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A Novel Ets-Related Transcription Factor, Elf-1, Binds to Human Immunodeficiency Virus Type 2 Regulatory Elements That Are Required for Inducible *trans* Activation in T Cells

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Human immunodeficiency virus type 1 (HIV-1) and HIV-2 are structurally related retroviruses which both cause AIDS in humans. Although both viruses establish latency in quiescent human-peripheral-blood T cells, the asymptomatic phase of HIV-2 infection may be more prolonged than that of HIV-1. The latent phases of both HIV-1 and HIV-2 infection have been shown to be disrupted by T-cell activation, a process that requires host cell transcription factors. In the case of HIV-1, the transcription factor NF-κB is sufficient for inducible transcriptional activation. In contrast, factors in addition to NF-κB are required to activate HIV-2 transcription in infected T cells. In this report, we demonstrate that a novel Ets-related transcription factor, Elf-1, binds specifically to two purine-rich motifs in the HIV-2 enhancer. Mutagenesis experiments demonstrated that these Elf-1 binding sites are required for induction of HIV-2 transcription following T-cell-receptor-mediated T-cell activation. Moreover, Elf-1 is the only factor present in activated T-cell nuclear extracts that binds to these sites in electrophoretic mobility shift assays. Thus, Elf-1 is a novel transcription factor that appears to be required for the T-cell-receptor-mediated *trans* activation of HIV-2 gene expression. These results may explain differences in the clinical spectra of diseases caused by HIV-1 and HIV-2 and may also have implications for the design of therapeutic approaches to HIV-2 infection.

Many RNA and DNA viruses that infect animal cells utilize host cell transcription factors to express genetic information (31). Thus, for example, transcription of the simian virus 40 genome is regulated by interactions between several host cell transcription factors, including Sp1, AP-1, and TEF-1 (32). Similarly, transcription of the human T-cell leukemia virus type I enhancer appears to be controlled, at least in part, by interactions between cellular transcription factors that belong to the Ets and CREB/ATF families (6, 29, 39). Previous studies have suggested that host cell transcription factors also play an important role in regulating the inducible expression of the human immunodeficiency viruses (HIVs) in T cells (1–3, 17, 25, 30, 31, 36, 42). Two different types of HIVs, HIV type 1 (HIV-1) and HIV-2, have thus far been described (4, 8–10, 17, 19, 23, 25, 31). The life cycles of both viruses are characterized by an initial viremia and infection of circulating $CD4^+$ T cells followed by a latent relatively asymptomatic phase which can last for as long as 10 years and which is characterized by a lack of transcription of the integrated proviral genome (11, 17, 25, 31). HIV latency is disrupted by the transcriptional activation of the viral genome during the process of T-cell-receptor (TCR)-mediated T-cell activation. The transcriptional activation of the proviral genome requires the participation of inducible host cell transcription factors that bind to the viral long terminal repeat (LTR) and activate viral gene expression (1–3, 17, 25, 30, 31, 36, 42). Previous studies have demonstrated significant differences in the patterns of T-cell latency during HIV-1 and HIV-2 infections (4, 25). Thus, it has been suggested that there may be fundamental differences in the host transcription factors involved in the *trans* activation of these two related human retroviruses (30). Binding of the inducible transcription factor NF-κB to two sites in the HIV-1 enhancer appears to be sufficient to activate HIV-1 gene expression in T cells (36). In contrast, it has been shown that factors in addition to NF-κB are needed to activate transcription of the HIV-2 LTR (30).

In the studies described in this report, we have identified two purine-rich sequences (purine boxes 1 and 2 [PuB1 and PuB2]) in the HIV-2 LTR that are required for transcriptional induction following TCR-mediated T-cell activation. We have also shown that these sequences bind a novel member of the Ets family of transcription factors, called Elf-1. Electrophoretic mobility shift assays (EMSAs) utilizing an Elf-1-specific antiserum were used to demonstrate that Elf-1 is the only factor in activated T-cell nuclear extracts that binds to these purine-rich sequence motifs in EMSAs. Taken together, these results show that the transcriptional activation of the HIV-2 LTR, unlike that of the HIV-1 LTR, requires at least two host cell transcription factors, NF-κB and Elf-1. These findings may be relevant to understanding some of the differences in the biological behavior of these two related retroviral pathogens.

MATERIALS AND METHODS

Cells and cell lines. Human peripheral blood mononuclear cells were isolated by using density gradient centrifugation of buffy coats obtained by leukophoresis of healthy donors aged 21 to 31 years. Purified T cells were then isolated from peripheral blood mononuclear cells by negative selection with immunoabsorption as described previously (24). Cell

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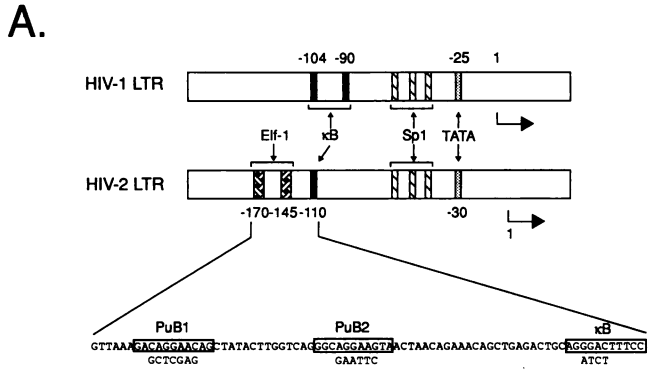
purification was routinely monitored by flow cytometry and histochemistry. Monocytes, B cells, and large granular lymphocytes were not detectable by immunofluorescence analysis. Viability was greater than 99% as measured by trypan blue exclusion. T cells were cultured at a concentration of 2 × 10^6/ml in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 2 mM L-glutamine, 100 U of penicillin G per ml, 100 μg of streptomycin per ml, and 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4; GIBCO). Freshly isolated T cells were activated by culture on plates coated with a saturating quantity of the anti-CD3 monoclonal antibody (MAb) G19-4 in the presence or absence of the anti-CD28 MAb 9.3 (1 μg/ml) for 6 to 8 h as described previously (24) or by treatment for 6 to 8 h with phorbol myristate acetate (PMA; 10 ng/ml), ionomycin (0.4 μg/ml), or both. Human Jurkat T-cell tumor cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and penicillin-streptomycin (GIBCO) as described previously (21).

Isolation of Elf-1 cDNA clones. Elf-1 cDNA clones were isolated by screening a λgt11 cDNA library from PMA-ionomycin-activated Jurkat T cells (22) as described previously (40).

Plasmids. The pRSVβgal reference plasmid in which the bacterial β-galactosidase gene is under the control of the Rous sarcoma virus LTR has been described previously (21). A truncated version of the human Ets-1 cDNA (bp 864 to 1347) containing a consensus eukaryotic initiation codon at the 5' end was prepared by the polymerase chain reaction using the following synthetic oligonucleotide primers: 5' primer, CCAAGCTTGGATCCCACCATGGAGGACTATC CGGCTGCCCTGCCC; 3' primer, CCAAGCTTTCCCCA GCCCCTTCAGTGC. This truncated cDNA was cloned into the *Hin*dIII site of pCDM7 for use in in vitro transcription and translation reactions. The full-length Elf-1 DNA was cloned into pcDNA1/neo (InVitrogen, San Diego, Calif.) for use in in vitro transcription and translation reactions. The wild-type HIV-2CAT plasmid containing bp −556 to +156 of the viral LTR from the ROD strain of HIV-2 has been described previously (13). Mutations were introduced into PuB1 and PuB2 (ΔPuB) or κB (ΔκB) as shown in Fig. 1A by using oligonucleotide-mediated gapped-heteroduplex site-directed mutagenesis (20). All mutations were confirmed by DNA sequence analysis. Plasmid DNA for use in transfections was prepared by cesium chloride density gradient centrifugation as described previously (16).

Preparation of nuclear extracts. Nuclei were isolated from cultured T cells by centrifugation at 14,000 × *g* for 2 min following cell lysis with 40 mM KCl, 10 mM HEPES (pH 7.0), 3 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 5% glycerol, 8 μg of aprotinin per ml, 2 μg of leupeptin per ml, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 0.2% Nonidet P-40 (vol/vol). Nuclei were resuspended in a solution of 20 mM HEPES (pH 7.9), 0.42 M KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, and 25% (vol/vol) glycerol for 30 min at 4°C. Extracts were cleared by centrifugation at 14,000 × *g* for 10 min at 4°C. The resulting supernatants were dialyzed for 4 h at 4°C against buffer containing 20 mM HEPES (pH 7.9), 0.1 M KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, and 20% (vol/vol) glycerol (buffer D) and frozen in aliquots at −70°C. Protein concentrations were determined with a commercially available kit (Bio-Rad, Richmond, Calif.).

EMSAs. The following double-stranded oligonucleotides containing overhanging *Bam*HI-*Bgl*II ends were synthesized on an Applied Biosystems model 380B DNA synthesizer and labeled with ^{32}P-nucleotides by fill in with the Klenow fragment of DNA polymerase I prior to use in EMSAs: Tα2, CCTCTTCTTTCCAGAGGATGTGGCTTCTGCGA; PuB1, CCATTTAGTTAAAGACAGGAACAGCTAT; PUB2, CA GCTATACTTGGTCAGGGCAGGAAGTAACTA. Binding reactions using T-cell nuclear extracts contained 1 to 1.5 μg of T-cell nuclear protein, 20,000 dpm (0.1 to 0.5 ng) of radiolabeled oligonucleotide probe, and 250 ng of poly(dI-dC) in 50 mM KCl–10 mM Tris (pH 7.5)–10 mM HEPES–1.25 mM DTT–1.1 mM EDTA–15% (vol/vol) glycerol in a final reaction volume of 20 μl. Following incubation at room temperature for 30 min, DNA-protein complexes were fractionated by electrophoresis in nondenaturing 4% polyacrylamide gels at 100 V for 2.5 h at room temperature in TGE buffer (0.05 M Tris, 0.5 M glycine, 0.5 mM EDTA) with 47 μM β-mercaptoethanol. Binding reactions using in vitro-transcribed and -translated Elf-1 and Ets-1 proteins contained 3 μl of in vitro-translated protein, 20,000 dpm of radiolabeled oligonucleotide probe, and 250 ng of poly(dI-dC) in 75 mM KCl–10 mM Tris (pH 7.5)–1 mM DTT–1 mM EDTA–4% Ficoll. Following incubation for 30 min at room temperature, DNA protein complexes were fractionated by electrophoresis in 4% nondenaturing polyacrylamide gels which were run in 0.25× TBE buffer (25 mM Tris, 25 mM boric acid, 0.5 mM EDTA) at 110 V for 4 h at 4°C. All gels were dried and subjected to autoradiography with intensifying screens as described previously (21).



B.

IL-2 Enh:	AGGAGGAAAAA (NFAT-1)
	AAGAGGAAAAA (IL-2B)
GM-CSF Pr:	CAGAGGAAATG
	CACAGGAACAT
IL-3 Pr:	GGGAGGAAGTA
HIV-2 LTR:	GACAGGAACAG (CD3R)
	GGCAGGAAGTA

FIG. 1. Identification of purine boxes in the HIV-2 enhancer. (A) Schematic illustration of HIV-1 and HIV-2 enhancers. The locations of the TATAA box (TATA), Sp1, and κB binding sites are from Markovitz et al. (30). The sequence of the HIV-2 LTR (bp −100 to −176) is from Guyader et al. (19). The purine boxes (PuB1 and PuB2) and the κB binding site are boxed. Nucleotide substitutions used to produce mutant enhancers are shown below the wild-type sequence. (B) Comparison of the purine boxes in four inducible T-cell enhancers. Purine boxes from the human IL-2 (12), human GM-CSF (34), and human IL-3 (35) promoter (Pr)-enhancers (Enh) are compared with those from the HIV-2 LTR. The shared purine-rich core pentanucleotide is shaded. Previous names for these elements are shown in parentheses at the right.

Transfections and CAT assays. Exponentially growing cultures containing 10^7 Jurkat T cells were transfected with 5 μg of reporter plasmid and 1 μg of the pRSVβgal reference plasmid by using DEAE-dextran as described previously (20). Thirty hours after transfection, cultures were treated with medium alone or with PMA (50 ng/ml), or they were cultured in plastic tissue culture dishes coated with 1 μg of the G19-4 anti-CD3 MAb per ml (43). Cells were harvested 12 h after stimulation, and cell extracts normalized for protein content with a commercially available kit (Bio-Rad) were assayed for chloramphenicol acetyltransferase (CAT) and β-galactosidase activities as described previously (21).

In vitro transcription and translation reactions. In vitro transcription reactions were carried out with a commercially available kit (InVitrogen) according to the manufacturer's instructions. In vitro translation reactions were performed with a commercially available rabbit reticulocyte system (Promega, Madison, Wis.) according to the manufacturer's instructions (40).

Production of recombinant Elf-1 protein and Elf-1-specific rabbit antiserum. For production of recombinant Elf-1 protein for immunization, the Elf-1 cDNA was excised from pcDNA1/neo by digestion with *Eco*RV and cloned into the *Bam*HI site of the pET-3b vector (38) (following blunting with the Klenow fragment of DNA polymerase I). The resulting plasmid was transformed into competent BL21(DE3) bacteria, and a single colony was grown in M9ZB medium containing 100 μg of carbenicillin per ml to an optical density at 600 nm of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 2 mM, and cells were grown at 37°C for 2.5 h with shaking. Cells were pelleted by centrifugation at 4,000 rpm in a microcentrifuge (Eppendorf) and lysed in 50 mM Tris (pH 8.0)–1 mM EDTA–1 mM DTT–1 mM PMSF–260 mg of lysozyme per ml. Inclusion bodies were pelleted by centrifugation at 4,000 rpm in a microcentrifuge. Proteins were denatured and solubilized in 6 M urea and then renatured by dialysis against buffer D overnight at 4°C prior to being frozen in aliquots at −70°C. Control extracts were prepared from BL21(DE3) bacteria containing the pET-3b plasmid without a cDNA insert. Rabbits were immunized with three sequential subcutaneous injections of 100 to 150 μg of renatured recombinant Elf-1 protein in complete (first immunization) or incomplete (second and third immunizations) Freund's adjuvant. Serum was collected 9 days after the third immunization. This serum was shown to specifically immunoprecipitate in vitro-translated Elf-1 but not Ets-1 or Ets-2 protein (data not shown). Moreover, in control experiments, this serum did not interfere with the binding of Ets-1 or members of the CREB/ATF family of transcription factors in EMSAs (data not shown).

Nucleotide sequence accession number. The complete nucleotide sequence of Elf-1 is available through GenBank, accession number M82882.

RESULTS

Two purine-rich sequence motifs are required for transcriptional activation of the HIV-2 LTR. A comparison of the sequences of the HIV-1 and HIV-2 enhancers (Fig. 1A) demonstrated the absence of one of the two NF-κB sites in HIV-2 compared with HIV-1. In addition, the HIV-2 enhancer contains two purine-rich sequence motifs, PuB1 and PuB2, corresponding to the recently described consensus binding site for the Ets family of transcription factors (18, 20, 26, 27, 41, 44). These two motifs are conserved in at least eight strains of HIV-2 but are absent from the HIV-1 enhancer. Similar motifs are also present in the transcriptional enhancers of several other inducible T-cell genes including those of the human interleukin 2 (IL-2), IL-3, and granulocyte macrophage–colony-stimulating factor (GM-CSF) genes (Fig. 1B). To test the importance of the purine boxes and the NF-κB site for inducibility of the HIV-2 enhancer, we prepared CAT reporter constructs containing either the wild-type HIV-2 LTR or mutant LTRs with nucleotide substitutions in the PuB1 and PuB2 (ΔPuB) or the NF-κB (ΔκB) binding sites. These reporter constructs were transfected into human Jurkat T cells. Portions of the transfected cultures were treated with medium alone, while other portions were activated by treatment with PMA or by cross-linking of the TCR complex by treatment with the anti-CD3 antibody G19-4. In agreement with previous results (30), the wild-type HIV-2 enhancer was induced 50- to 100-fold following T-cell activation with either PMA or the anti-CD3 MAb (Fig. 2). Mutation of the NF-κB site reduced PMA inducibility by more than 90% (Fig. 2) and had a similar effect on inducibility following TCR cross-linking (30 and data not shown). Mutation of both purine boxes abolished the inducibility of the HIV-2 enhancer (Fig. 2). In contrast, mutation of either purine box alone decreased but did not abolish inducible enhancer activity following either PMA treatment or TCR cross-linking (30a). Thus, both the NF-κB binding site and at least one purine box are required for the inducibility of the HIV-2 enhancer following signaling through the TCR complex or activation with phorbol esters.

A novel Ets family member, Elf-1, binds to the purine boxes of the HIV-2 LTR. Two Ets family members, Ets-1 and Ets-2, were known to be expressed in T cells (5). Several types of experiments were performed to determine whether either of these proteins bound to the purine boxes in the HIV-2 enhancer. First, recombinant and in vitro-transcribed and -translated Ets-1 and Ets-2 proteins were used in EMSAs with radiolabeled probes containing PuB1 and PuB2. Neither Ets-1 nor Ets-2 bound efficiently to the purine boxes from the HIV-2 enhancer despite the fact that these same protein preparations bound with high affinity to the Tα2 (20) site from the TCR α enhancer (see Fig. 4; data not shown). In a second series of experiments, anti-Ets-1 and anti-Ets-2 MAbs were used in EMSA experiments utilizing radiolabeled PuB1 and PuB2 probes and nuclear extracts from PMA-activated normal human peripheral blood T cells. Neither of these antibodies caused alterations in the binding and/or mobility of the major T-cell nuclear protein complex that bound to PuB1 and PuB2 (see Fig. 4).

Taken together, these experiments demonstrated that neither Ets-1 nor Ets-2 is involved in regulating the inducibility of the HIV-2 enhancer and suggested the possibility that there are novel Ets-related nuclear proteins that interact with PuB1 and PuB2 in T cells. To test this hypothesis, we screened an activated Jurkat T-cell cDNA library by low-stringency hybridization with a probe from the DNA-binding domain of Ets-1. One cDNA clone isolated by this approach was shown to contain an Ets basic domain with 39 of 42 amino acids identical to those of the ecdysone-inducible transcription factor E74, which is required for *Drosophila* metamorphosis (7). We therefore named this clone E74-like factor-1 (Elf-1) (40). As shown in Fig. 3, the full-length 3.6-kb Elf-1 cDNA contains a 315-bp 5′ untranslated region, a single long open reading frame of 1,857 bp, and a 1,392-bp 3′ untranslated region. The predicted amino acid sequence of Elf-1 includes a basic domain that is related to those of the other Ets family members as well as an α-helical domain

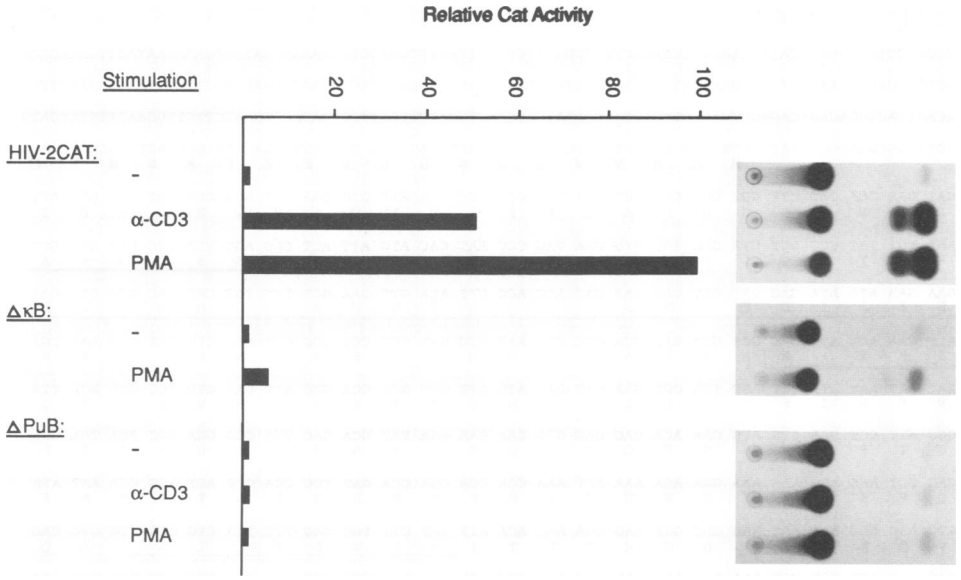


FIG. 2. Functional significance of the κB and PuB sites in the HIV-2 enhancer. Jurkat human T cells were transfected with reporter plasmids by using a modification of the DEAE-dextran protocol. Thirty hours after transfection, cultures were treated with medium alone (−) or PMA (50 ng/ml) or were cultured in plastic tissue culture dishes coated with 1 μg of the G19-4 anti-CD3 MAb per ml as described in Materials and Methods. Cells were harvested 12 h after stimulation, and cell extracts normalized for protein content by using a commercially available kit (Bio-Rad) were assayed for CAT and β-galactosidase activities. The data are shown as CAT activity relative to that of the medium-treated control following normalization for differences in transfection efficiencies. Autoradiograms of representative CAT assays are shown at the right.

immediately 5′ of the basic region that is also conserved in most of the other Ets proteins (26). Together, these two regions, previously termed the ETS domain, represent the DNA-binding domain of the Ets family of transcription factors (26, 45).

Previous studies have demonstrated that E74 binds to a purine-rich sequence (ACGAATCAGGAAAACTG) that contains a core pentanucleotide that is identical to PuB1 and PuB2 (44). We therefore reasoned that Elf-1 might bind preferentially to the purine boxes from the HIV-2 enhancer. As shown in Fig. 4, in vitro-transcribed and -translated Elf-1 protein bound to PuB1 and PuB2 but not to the Tα2 Ets-1 binding site from the human TCR α enhancer. Conversely, Ets-1 bound to Tα2 but failed to bind to PuB1 or PuB2. Binding of Elf-1 to PuB2 was efficiently inhibited by unlabeled PuB2 and PuB1 oligonucleotide competitors but not by competitors containing nucleotide substitutions within the core of PuB1 or PuB2 (see Fig. 1A for the nucleotide substitutions) (Fig. 4C). Finally, an antiserum raised against recombinant Elf-1 protein specifically inhibited binding of in vitro-transcribed and -translated Elf-1 to PuB2 and also inhibited binding of the major nuclear protein complex from PMA-activated normal human peripheral blood T cells to this same probe (Fig. 4B). Of note, the mobility of the PuB2 binding complex from activated T-cell nuclear extracts was identical to that produced by in vitro-translated Elf-1 protein (Fig. 4B). In control experiments, preimmune rabbit serum as well as anti-Ets-1, anti-Ets-2, and anti-IL-2-receptor antibodies had no effect on Elf-1 binding (Fig. 4). Taken together, these experiments demonstrated that Elf-1 present in activated T-cell nuclear extracts binds preferentially and specifically to the purine boxes from the HIV-2 enhancer. Furthermore, these Elf-1 binding sites are required for *trans* activation of the enhancer following T-cell activation.

DISCUSSION

In the studies described in this report, we have identified two purine-rich sequence motifs corresponding to Ets-binding sites that are required for the inducible expression of the HIV-2 LTR. These sites bind a novel Ets-related family member, Elf-1, but fail to bind the closely related Ets-1 transcription factor. Importantly, Elf-1 appears to be the only protein in activated T-cell nuclear extracts that binds to these sites in EMSAs. In agreement with previous reports (30), we have shown that in addition to the Elf-1-binding sites, HIV-2 activation also requires an intact NF-κB binding site. These results have several implications for the molecular mechanisms underlying HIV-2 activation in T cells and for understanding the role of Ets family members in regulating inducible gene expression during T-cell activation.

The preponderance of evidence from several experimental systems suggests that Ets family members activate transcription by working in concert with other non-Ets DNA-binding proteins. Thus, for example, Ets-1 has been shown to cooperate with AP-1 to activate transcription of the polyomavirus enhancer (46). Similarly, GABPα, a novel Ets family member, has recently been shown to cooperate with GABPβ and VP16 to activate HSV-1 immediate-early genes (41). The results presented in this report suggest that Elf-1 can also function cooperatively with the NF-κB family of transcription factors to activate transcription of the HIV-2 LTR during T-cell activation. The requirement for both proteins may explain in part why overexpression of Elf-1 alone fails to activate the HIV-2 LTR (data not shown). In addition, it is worth noting that recent studies have demonstrated that Elf-1 interacts with the product of the retinoblastoma gene (45a). Thus, the regulation of Elf-1-dependent transcriptional activation is complex and cannot be dupli-

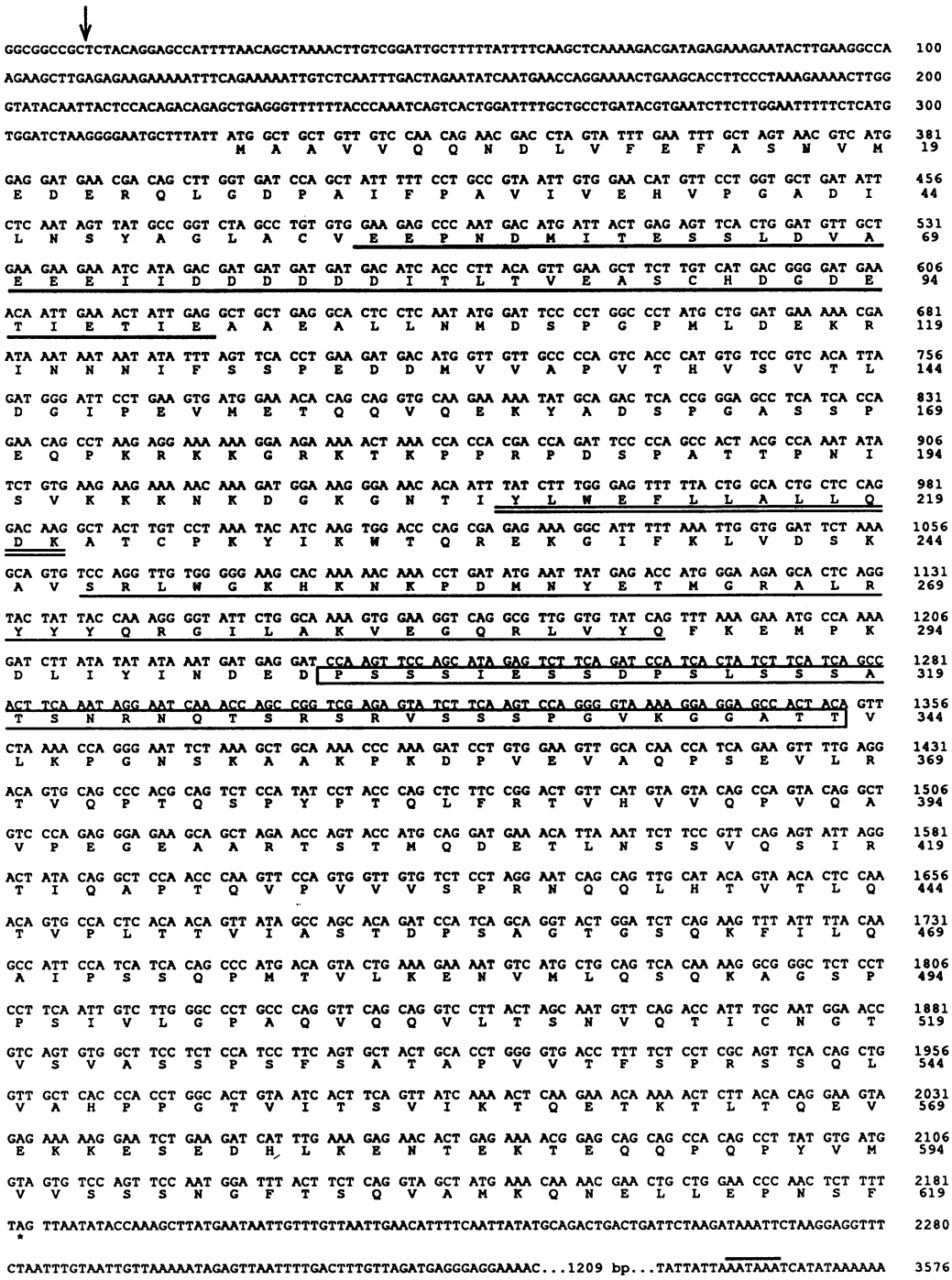


FIG. 3. Nucleotide and predicted amino acid sequences of the full-length human Elf-1 cDNA. The NH_2-terminal acidic region is denoted by the bold underline. The conserved α-helical region of the DNA-binding domain is denoted by the double underline. The basic region of the DNA-binding domain is denoted by the single underline. The serine-threonine-rich region immediately COOH terminal to the DNA-binding domain is boxed. The polyadenylation signal is overlined. Nucleotide and amino acid numbers are shown at the right. The 315-bp 5′ untranslated region begins at the arrow. Only a part of the 1,392-bp 3′ untranslated region is shown.

cated by simple overexpression in T cells. The molecular mechanisms underlying cooperative interactions between Ets family members and other transcription factors have not yet been fully defined. However, structural comparisons of the different Ets family members and their cooperative partners suggest that multiple distinct interactive domains may be responsible for this cooperativity (15, 28, 37).

The results presented in this report also suggest that different Ets family members display distinct DNA-binding specificities and may thereby regulate the expression of

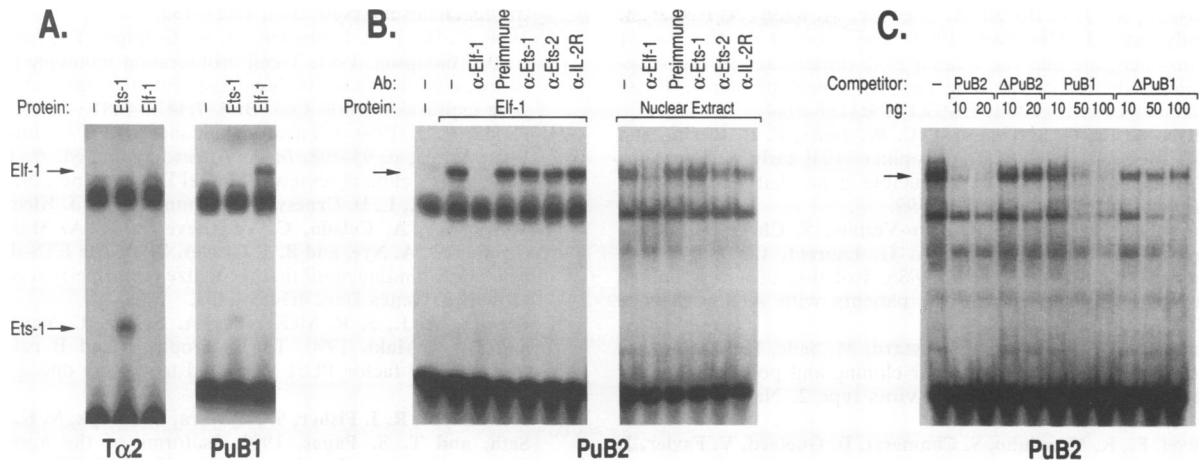


FIG. 4. Binding of Elf-1 to purine boxes in the HIV-2 enhancer. (A) Binding of in vitro-transcribed and -translated Elf-1 and Ets-1 proteins to radiolabeled PuB1 and Tα2 (22) probes was as described previously (40, 45). The positions of the Elf-1 and Ets-1 bands are shown by arrows. (B) Antibody blocking of Elf-1 binding. Binding reaction mixtures containing in vitro-transcribed and -translated Elf-1 (left panel) or nuclear extracts from PMA-ionomycin-activated normal human peripheral blood T cells (right panel) were pretreated with 1 μl of preimmune or α-Elf-1 rabbit antiserum or 1 μl of the previously described α-Ets-1 (22), α-Ets-2 (14), or a commercially available (Becton Dickinson, Mountain View, Calif.) α-IL-2 receptor (α-IL-2R) MAb at 4°C for 15 min prior to the addition of radiolabeled PuB2 probe. The position of the Elf-1 band is indicated with an arrow. The faint band remaining in the nuclear extract lane following pretreatment with the α-Elf-1 antiserum and competitions with PuB1 and PuB2 oligonucleotides represents a nonspecific DNA-binding activity with slightly faster mobility than that of the wild-type Elf-1 protein. (C) Cold competitor experiments. Radiolabeled PuB2 probe was incubated with nuclear extract from PMA-ionomycin-activated normal human peripheral blood T cells in the absence (−) or presence of unlabeled competitor oligonucleotides. The nucleotide substitutions used to make the mutant PuB1 (ΔPuB1) and PuB2 (ΔPuB2) oligonucleotides are shown in Fig. 1A. The Elf-1 band is indicated by the arrow. The two lower bands represent non-sequence-specific DNA-binding activities in that they are inhibited equally by wild-type and mutant PuB1 and PuB2 cold competitor oligonucleotides as well as by several unrelated oligonucleotide competitors.

different sets of genes in T cells. Thus, for example, Ets-1 binds to the TCR α enhancer but fails to bind to the HIV-2 enhancer. Conversely, Elf-1 binds to the HIV-2 but not to the TCR α enhancer. Furthermore, despite the fact that multiple Ets family members are expressed in T cells (40), Elf-1 is the only protein in activated T-cell nuclear extracts that can be shown by EMSA to bind to the purine boxes in the HIV-2 enhancer. Thus, transcription factors containing Ets domains appear to display pleiomorphic regulatory activities by virtue of their abilities to cooperate with multiple other DNA-binding proteins and to bind with different specificities to purine-rich enhancer motifs. In a separate report, we have recently demonstrated that nucleotides at the 3′ ends of the Ets binding sites determine the fine specificities of binding of Ets-1 and Elf-1 to naturally occurring Ets binding sites in the TCR α, IL-2, and HIV-2 enhancers (45). Finally, we cannot rule out the possibility that additional Ets or non-Ets transcription factors bind to the HIV-2 enhancer in vivo but fail to bind in in vitro EMSAs.

Although the organization of HIV-2 is quite similar to that of HIV-1, the nucleotide sequences of the two viruses differ significantly (19). This sequence divergence involves both the structural genes of the viruses and their transcriptional enhancers. These differences in primary structure are reflected in the distinct immunodeficiency syndromes caused by HIV-1 and HIV-2. In particular, the asymptomatic latent period of HIV-2 infection appears to be significantly longer than that associated with HIV-1 infection (4, 25). The studies described in this report suggest a molecular mechanism that may account for these differences. Unlike HIV-1 *trans* activation, which appears to require only NF-κB for induction from latency (30, 36), HIV-2 *trans* activation requires both NF-κB and Elf-1, a novel Ets-related family member present in activated T cells. Thus, these results suggest that, as is the case for avian and murine retroviruses, human retroviruses have evolved to utilize different sets of host factors for transcriptional regulation. These results also suggest Elf-1 as a potential target for therapeutic approaches designed to block the activation of HIV-2 from latency.

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