pHIVCAT3	* * * * * * T * * * * * * * * * * * * * * *	0.44	0.27
pHIVCAT4	* * * * * * * T * * * * * * * * * * * * * *	0.30	0.13
pHIVCAT5	* * * * * * * * T * * * * * * * * * * * * *	0.35	0.13
pHIVCAT6	* * * * * * * * * C * * * * * * * * * * * *	0.38	0.10
pHIVCAT11	* * * * * * * * * * C * * * * * * * * * * *	0.43	0.37
pHIVCAT9	* * T C G * * * * * C G A * * * * * * * * * *	0.32	0.08
pHIVCAT14	* * * * * * * * * _ _ _ * * * * * * * * * *	0.29	0.03
pHIVCAT12	* * * * * * * * * * * * G A G T T * * * * * *	1.05	0.37
pHIVCAT13	* G C C T G	0.95	0.80
NF-κB site	$^{+31}$G G G A C T C T C^{+40}		
NF-κB consensus	G G G R R N Y Y Y Y		

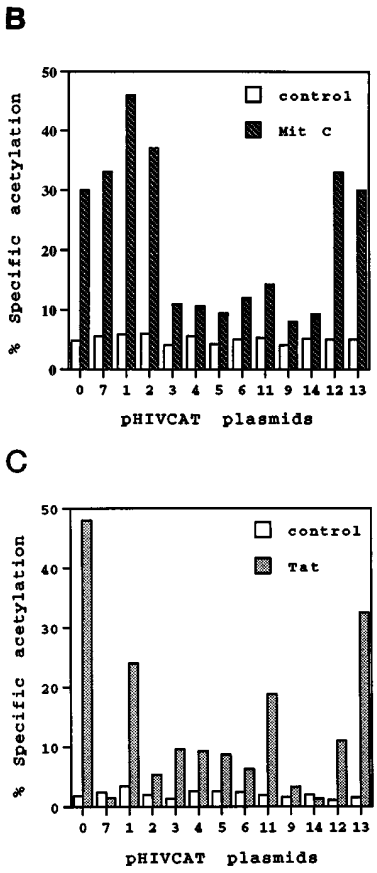


FIG. 1. ***trans*-Activation of TAR mutant plasmids by mitomycin C.** *A*, the sequence from +24 to +51 nucleotide of TAR in pHIVCAT plasmids is shown. *Asterisks* indicate the complete homology with the sequence of the wild-type plasmid, pHIVCAT0. *Dashes* indicate deleted bases. The NF-κB site from +31 to +40 nucleotide is shown. *R* = purine; *Y* = pyrimidine. *trans*-Activation by mitomycin C (*Mit C*) or by Tat is reported as fold induction of each mutant plasmid respect to the fold induction of the wild-type pHIVCAT0, which is defined as 1.00. The values are calculated from the representative experiments shown in *B* and *C*. *B*, induction of pHIVCAT plasmids by mitomycin C. MC3 cells were transfected with the indicated pHIVCAT plasmids (10 μg) and the pRSV-β-gal plasmid (2 μg), and 12 h later they were divided into aliquots treated with 10 μM mitomycin C or left untreated. The CAT activity was evaluated 48 h after treatment and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 1 μg of protein per 3 h. Transfection efficiency was normalized by determining the β-galactosidase activity. A representative experiment of four independent experiments giving similar results is shown. *C*, induction of pHIVCAT plasmids by Tat. MC3 cells were transfected with the indicated pHIVCAT plasmids (5 μg) and the pRSV-β-gal plasmid (2 μg) with or without pSVTat plasmid (2 μg). The CAT activity was evaluated 48 h after transfection and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 1 μg of protein per 3 h. Transfection efficiency was normalized by determining the β-galactosidase activity. A representative experiment of four independent experiments giving similar results is shown.

NF-κB complex and enhances the HIV-1 expression either in cooperation with or in absence of the NF-κB enhancer upstream of the TATA box.

The characterization of a novel NF-κB enhancer in the HIV-1 LTR provides additional information to understand the NF-κB-mediated regulation of HIV-1 transcription.

MATERIALS AND METHODS

Plasmids—The plasmid pHIVCAT0, carrying the HIV-1 LTR from positions −644 to +78 upstream of the *cat* gene, and the derivative base pair substitution TAR mutants pHIVCAT1, pHIVCAT2, pHIVCAT3, pHIVCAT4, pHIVCAT5, pHIVCAT6, pHIVCAT7, pHIVCAT9, and pHIVCAT11 (40) were obtained from Dr. R. W. Davis, Stanford University, Stanford, CA. The TAR mutants +39/+43 and +45/+49 (5), here referred as pHIVCAT12 and pHIVCAT13, carrying 5-base pair substitutions of TAR, were obtained from Dr. K. A. Jones, The Salk Institute, La Jolla, CA. The pTAR (41), here referred as HIVCAT14, carrying the +34/+37 base pair deletion of TAR, and pSVTat (41) were obtained from Dr. A. Rabson, Center for Advanced Biotechnology and Medicine, Piscataway, NJ. The TAR mutations are listed in Fig. 1*A*. The pTARTK and pmTAR1TK plasmids were generated by ligating the synthetic +24/+47 TAR and mTAR1 oligonucleotides, respectively, to pBLCAT2 plasmid (42) linearized by *Sal*I digestion and filled in. In the resulting plasmids, the TAR oligonucleotide is inserted upstream of the herpes simplex virus *tk* minimal promoter fused to the *cat* gene. The correct insertion and orientation of TAR fragment was checked by sequencing. The pCD23, pCD52, and pCD54 plasmids (19) were obtained from Dr. S. Josephs through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. The derivative pCD23ΔTAR and pCD52ΔTAR were constructed by ligating the mTAR1 oligonucleotide to pCD23 and pCD52, respectively, linearized by *Xba*I and filled in. pCD plasmids are shown in Fig. 6*A*. The Rc/CMVp50 and Rc/CMVp65 plasmids (43) were obtained from Dr. N. Rice, Frederick Cancer Research and Development Center, Frederick, MD.

Cells, Transfections, and Chemical Treatment—MC3 cells (34) and NTera-2 cells (44) were cultured in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (Flow Laboratories, Italy), 3 mM glutamine, and 10 mM Hepes buffer, pH 7.2 (Life Technologies, Inc., Italy). Cells were transfected by electroporation as described previously (34). Briefly, cells (3×10^6) in exponential growth phase were washed and resuspended in 0.3 ml of RPMI 1640 plus 20% fetal calf serum in presence of the reporter plasmid DNA (5–10 μg). Cells were subjected to two electrical pulses (0.2 kV, 960 microfarads) using a Bio-Rad apparatus, recovered, cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, and 2 days later collected for CAT assay. To measure transfection efficiency, pRSV-β-gal (2 μg) was cotransfected, and β-galactosidase assays were performed as described previously (34). For chemical treatments, 12 h after transfection cells were divided in equivalent aliquots to be treated or left untreated. Two days post-treatment the cells were harvested, washed with phosphate-buffered saline, and collected for CAT assay. At least four independent experiments with different plasmid preparations were performed to evaluate the transient expression of the *cat* gene. The chemicals used were mitomycin C (Kyowa, Japan), PMA (Sigma), and human recombinant TNF-α (Boehringer Mannheim, Germany).

Assay of CAT Activity—Cell extracts were prepared by three cycles of freeze-thawing in 0.2 ml of 0.25 M Tris, pH 7.8, and the CAT assay was performed as described previously (34). Briefly, each CAT assay contained 1–50 μg of proteins, 20 μl of 4 mM acetyl coenzyme A (Boehringer Mannheim, Germany), 1 μl (0.5 μCi) of [^{14}C]chloramphenicol (DuPont NEN) in a final volume of 150 μl of 0.25 M Tris, pH 7.8. The cell extracts were used at protein concentrations ensuring linear conversion of substrate in each reaction. Reactions were incubated at 37 °C for 3 h, extracted with ethyl acetate, dried, and spotted on Polygram Sil G silica gel plates (Macherey-Nagel, Germany). Plates were run in a TLC tank containing chloroform:methanol (95:5). After a 16-h autoradiography, the TLC plates were cut, and samples were counted in a Beckman LS5000TD scintillation counter. The percent acetylation of [^{14}C]chloramphenicol was determined by scintillation counting the unacetylated and the acetylated forms resolved by thin layer chromatography. The

CAT activity was expressed as the percent acetylated chloramphenicol per μg of protein per 3 h.

Electrophoretic Mobility Shift Assays—Nuclear extracts and gel retardation assays were performed as described previously (34). Briefly, cells were harvested, washed twice in cold phosphate-buffered saline, and resuspended in lysing buffer (10 mM Hepes, pH 7.9, 1 mM EDTA, 60 mM KCl, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.2% v/v Nonidet P-40) for 5 min. Nuclei were collected by centrifugation (500 × *g*, 5 min), rinsed with Nonidet P-40-free lysing buffer, and resuspended in 150 μl of buffer containing 250 mM Tris-HCl, pH 7.8, 20% glycerol, 60 mM KCl, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride. Nuclei were then subjected to three cycles of freezing and thawing. The suspension was cleared by centrifugation (7000 × *g*, 15 min), and aliquots were immediately tested in gel retardation assay or stored in liquid phase N_2 until use. TAR oligonucleotide probes used are represented in Fig. 2*A*. HIV-1 NF-κB oligonucleotide was 5′-CAAGGGACTTTCCGCTGGGGACTTTCCAG-3′ and Sp1 oligonucleotide was 5′-GGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCG-3′. The TAR probe was annealed to its complementary strand and end-labeled with [γ-^{32}P]ATP (Amersham Int., Buckinghamshire, UK) using polynucleotide kinase (New England Biolabs, Beverly, MA). Equal amounts (5 μg) of cell extracts were incubated in a 20-μl reaction mixture containing 10% glycerol, 60 mM KCl, 1 mM EDTA, 1 mM DTT, and 2 μg of poly[d(G-C)] (Boehringer Mannheim, Germany) for 5 min on ice. One μl of γ-^{32}P-labeled double-stranded probe (0.2 ng, 4–6 × 10^4 cpm) was then added with or without a 25–200-fold molar excess of competitor oligonucleotide. The reactions were incubated at room temperature for 15 min and run on a 6% acrylamide:bisacrylamide (30:1) gel in 22.5 mM Tris borate, 0.5 mM EDTA. Gels were dried and autoradiographed.

DNA Affinity Chromatography and Immunoblotting—Nuclear extracts (100 μg) obtained from MC3 cells treated for 1 h with mitomycin C (10 μM) were incubated with 100 ng of streptavidin-conjugated paramagnetic beads (Dynabeads M-280 Streptavidin, Dynal, Norway) bound to the biotinylated double-stranded oligonucleotide according to the manufacturer's instructions in a 20-μl binding solution containing 10% glycerol, 60 mM KCl, 1 mM EDTA, 1 mM DTT, 0.5% v/v Nonidet P-40, and 10 μg of poly[d(G-C)] for 30 min at room temperature. As negative control, equal amounts of extracts were also incubated with unconjugated beads. Binding proteins were extensively washed with the binding solution and eluted with 20 μl of elution buffer containing 125 mM Tris-HCl, 1% 2-mercaptoethanol, 10% glycerol, and 2% SDS at 100 °C for 5 min. The eluate was separated by electrophoresis in 10% SDS-polyacrylamide gel. The immunoblotting was performed with rabbit polyclonal antibodies raised against the N-terminal of human p50 or C-terminal of human p65 followed by enhanced chemiluminescence detection (Amersham, United Kingdom). The rabbit anti-p50 and anti-p65 antiserum (43) were kindly provided by Dr. N. Rice, Frederick Cancer Research and Development Center, Frederick, MD.

RESULTS

An NF-κB-binding Site Is Present in the 5′-Untranslated Leader Region of HIV-1—In the absence of Tat, the TAR region functionally cooperates with the NF-κB enhancer upstream of the TATA box of HIV-1 to confer the maximal induction of HIV-1 gene expression by DNA alkylating agents (34). To identify the mutagen-responsive sequence within TAR, the pHIV-CAT0 plasmid, carrying the HIV-1 LTR fused to the *cat* reporter gene, and the derivative plasmids, carrying base pair substitutions within the region +28 to +51, were used in transient expression assays (Fig. 1*A*). For this purpose, MC3 cells, an Epstein-Barr virus-immortalized B cell line (34), were transfected with these plasmids and treated with the DNA alkylating agent mitomycin C. MC3 cells constitutively express a low level of NF-κB activity that is rapidly increased by NF-κB activating stimuli (45). Moreover, they are representative of *in vivo* target cells for HIV-1 infection (46, 47). As shown in Fig. 1, *A* and *B*, base pair substitutions of the GGGAGCT sequence extending from +31 to +37 (pHIVCAT 3, 4, 5, 6, 9, 11) significantly reduced the activation by mitomycin C, whereas mutations flanking this sequence (pHIVCAT 1, 2, 7, 12, 13) were irrelevant. These findings confirm the results previously obtained with pHIV14, a mutant in which the +34/+37 sequence had been deleted (Fig. 1, *A* and *B*) (34), and they indicate that the mutagen-responsive element maps from +31 to +37. This

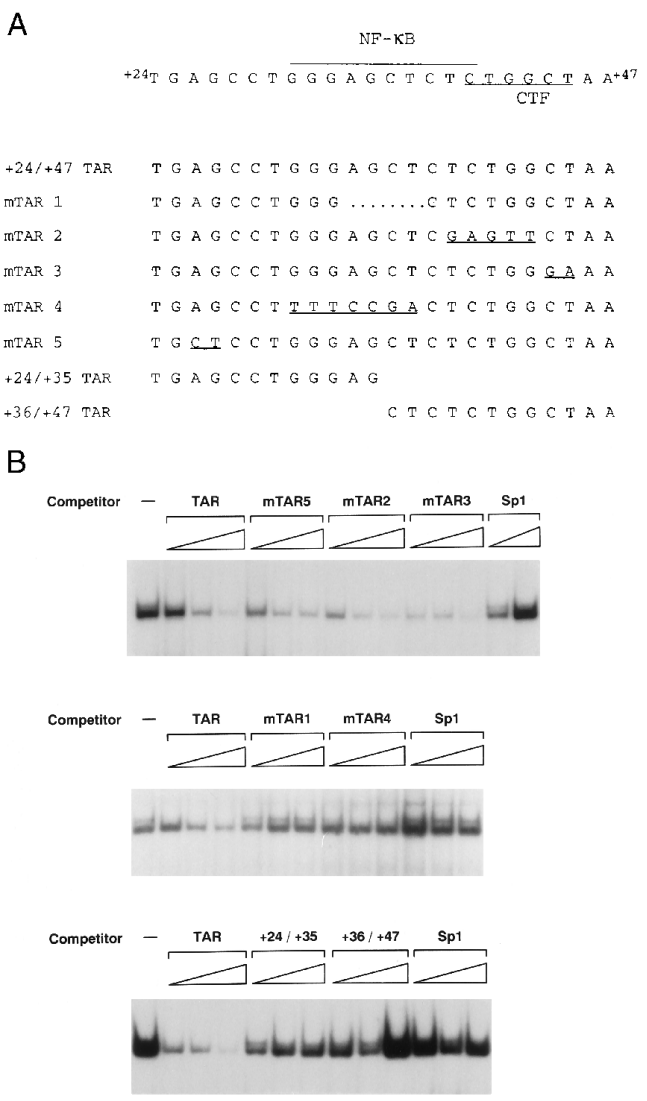


FIG. 2. **An NF-κB consensus in the +24/+47 TAR DNA sequence is required for binding to nuclear proteins induced by mitomycin C.** *A*, sequence of TAR DNA oligonucleotides. The nucleotides are numbered according to the position in the HIV-1 TAR sequence. Base pair substitutions are *underlined*. Deletion is indicated by a *dotted line*. The NF-κB- and CTF-binding sites are indicated. *B*, competition of the binding activity to +24/+47 TAR DNA with mutant TAR oligonucleotides. Nuclear extracts (5 μg) from MC3 cells activated by mitomycin C (10 μM for 1 h) were incubated with a double-stranded ^{32}P-labeled oligonucleotide spanning the +24/+47 TAR sequence. Competitions were performed with 50-, 100-, and 200-fold molar excess of the indicated unlabeled oligonucleotides.

tract overlaps the potential NF-κB site GGGAGCTCTC located from nucleotides +31 to +40 (Fig. 1*A*). Moreover, the +31/+37 sequence is contained in the TAR region from +19 to +42 that generates the upper stem-loop RNA structure required for an efficient transactivation by Tat (Fig. 1, *A* and *C*) (2, 26, 40). These evidences suggest a dual role of the TAR sequence from +31 to +37 in both the mutagen-mediated and the Tat-mediated transactivation of HIV-1 LTR.

DNA alkylating agents induced a binding activity to the double-stranded DNA oligonucleotide representing the +24/+47 TAR sequence (34). This sequence contains a CTF/NF-1-binding site (5), as well as a potential NF-κB site (Fig. 2*A*). To identify the minimal sequence required for the binding activity to TAR DNA, EMSA was performed by using mutant TAR oligonucleotides to compete the TAR DNA-binding activity. The mutant oligonucleotides contained deletions or base pair sub-

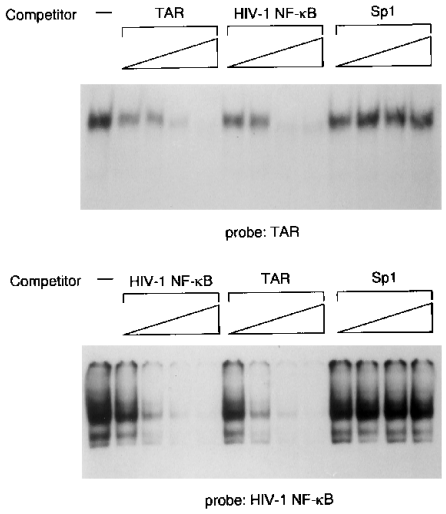


FIG. 3. **The binding activities to the +24/+47 TAR oligonucleotide and to the HIV-1 NF-κB oligonucleotide are reciprocally competed.** Nuclear extracts (5 μg) from MC3 cells treated with mitomycin C (10 μM for 1 h) were incubated with a double-stranded ^{32}P-labeled oligonucleotide representing the +24/+47 TAR sequence or the HIV-1 NF-κB enhancer. Competitions were performed with 25-, 50-, 100-, and 200-fold molar excess of the indicated unlabeled oligonucleotides.

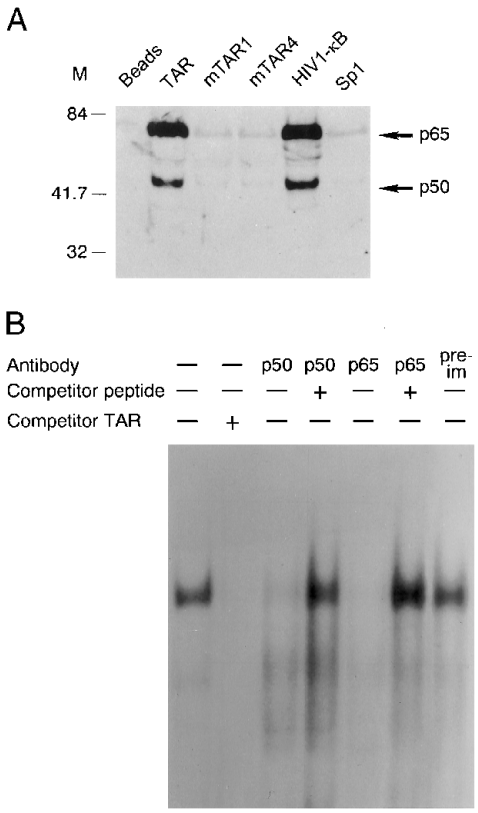


FIG. 4. **NF-κB/Rel proteins bind to +24/+47 TAR DNA following activation by mitomycin C.** *A*, nuclear extracts (100 μg) from MC3 cells treated with mitomycin C (10 μM for 1 h) were separated by DNA affinity chromatography using biotinylated TAR, mTAR1, or mTAR4 double-stranded oligonucleotides bound to streptavidin-conjugated paramagnetic beads. As controls, the same extracts were incubated with beads alone or bound to oligonucleotides representing the NF-κB or Sp1 sequences upstream of the TATA box of HIV-1. Immunoblotting analysis of eluted proteins was sequentially performed with anti-p50 and anti-p65 antiserum followed by enhanced chemiluminescence detection. *B*, inhibition of TAR DNA binding activity by anti-p50 and anti-p65 antiserum. Nuclear extracts (5 μg) were incubated with ^{32}P-labeled +24/+47 TAR oligonucleotide in presence or absence of anti-p50 or anti-p65 antiserum (2 μl). Competitions with the antagonist peptides were performed by preincubating each antiserum with the relative competitor peptide (150 ng/ml) for 20 min. The protein-TAR DNA oligonucleotide complexes were analyzed by EMSA. Competitor TAR oligonucleotide was 100-fold molar excess with respect to the ^{32}P-labeled TAR oligonucleotide.

stitutions in the +24/+47 region (Fig. 2*A*). In particular, competitor oligonucleotides were mutated either at the NF-κB site (mTAR1, mTAR4) or in the flanking regions (mTAR2, mTAR3, mTAR5) (Fig. 2*A*). Base pair substitutions outside the NF-κB consensus competed for the TAR DNA-binding activity (Fig. 2*B*). In contrast, base pair mutations of the +31/+37 sequence (mTAR4) or the deletion of the +34/+37 sequence (mTAR1), which both eliminate the NF-κB site, abolished the ability to compete for the binding to TAR DNA (Fig. 2*B*). The +24/+35 and +36/+47 oligonucleotides, which do not contain the NF-κB consensus, were also unable to compete for the TAR binding activity (Fig. 2, *A* and *B*). These results indicate that the sequence from nucleotide +31 to +38 of TAR, overlapping the potential NF-κB site, was required for the TAR DNA-binding activity. Moreover, the TAR DNA-binding activity was not due to the binding of CTF/NF-1 since it was still observed when the CTF site located from +40 to +45 was mutated (see mTAR2 and mTAR3 in Fig. 2, *A* and *B*).

The possibility that NF-κB factors were involved in the TAR DNA-binding activity was further supported by the evidence that the oligonucleotide representing the HIV-1 NF-κB enhancer competed for the TAR DNA binding as efficiently as the TAR oligonucleotide (Fig. 3). Accordingly, the binding activity to the HIV-1 NF-κB probe was competed by the TAR oligonucleotide (Fig. 3).

NF-κB/Rel Proteins Bind to the Mutagen-responsive Element of TAR—Next, we tested whether NF-κB/Rel proteins could bind to the NF-κB site within the TAR region. For this purpose, nuclear extracts from MC3 cells treated with mitomycin C were incubated with streptavidin-conjugated beads bound to biotinylated oligonucleotides including either the wild-type +24/+47 TAR or the mutants mTAR1 and mTAR4, lacking the NF-κB consensus (Fig. 2*A*). The retained proteins were eluted and analyzed by immunoblotting using antisera raised against p50 and p65 Rel proteins. Both p50 and p65 proteins were specifically retained by the wild-type TAR oligonucleotide, whereas they were not recovered from mTAR1, mTAR4, or from an unrelated oligonucleotide (Fig. 4*A*). The presence of p50 and p65 proteins in the TAR binding complex was further analyzed in EMSA by the use of antisera raised against p50 or p65 Rel proteins. As shown in Fig. 4*B*, both antisera inhibited the TAR DNA-binding activity, whereas they were ineffective in the presence of the relative antagonist peptides. The observation of inhibition rather than supershift by anti-p50 and anti-p65 antiserum was peculiar of DNA band shift using as a probe the TAR oligonucleotide. In fact, the same antisera were able to supershift the HIV-1 NF-κB oligonucleotide (not shown). This is possibly due to the conformation of p50·p65 complex bound to different DNA probes that may affect the accessibility to supershifting antibodies.

Altogether these results indicate that the NF-κB site located between +31 to +40 of TAR region is efficiently recognized by p50·p65 NF-κB complex. From here on, for simplicity we will refer to this NF-κB site as to TAR NF-κB.

The TAR NF-κB Site Acts as a DNA Enhancer in Response to NF-κB-activating Stimuli—In the Tat-dependent *trans*-activation of HIV-1 LTR, TAR acts as an RNA enhancer that binds to the viral *trans*-activator Tat and to cellular RNA-binding proteins (reviewed in Ref. 2). To test the function of TAR as a DNA enhancer, the TAR activity was examined in a context where

TABLE I
Transactivation of pTARTK plasmids by mitomycin C

MC3 cells (3×10^6) were electroporated with the indicated plasmid (10 μg) and pRSV-β-gal (2 μg) or lett untreated. CAT activities were determined 48 h after treatment and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 1 μg of protein per 3 h. Transfection efficiency was normalized by determining the β-galactosidase activity. The results of three independent experiments are reported.

Plasmid	Acetylation		Activation
	−Mit C	−Mit C	
	%	−fold	
pBLCAT2			
Exp. 1	0.37	0.40	1.1
Exp. 2	0.20	0.26	1.3
Exp. 3	0.30	0.50	1.7
pTARTK-sense			
Exp. 1	1.00	8.80	8.8
Exp. 2	0.87	11.50	13.2
Exp. 3	1.35	11.00	8.2
pTARTK-antisense			
Exp. 1	0.50	5.30	10.6
Exp. 2	0.27	13.20	48.9
Exp. 3	0.56	7.75	13.8
pmTAR1TK			
Exp. 1	0.20	0.40	2.0
Exp. 2	0.20	0.45	2.3
Exp. 3	0.20	0.40	2.0

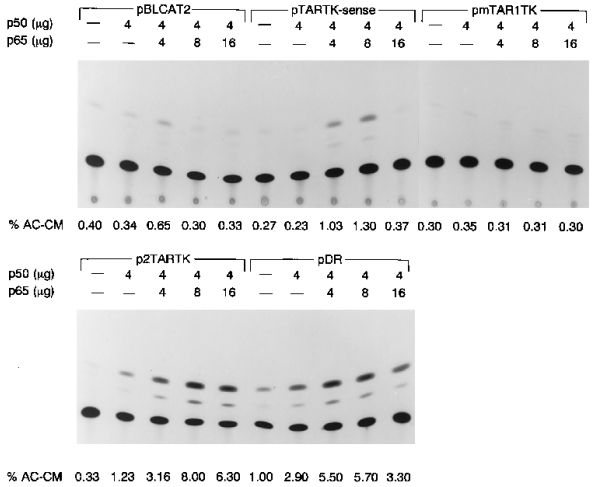


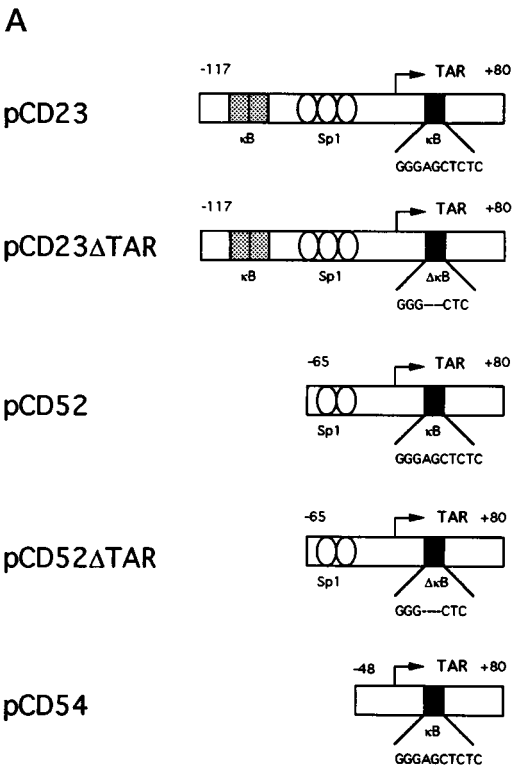
FIG. 5. **The +24/+47 TAR sequence enhances the *tk* minimal promoter-directed *cat* expression in response to NF-κB proteins.** NTera-2 cells (5×10^6) were electroporated with the indicated reporter plasmid (10 μg) alone or together with p50- and p65-expressing plasmids at the indicated doses. Transfection efficiency was monitored by cotransfecting pRSV-β-gal (2 μg) and measuring the β-galactosidase activity. The CAT activity was evaluated 48 h later and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 50 μg of protein per 3 h (*% AC-CM*). A representative experiment of three independent experiments giving similar results is shown.

TAR was moved upstream of the TATA box and acted exclusively at the DNA level. For this purpose, a single copy of the +24/+47 TAR oligonucleotide was inserted in direct or inverse orientation upstream of the herpes simplex virus *tk* minimal promoter fused to the *cat* reporter gene to generate the pTARTK-sense and pTARTK-antisense plasmids, respectively. MC3 cells were transiently transfected with these plasmids and treated with mitomycin C. As shown in Table I, the insertion of TAR DNA in both orientations conferred an 8−40-fold increase in CAT activity in response to mitomycin C. To verify whether the TAR NF-κB site was responsible for the enhancer activity, the mTAR1 oligonucleotide deleted of the NF-κB site was inserted upstream of the *tk* promoter to generate the pmTAR1TK plasmid. As shown in Table I, the deletion of the NF-κB consensus abolished the induction of CAT activity by mitomycin C, thus confirming the requirement of an integral NF-κB-binding site for responsiveness to the genotoxic treatment.

To verify whether proteins of the NF-κB/Rel family directly *trans*-activate *in vivo* the expression of pTARTK plasmids, NTera-2 cells, which constitutively show very low or no NF-κB activity (44), were transiently transfected with pBLCAT2, pTARTK-sense, or pmTAR1TK plasmids together with plasmids expressing p50 and p65. The TAR-driven CAT activity increased up to 5-fold by cotransfecting p50 and p65 (Fig. 5). This increase was not observed in pBLCAT2, lacking the TAR insert, and pmTAR1, deleted of the NF-κB site in the TAR insert (Fig. 5). Moreover, the CAT activity increased up to 20-fold with p2TARTK, a plasmid that carries two copies of the TAR oligonucleotide (Fig. 5). This induction was comparable to the one observed with pDR (Fig. 5), a plasmid that carries the two tandem NF-κB sites of HIV-1 enhancer upstream of the *tk* promoter (48). These results indicate that the +24/+47 TAR region contains a functional NF-κB site that is required both for the induction by the NF-κB activator mitomycin C and for the *trans*-activation by p50·p65 NF-κB complexes.

The TAR NF-κB Enhancer Acts Either Cooperatively with or Independently of the NF-κB Enhancer Upstream of the TATA Box of HIV-1 LTR—Next, we investigated the role of TAR NF-κB in the context of HIV-1 LTR in response to different NF-κB-activating stimuli. For this purpose, MC3 cells were transfected with the wild-type HIV-1 LTR upstream of the *cat* gene (pCD23) or with the derivative mutant plasmids carrying a deletion of the TAR NF-κB site (pCD23ΔTAR), or a deletion of the two NF-κB and the 5′ Sp1 sites upstream of the TATA box (pCD52), or deletions of the NF-κB and 5′ Sp1 sites upstream of the TATA box and of the TAR NF-κB site (pCD52ΔTAR), or deletions of the two NF-κB sites and three Sp1 sites upstream of the TATA box (pCD54) (Fig. 6*A*). Then the transfected cells were treated with PMA, TNF-α, or mitomycin C, which activate NF-κB (45, 49). The CAT activity driven by the wild-type HIV-1 LTR was significantly induced by the chemical treatments (Fig. 6*B*, *pCD23*). This activation was reduced by deletion of the NF-κB sites upstream of the TATA box or deletion of the TAR NF-κB site (Fig. 6*B*, *pCD52* and *pCD23ΔTAR*, respectively), and it was abolished by deletion of both the NF-κB sites upstream of the TATA box and the TAR NF-κB site (Fig. 6*B*, *pCD52ΔTAR*). Moreover, the TAR NF-κB-directed activation of HIV-1 minimal promoter was abolished by deletion of the Sp1 sites upstream of the TATA box (Fig. 6*B*, *pCD 54*). These results indicate that the TAR NF-κB site cooperates with the upstream NF-κB enhancer to induce the maximal activation of HIV-1 expression, and it can exert a residual enhancer activity in the absence of the two NF-κB sites upstream of the TATA box. However, the TAR NF-κB activity requires the presence of the Sp1 sites upstream of the TATA box. This suggests a functional cooperation between the Sp1 and the TAR NF-κB complex from their positions upstream and downstream of the TATA box, respectively.

The TAR NF-κB-driven activation of HIV-1 LTR was further investigated in response to p50 and p65 Rel proteins by transient expression assays. For this purpose, NTera-2 cells were transfected with pCD23, pCD23ΔTAR, pCD52, and pCD52ΔTAR plasmids alone or in combination with p50- and p65-expressing vectors. The CAT activity driven by HIV-1 LTR containing the NF-κB sites upstream of the TATA box and the TAR NF-κB site was significantly increased by p65 alone or in combination with p50, whereas it was not affected by p50 alone (pCD23 in Fig. 7). A similar responsiveness was observed when the HIV-1 LTR was deleted of the TAR NF-κB site (Fig. 7,



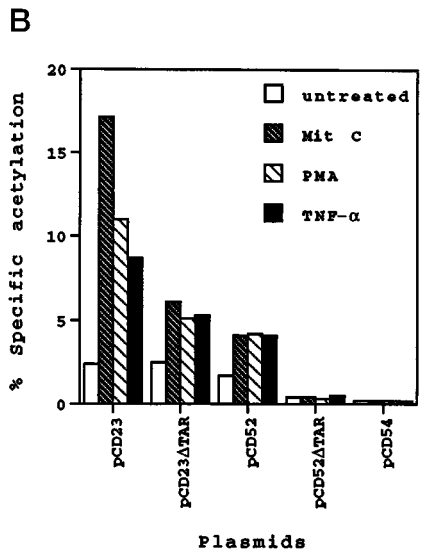


FIG. 6. **Activation of wild-type and mutant HIV-1 LTR by NF-κB activating stimuli.** *A*, schematic representation of the HIV-1 LTR fused to the *cat* gene in pCD plasmids. *B*, CAT activity expressed from pCD plasmids following the treatment with mitomycin C, PMA, or TNF-α. MC3 cells were transfected with the indicated pCD plasmids (10 μg) and pRSV-β-gal (2 μg) and 12 h later divided into aliquots and treated with mitomycin C (10 μM), PMA (50 ng/ml), TNF-α (100 units/ml), or left untreated. The CAT activity was evaluated 48 h after treatment and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 1 μg of protein per 3 h. Transfection efficiency was normalized by determining the β-galactosidase activity. A representative experiment of four independent experiments giving similar results is shown.

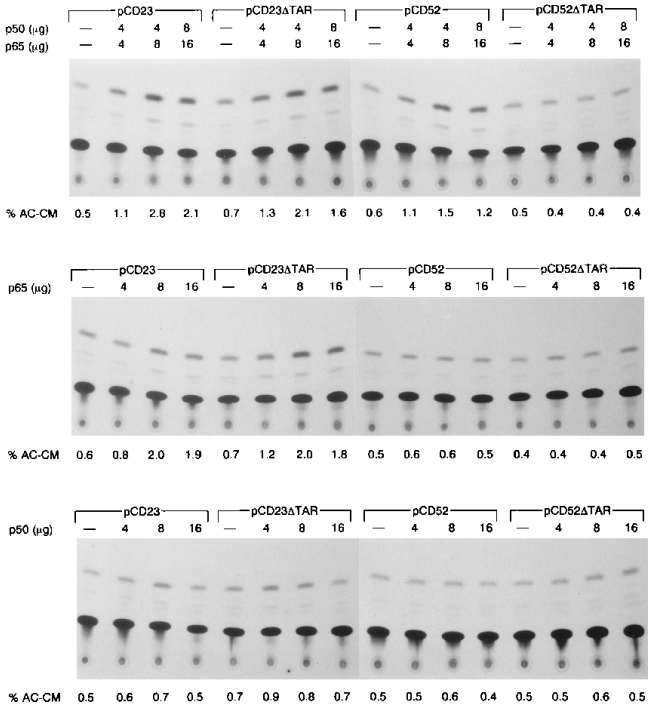


FIG. 7. **Activation of wild-type and mutant HIV-1 LTR by p50 and p65 Rel proteins.** NTera-2 cells (5×10^6) were electroporated with the indicated plasmids (10 μg) alone or together with p50- and/or p65-expressing plasmids at the indicated dose. Transfection efficiency was monitored by cotransfecting pRSV-β-gal (2 μg) and measuring the β-galactosidase activity. The CAT activity was evaluated 48 h later and expressed as percent specific acetylation of [^{14}C]chloramphenicol per 50 μg of protein per 3 h (*% AC-CM*). A representative experiment of four independent experiments giving similar results is shown.

pCD23ΔTAR). The HIV-1 LTR deleted of the NF-κB enhancer upstream of the TATA box was still activated by increasing doses of p65 in combination with p50, whereas it was uninduced by p65 or p50 alone (Fig. 7, *pCD52*). The deletion of both the upstream NF-κB enhancer and the TAR NF-κB site abrogated the activation of HIV-1 LTR by NF-κB proteins (Fig. 7, *pCD52ΔTAR*), thus indicating that the examined NF-κB elements were strictly required to confer responsiveness of HIV-1 LTR to NF-κB complex. These results indicated that the TAR NF-κB site was specifically induced by p50·p65 NF-κB heterodimers, whereas the NF-κB enhancer upstream of the TATA box was responsive to both p50·p65 heterodimers and p65 homodimers. This is consistent with the ability of p50·p65 complex to bind to the TAR NF-κB site (Fig. 4*B*).

DISCUSSION

The 5′-untranslated leader region of HIV-1 interacts with several cellular *trans*-acting factors, whose role in the regulation of HIV-1 gene expression is not completely understood (2). We now describe the presence of an NF-κB responsive element in the TAR region that is required for the maximal induction of HIV-1 gene expression in response to NF-κB activating stimuli. The NF-κB site GGGAGCTCTC spans from nucleotide +31 to +40 (Fig. 1*A*), and it was initially identified by transient expression assays of plasmids carrying the wild-type or mutant HIV-1 LTR fused to the reporter *cat* gene. The mutant plasmids contained base pair substitutions within the +24/+47 sequence of TAR that allowed the mapping of the minimal DNA segment required for the mutagen-mediated activation of HIV-1 LTR. The mutagen-responsive element overlapped a potential NF-κB site that was shown to bind to p50·p65 NF-κB complex. When inserted upstream to the herpesvirus *tk* minimal promoter, TAR NF-κB acted as DNA enhancer in response to NF-κB activating stimuli, as well as to p50·p65 complex. The TAR NF-κB-dependent *trans*-activation of HIV-1 LTR was further investigated in the presence or absence of the regulatory sequences upstream of the TATA box, such as NF-κB and Sp1, and in response to different NF-κB inducers, such as mitomycin C, PMA, and TNF-α. The maximal activation of HIV-1 LTR was observed in presence of both the NF-κB enhancer up-

stream of the TATA box and the TAR NF-κB site. A still significant activation was observed when the NF-κB enhancer upstream of the TATA sequence was deleted, thus indicating a possible role for TAR NF-κB in the HIV-1 LTR activation. This was confirmed by the lack of activation when, in addition to the upstream NF-κB enhancer, the TAR NF-κB site was also deleted. These results indicate that the TAR NF-κB site cooperates with the NF-κB sites upstream of the TATA box in the NF-κB-mediated induction of HIV-1 LTR, and it can still provide a significant activation of HIV-1 LTR in the absence of the upstream NF-κB enhancer. The TAR NF-κB activity requires the presence of the Sp1 sites upstream of the TATA box to *trans*-activate the HIV-1 gene expression. A similar requirement for Sp1 sites was previously shown for the enhancer activity of NF-κB sites upstream of the TATA box (17, 50, 51). Interestingly, the TAR NF-κB activity is mediated by the binding of p50·p65 heterodimers and not by p50 or p65 homodimers. In contrast, the NF-κB enhancer upstream of the TATA box is responsive to both p50·p65 heterodimers and p65 homodimers. A physical interaction between the p65 Rel protein and the transcription factors TATA-binding protein and TFIIB was described (52, 53). Thus, the NF-κB TAR site could allow the p50·p65 NF-κB complex to reside downstream of the TATA box and, from this position, to interact with the initiation complex in order to increase the rate of transcription from the HIV-1 minimal promoter in concert with the Sp1 *trans*-acting factors.

The TAR NF-κB site is included in a region of HIV-1 LTR that contains a complex array of putative regulatory elements. In fact, the TAR NF-κB sequence overlaps the upper stem-loop sequence of TAR RNA that binds to cellular proteins cooperating with the viral *trans*-activator Tat (reviewed in Ref. 2). In addition, the TAR NF-κB site partially overlaps the binding site for a cellular factor called UBP-2, not yet purified and functionally characterized (26). The putative initiator element Inr 2 was identified at positions +29 to +42 (21), which encompass the TAR NF-κB site. In synergy with the upstream Inr 1 element, Inr 2 conferred full promoter activity by interacting with the USF protein (21). Mutations of Inr 2 abrogating the NF-κB sequence affected neither the initiation start site position nor the basal and USF-mediated transcription of HIV-1 (5, 21). The TAR NF-κB site is flanked by the inducer of short transcripts element that is located at positions −5/+26 and +40/+59 and mediates the synthesis of short transcripts of HIV-1 (24). Mutations of TAR NF-κB site reduced the production of full-length rather than short-length RNAs (24), thus excluding the involvement of the NF-κB sequence in the inducer of short transcripts activity.

The presence of a NF-κB enhancer in TAR may provide an additional explanation for the conflicting results concerning the ability to replicate of HIV-1 proviral strains deleted of the NF-κB enhancer upstream of the TATA box (41, 54–56). In primary cells, such as phytohemagglutinin-stimulated PBLs, NF-κB-deleted proviral strains were shown either to replicate similarly to the wild-type strain (41, 54) or to be unable to replicate (56). Indeed, the TAR NF-κB site is conserved in the mutant HIV-1 provirus lacking the NF-κB enhancer upstream of the TATA box, and it could play a role in the viral transcription and replication in response to efficient activation of NF-κB. Accordingly, the reduced transcription and replication of the +31/+34 TAR mutant virus (57), lacking the TAR NF-κB site, could be attributed not only to the absence of Tat-mediated *trans*-activation but also to the inability of NF-κB complex to bind to the TAR NF-κB enhancer. The difficulty to show a biological role for TAR DNA in HIV-1 replication depends on the additional regulatory role of TAR as a stem-loop RNA structure responsive to Tat. In fact, TAR mutations abrogating the NF-κB site also affect the Tat-mediated activation of HIV-1 LTR (Fig. 1) (reviewed in Ref. 2). For this purpose, the role of TAR DNA needs to be examined in Tat-defective HIV-1 strains where the HIV-1 gene expression depends exclusively on cellular transacting factors. Indeed, Tat-defective HIV-1 can be expressed and replicated in T-cell lines and primary mononuclear cells in response to a NF-κB-activating stimulus, such as TNF-α (58). This suggests that, in the absence of Tat, NF-κB may still provide a sufficient HIV-1 gene expression and replication.

The identification of an additional NF-κB enhancer in TAR points to the relevance of NF-κB transacting factors in the HIV-1 gene regulation. The TAR NF-κB sequence is well conserved in different primary isolates of HIV-1 (59) and in the same HIV-1 strain through several rounds of viral replication (60). Moreover, a putative NF-κB site can be identified at positions +37/+46 of HIV-2 TAR DNA (61), and similar to TAR NF-κB of HIV-1, it overlaps the upper stem-loop sequence in the hairpin of HIV-2 TAR RNA. These evidences suggest a natural selection in favor of viral genomes containing NF-κB-binding sites in the TAR region. The NF-κB activity is induced in response to different stimuli such as cytokines and DNA damaging agents (49). Indeed, all these treatments increase the cellular production of free radicals, which may represent a sort of a second messenger of NF-κB activation (62). In the realm of hypothesis, HIV-1 genome could have acquired regulatory regions, such as NF-κB sites, which provide an efficient viral expression and replication in order to escape from cells damaged by free radicals and possibly destined to death. An analogous mechanism is used by the bacteriophage lambda to escape from *Escherichia coli* in SOS signaling (63). In this view, efforts should be developed to inhibit the NF-κB activation in order to suppress the HIV-1 expression and replication.

Acknowledgments—We thank Dr. R. W. Davis for the kind gift of pHIVCAT plasmids, Dr. K. A. Jones for the +39/+43 and +45/+49 TAR mutants, Dr. A. Rabson for pTAR and pSVTat, N. Rice for anti-p50 and anti-p65 antiserum and the Rc/CMVp50 and Rc/CMVp65 plasmids, Dr. U. Siebenlist for NTera-2 cells, and the AIDS Research and Reference Reagent Program for the pCD plasmids. We also thank Dr. P. Di Nocera for helpful discussion and A. Wilcocks for editorial revision.

REFERENCES

1. Pantaleo, G., Graziosi, C., and Fauci, A. S. (1993) *N. Engl. J. Med.* **328,** 327–335
2. Jones, K. A., and Peterlin, B. M. (1994) *Annu. Rev. Biochem.* **63,** 717–743
3. Rosen, C. A., Sodroski, J. G., and Haseltine, W. A. (1985) *Cell* **41,** 813–823
4. Garcia, J. A., Wu, F. K., Mitsuyasu, R., and Gaynor, R. B. (1987) *EMBO J.* **6,** 3761–3770
5. Jones, K. A., Luciw, P. A., and Duchange, N. (1988) *Genes Dev.* **2,** 1101–1114
6. Wu, F. K., Garcia, J. A., Harrich, D., and Gaynor, R. B. (1988) *EMBO J.* **7,** 2117–2129
7. Kato, H., Horikoshi, M., and Roeder, R. G. (1991) *Science* **251,** 1476–1479
8. Yoon, J. B., Li, G., and Roeder, R. G. (1994) *Mol. Cell. Biol.* **14,** 1776–1785
9. Jones, K. A., Kadonaga, J. T., Luciw, P. A., and Tjian, R. (1986) *Science* **232,** 755–759
10. Nabel, G., and Baltimore, D. (1987) *Nature* **326,** 711–713
11. Waterman, M. L., Fischer, W. H., and Jones, K. A. (1991) *Genes Dev.* **5,** 656–669
12. Kim, J. Y. H., Gonzales-Scarano, F., Zeichner, S. L., and Alwine, J. C. (1993) *J. Virol.* **67,** 1658–1662
13. Holzmeister, J., Ludewig, B., Pauli, G., and Simon, D. (1993) *Biochem. Biophys. Res. Commun.* **197,** 1229–1233
14. Lu, Y., Touzjian, N., Stenzel, M., Dorfman, T., Sodroski, J. G., and Haseltine, W. A. (1990) *J. Virol.* **64,** 5226–5229
15. d'Adda di Fagagna, F., Marzio, G., Gutierrez, M. I., Kang, L. Y., Falaschi, A., and Giacca, M. (1995) *J. Virol.* **69,** 2765–2775
16. Henderson, A. J., Zou, X., and Calame, K. L. (1995) *J. Virol.* **69,** 5337–5334
17. Li, Y., Mak, G., and Franza, B. R. (1994) *J. Biol. Chem.* **269,** 30616–30619
18. Ladias, J. A. A. (1994) *J. Biol. Chem.* **269,** 5944–5951
19. Siekevitz, M., Josephs, S. F., Dukovich, M., Peffer, N., Wong-Staal, F., and Greene, W. C. (1987) *Science* **238,** 1575–1578
20. Zeichner, S. L., Kim, J. Y. H., and Alwine, J. C. (1991) *J. Virol.* **65,** 2436–2444
21. Du, H., Roy, A. L., and Roeder, R. G. (1993) *EMBO J.* **12,** 501–511
22. Zenzie-Gregory, B., Sheridan, P., Jones, K. A., and Smale, S. T. (1993) *J. Biol. Chem.* **268,** 15823–15832
23. Ratnasabapathy, R., Sheldon, M., Johal, L., and Hernandez, N. (1990) *Genes Dev.* **4,** 2061–2074
24. Sheldon, M., Ratnasabapathy, R., and Hernandez, N. (1993) *Mol. Cell. Biol.*

13, 1251–1263
25. Roy, A. L., Meisterernst, M., Pognonec, P., and Roeder, R. G. (1991) *Nature* **354,** 245–248
26. Garcia, J. A., Harrich, D., Soultanakis, E., Wu, F., Mitsuyasu, R., and Gaynor, R. (1989) *EMBO J.* **8,** 765–778
27. Margolis, D. M., Ostrove, J. M., and Straus, S. E. (1993) *Virology* **192,** 370–374
28. Ou, S.-H. I., Wu, F., Harrich, D., García-Martínez, L. F., and Gaynor, R. (1995) *J. Virol.* **69,** 3584–3596
29. Folks, T. M., Justement, J., Kinter, A., Dinarello, C. A., and Fauci, A. S. (1987) *Science* **238,** 800–802
30. Duh, E. J., Maury, W. J., Folks, T. M., Fauci, A. S., and Rabson, A. B (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 5974–5978
31. Osborn, L., Kunkel, S., and Nabel, G. J. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2336–2340
32. Poli, G., Bressler, P., Kinter, A., Duh, E., Timmer, W. C., Rabson, A. B., Justement, J. S., Stanley, S., and Fauci, A. S. (1990) *J. Exp. Med.* **172,** 151–158
33. Valerie, K., Delers, A., Bruck, C., Thiriart, C., Rosenberg, H., Debouck, C., and Rosenberg, M. (1988) *Nature* **333,** 78–81
34. Quinto, I., Ruocco, M. R., Baldassarre, F., Mallardo, M., Dragonetti, E., and Scala, G. (1993) *J. Biol. Chem.* **268,** 26719–26724
35. Gendelman, H. E., Phelps, W., Feigenbaum, L., Ostrove, J. M., Adachi, A., Howley, P. M., Khoury, G., and Ginsberg, H. S. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 9759–9763
36. Davis, M. G., Kenney, S. C., Kamine, J., Pagano, J. S., and Huang, E. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 8642–8646
37. Gimble, J. M., Duh, E., Ostrove, J. M., Gendelman, H. E., Max, E. E., and Rabson, A. B. (1988) *J. Virol.* **62,** 4104–4112
38. Mallon, R., Borkowski, J., Albin, R., Pepitoni, S., Schwartz, J., and Kieff, E. (1990) *J. Virol.* **64,** 6282–6285
39. Scala, G., Quinto, I., Ruocco, M. R., Mallardo, M., Ambrosino, C., Squitieri, B., Tassone, P., and Venuta, S. (1993) *J. Virol.* **67,** 2853–2861
40. Feng, S., and Holland, E. C. (1988) *Nature* **334,** 165–167
41. Leonard, J., Parrott, C., Buckler-White, A. J., Turner, W., Ross, E. K., Martin, M. A., and Rabson, A. B. (1989) *J. Virol.* **63,** 4919–4924
42. Luckow, B., and Schütz, G. (1987) *Nucleic Acids Res.* **15,** 5490
43. Rice, N. R., Mackichan, M. L., and Israel, A. (1992) *Cell* **71,** 243–253
44. Franzoso, G., Bours, V., Park, S., Tomita-Yamaguchi, M., Kelly, K., and Siebenlist, U. (1992) *Nature* **359,** 339–342
45. Baldassarre, F., Mallardo, M., Mezza, E., Scala, G., and Quinto, I. (1995) *J. Biol. Chem.* **270,** 31244–31248
46. Monroe, J. E., Calender, A., and Mulder, C. (1988) *J. Virol.* **62,** 3497–3500
47. De Rossi, A., Roncella, S., Calabro, M. L., D'Andrea, E., Pasti, M., Panozzo, M., Mammano, F., Ferrarini, M., and Chieco-Bianchi, L. (1990) *Eur. J. Immunol.* **20,** 2041–2049
48. Lowenthal, J. W., Ballard, D. W., Böhnlein, E., and Greene, W. C. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2331–2335
49. Baeuerle, P. A., and Henkel, T. (1994) *Annu. Rev. Immunol.* **12,** 141–179
50. Parrott, C., Seidner, T., Duh, E., Leonard, J., Theodore, T. S., Buckler-White, A., Martin, M. A., and Rabson, A. B. J. (1991) *Virology* **65,** 1414–1419
51. Perkins, N. D., Edwards, N. L., Duckett, C. S., Agranoff, A. B., Schmid, R. M., and Nabel, G. J. (1993) *EMBO J.* **12,** 3551–3558
52. Kerr, L. D., Ransone, L. J., Wamsley, P., Schmitt, M. J., Boyer, T. G., Zhou, Q., Berk, A. J., and Verma, I. M. (1993) *Nature* **365,** 412–419
53. Schmitz, M. L., Stelzer, G., Altmann, H., Meisterernst, M., and Baeuerle, P. A. (1995) *J. Biol. Chem.* **270,** 7219–7226
54. Ross, E. K., Buckler-White, A., J., Rabson, A. B., Englund, G., and Martin, M. A. (1991) *J. Virol.* **65,** 4350–4358
55. Antoni, B. A., Rabson, A. B., Kinter, A., Bodkin, M., and Poli, G. (1994) *Virology* **202,** 684–694
56. Alcamì, J., Laìn de Lera, T., Folgueira, L., Pedraza, M. A., Jacqué, J. M., Bachelerie, F., Noriega, A. R., Hay, R. T., Harrich, D., Gaynor, R. B., Virelizier, J. L., and Arenzana-Seisdedos, F. (1995) *EMBO J.* **14,** 1552–1560
57. Harrich, D., Hsu, C., Race, E., and Gaynor, R. (1994) *J. Virol.* **68,** 5899–5910
58. Popik, W., and Pitha, P. M. (1993) *J. Virol.* **67,** 1094–1099
59. Myers, G., Korber, B., Wain-Hobson, S., Smith, R. F., and Pavlakis, G. N. (1993) *Human Retroviruses and AIDS 1993,* pp. I-A-22–I-A-23, Los Alamos National Laboratory, Los Alamos, NM
60. Michael, N. L., D'Arcy, L., Ehrenberg, P. K., and Redfield, R. R. (1994) *J. Virol.* **68,** 3163–3174
61. Guyader, M., Emerman, M., Sonigo, P., Clavel, P., Montagnier, L., and Alizon, M. (1987) *Nature* **326,** 662–669
62. Schreck, R., Rieber, P., and Baeuerle, P. A. (1991) *EMBO J.* **10,** 2247–2258
63. Quinto, I., Mallardo, M., Ruocco, M. R., Arcucci, A., and Scala, G. (1991) in Grandolfo, M., Rindi, A., and Sliney, D. H. (ed) *Light, Lasers, and Synchrotron Radiation,* pp. 247–258, Plenum Press, New York