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Transcription Factor PRDII-BF1 Activates Human Immunodeficiency Virus Type 1 Gene Expression

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Gene expression of human immunodeficiency virus (HIV) is modulated by both cellular transcription factors, which bind to *cis*-acting regulatory elements in the HIV-1 long terminal repeat (LTR) and the viral transactivator, *tat*. The enhancer element in the HIV-1 LTR which extends from −103 to −82 is critical for gene expression. This region contains two identical 10-bp direct repeats which serve as binding sites for members of the NF-κB family of transcription factors. However, several other cellular transcription factors, including a group of zinc finger DNA-binding proteins, also bind to NF-κB and related motifs. A member of this family of transcription factors, designated PRDII-BF1 or MBP-1, is a 300-kDa cellular protein which contains two widely separated zinc finger DNA binding domains. Each of these binding domains is capable of binding to NF-κB or related recognition motifs. Since no functional role for this protein has been demonstrated in the regulation of viral and cellular promoters, we began studies to determine whether PRDII-BF1 could modulate HIV-1 gene expression. DNase I footprinting of the HIV-1 LTR indicated that PRDII-BF1 bound to both NF-κB and TAR transactivation response DNA elements. Both in vitro translation and vaccinia virus expression of PRDII-BF1 cDNA resulted in the synthesis of the full-length 300-kDa PRDII-BF1 protein. Transfection experiments, using both eucaryotic expression vectors and antisense constructs, indicated that PRDII-BF1 activated HIV-1 gene expression in both the presence and absence of *tat*. These results are consistent with a role for PRDII-BF1 in activating HIV-1 gene expression.

Gene expression from the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) is modulated by the viral transactivator *tat* protein, in addition to cellular factors that bind to both *cis*-acting DNA and RNA regulatory regions (reviewed in reference 11). The DNA regulatory elements that are important in modulating the levels of basal and *tat*-induced gene expression include the enhancer, SP1, and TATA elements (11). The enhancer element in the HIV-1 LTR extends from −103 to −82 and contains two NF-κB motifs (31). NF-κB elements, in both viral and cellular promoters, mediate changes in gene expression response to a variety of signaling pathways, including those induced by viral proteins, mitogens, and cytokines (1, 26, 38). Thus, a study of cellular factors that bind to the NF-κB motifs is important in determining the mechanisms that activate viral and/or cellular gene expression.

Several different cellular factors are capable of binding specifically to NF-κB elements in the HIV-1 LTR (6, 31, 47). Cellular proteins, known as NF-κB (39), comprise a family of proteins which are highly homologous to the products of the v-*rel*, c-*rel*, and *Drosophila dorsal* genes (4, 7, 15, 23, 28, 31, 34, 37, 41). These proteins are capable of forming both homodimers and heterodimers which bind to NF-κB motifs (25). Both transfection and in vitro transcription experiments indicate that members of the *rel* family are able to activate gene expression from the HIV-1 LTR (22, 24, 37).

Another family of transcription factor genes which contain zinc finger DNA binding domains is also able to bind to NF-κB and related sequence motifs (5, 10, 13, 30, 32, 34). One member of this gene family, known as MBP-1 or PRDII-BF1, encodes a 300-kDa cellular protein with two widely spaced zinc fingers. Each of these domains binds specifically to NF-κB or related recognition motifs (5, 10, 30). Alternative splicing of the PRDII-BF1 gene generates proteins of 200 and 68 kDa, respectively, which contain either of the two zinc finger DNA binding domains. The level of PRDII-BF1 mRNA is induced in Jurkat cells by treatment with phorbol esters (5) in a manner similar to that seen with members of the NF-κB gene family (28). However, no functional role for PRDII-BF1 in the gene expression of promoters containing NF-κB motifs has been demonstrated.

To determine the role of the PRDII-BF1 protein on HIV-1 gene expression, a detailed analysis of both its DNA binding properties and transcriptional modulatory properties was undertaken. We demonstrate that each of the PRDII-BF1 binding domains interacts specifically with the two NF-κB motifs in the enhancer. Surprisingly, we also demonstrate that PRDII-BF1 is able to bind to a DNA element encoding the so-called loop sequences present in transactivation response (TAR) RNA. Both in vitro translation and vaccinia virus expression of the PRDII-BF1 cDNA confirm the synthesis of a full-length 300-kDa PRDII-BF1 protein. Transfection experiments indicate that PRDII-BF1 is an activator of HIV-1 gene expression. Thus, like the Rel proteins, PRDII-BF1 is capable of activating HIV-1 gene expression from NF-κB sites in the enhancer element.

MATERIALS AND METHODS

Cloning of PRDII-BF1. Cloning of the cDNA encoding the full-length PRDII-BF1 protein was previously described (10, 30). Briefly, clones of the PRDII-BF1 cDNA spanning nucleotides 564 to 9010 and 417 to 6350 were joined by using the *Csp*45I site at nucleotide 5936. The complete coding sequence of PRDII-BF1 was obtained by cloning a PCR fragment

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spanning nucleotides 280 to 417. This construct was cloned downstream of the Rous sarcoma virus (RSV) promoter in the eucaryotic expression vector pDP18 (45).

The PRDII-BF1 cDNA was cloned into the expression vector pTM1 for expression by in vitro transcription and translation in reticulocyte lysate or following transfection into HeLa cells and infection with a recombinant vaccinia virus producing T7 polymerase (9). The pTM1 vector contains a T7 promoter upstream of an encephalomyocarditis virus 5′ leader sequence and a T7 RNA polymerase terminator sequence downstream of the polylinker (9). The initiating ATG codon of the recombinant insert is cloned into an in-frame *Nco*I site at the 5′ end of the polylinker. PCR was used to introduce an *Nco*I site at the initiating ATG of PRDII-BF1, using synthetic oligonucleotide primers with the sequences 5′-GCCAAAGA CCATGGCTCGAACTAAACAAATTCATCCC-3′ (forward, nucleotides 315 to 351) and 5′-GCTGACTGAATTCCCCCT TGCTCATTCTTCTGAGC-3′ (reverse, nucleotides 1129 to 1161). The resulting PCR fragment was restricted with *Nco*I and *Eco*RI and cloned into pTM1 restricted with *Nco*I and *Eco*RI. An *Nsi*I-*Xho*I fragment (nucleotides 858 to 4553) and a 3′ *Xho*I-*Xho*I fragment (nucleotides 4553 to 9010, including the pUC polylinker) were inserted sequentially into the construct described above to complete the PRDII-BF1 coding sequence.

Antisense expression constructs. To construct an antisense PRDII-BF1 expression construct, a *Ban*II (nucleotide 5737)-*Sca*I (nucleotide 7006) fragment cloned in pUC18 was restricted with *Sca*I (in the coding sequence) and *Bam*HI (in the pUC18 polylinker). This fragment was cloned into pDP18 which was restricted with *Sma*I and *Bgl*II, placing this portion of PRDII-BF1-cDNA immediately downstream of the RSV promoter at the *Sma*I site.

To construct an antisense CREB expression construct, a CREB cDNA cloned in pUC18 (29) was first digested with *Stu*I (nucleotide 474 of cDNA sequence) and then partially digested with *Asp* 718 to cleave the *Asp* 718 site in the polylinker downstream of the 3′ end of the cDNA. The resulting fragment was cloned into pDP18 which had been restricted with *Asp* 718 and *Sma*I, thus placing this portion of the CREB cDNA downstream of the RSV promoter *Asp* 718 site.

Bacterial expression constructs. The first binding domain (amino acids 342 to 551) and the second binding domain (amino acids 2045 to 2046) of PRDII-BF1 were cloned in frame in pGEX-2T (Pharmacia), and the glutathione *S*-transferase (GST) fusion proteins were purified by glutathione-agarose chromatography (40). These constructs and a control pGEX-2T plasmid without insert were transformed into *Escherichia coli* JM109. Cultures (500 ml) were grown to an optical density at 600 nm of 0.7 to 0.9 and induced with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h. Cells were pelleted and resuspended in 10 ml of phosphate-buffered saline (PBS) plus 1% Triton X-100 (Sigma). Cells were mildly sonicated, debris was pelleted, and the supernatant was incubated with 1 ml of glutathione-Sepharose for 90 min at 4°C. The beads were then washed five times with 10 ml of PBS. Fusion protein was eluted in 3 ml of 50 mM Tris-HCl (pH 8.0)–10 mM glutathione for 90 min at 4°C and dialyzed against protein storage buffer (10 mM Tris-HCl [pH 8.0], 20% glycerol, 0.2 mM EDTA, 1.0 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride).

Gel retardation assays. DNA binding assays were performed with 50 ng of bacterially produced GST–PRDII-BF1 binding domain 1, GST–PRDII-BF1 binding domain 2, or GST alone. The bacterial GST fusion protein extracts were incubated with 1 μg of co-poly(dI-dC) in binding buffer (10 mM Tris [pH 7.4], 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, 50 mM NaCl) for 10 min. When competition was performed, proteins were incubated for a further 10 min with 50 ng of unlabeled oligonucleotides. Labeled probe (50,000 cpm) was added to the mixture, and after 20 min of incubation at room temperature, the samples were loaded on a 4% native polyacrylamide gel. The oligonucleotides used for labeling and competition (with mutations indicated by underlining) were the wild-type and mutated HIV enhancer extending from −114 to −72 (5′-CTTTCTACAAGGGACTTTCCGCTGGG GACTTTCCAGGGAGGCG-3′ and 5′-CTTTCTACTTTAA AGTTTCCGCTTTAAAGTTGCCAGGGAGGCG-3′, respectively) and wild-type and mutated TAR DNA extending from +10 to +52 (5′-GGTTAGACCAGATCTGAGCCTG G G A G C TCTCTGGCTAACTAG-3′ and 5′-GGTTAGAC CAGATCTGAGCCCAAAAGCTCTCTGGCTAACTAG-3′, respectively).

DNase I footprinting. DNase I footprinting was carried out as described previously (46). An HIV-1 LTR chloramphincol acetyltransferase (CAT) construct spanning the region in the LTR between −179 and +80 was digested first with *Eco*RI in a polylinker site upstream HIV-1 LTR *Ava*I site, dephosphorylated with calf intestinal alkaline phosphatase, digested at an *Xho*I site in the CAT gene, and end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP to produce a labeled probe for the coding strand. The noncoding strand was labeled by end labeling a fragment generated by restriction with *Xho*I followed by treatment with alkaline phosphatase and subsequent *Eco*RI digestion. Approximately 2 ng of probe (25,000 cpm) and up to 60 μg of purified bacterial GST–PRDII-BF1 (binding domain 1 or 2) or purified bacterial GST were used in the reactions. DNase I-digested fragments, along with Maxam-Gilbert G+A and C+T sequencing reactions of the same probes, were resolved on 10% denaturing polyacrylamide gels and visualized by autoradiography with an intensifying screen.

Expression of PRDII-BF1 in vitro and in vivo. [^{35}S]methionine-labeled PRDII-BF1 protein was produced from pTM1, using the Promega TNT coupled in vitro transcription/translation system with T7 RNA polymerase according to the manufacturer's instructions. Vaccinia virus-directed protein expression with this construct was achieved by infection of 100-mm-diameter HeLa cell plate cultures at 80% confluence at a multiplicity of infection of 5 with the T7 RNA polymerase-encoding helper virus followed by calcium phosphate transfection of 10 μg of pTM1 expression construct (9) per 100-mm-diameter plate. Cells were lysed and harvested in protein gel loading dye, and cleared whole-cell lysates from approximately 10^6 cells were used in Western immunoblotting. Immunoprecipitation and Western blot analysis were performed as described previously (9), using a rabbit polyclonal antiserum directed against protein prepared from the GST–PRDII-BF1 binding domain 2 (30) or a GST-CREB construct (29).

Eucaryotic expression and reporter constructs. The wild-type HIV-1 LTR CAT construct contains an *Eco*RV (−337)-*Hin*dIII (+80) fragment cloned into *Hin*dII-*Hin*dIII-cut pJGFCAT18, while the enhancer mutant contains a *Hae*III (−70)-*Hin*dIII (+80) fragment cloned into pJGFCAT18. The *tat* expression vector encodes amino acids 1 to 72 cloned downstream of the RSV promoter in pDP18 (46).

Jurkat cells were maintained in RPMI–10% fetal bovine serum with penicillin and streptomycin. Cells were diluted 1:3 in fresh medium on the day prior to transfection. A total of 1.5 × 10^7 cells were transfected by electroporation in a volume 250 μl, using a capacitance of 1,180 μF loaded at 250 V. Cells were stimulated by incubation with tetradecanoyl phorbol

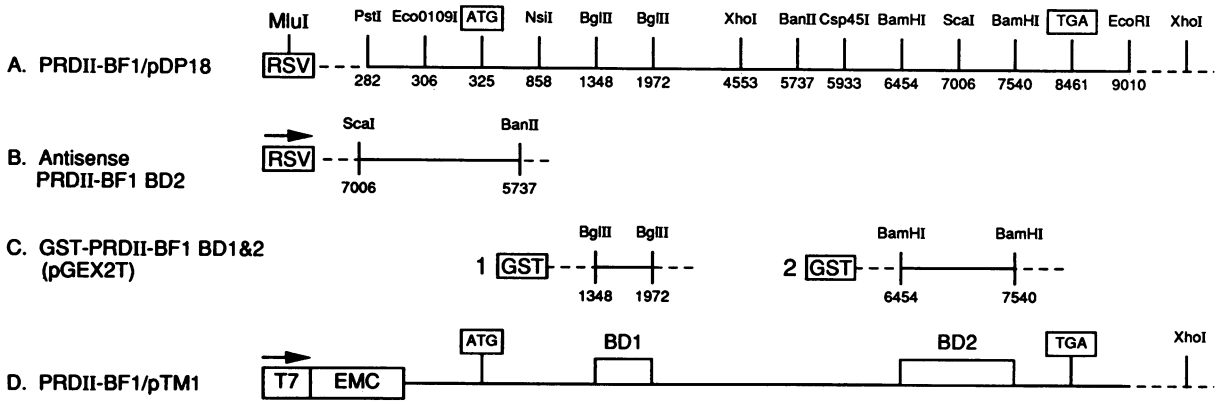

FIG. 1. Diagram of PRDII-BF1 cDNA constructs. (A) Restriction sites in the PRDII-BF1 cDNA extending from nucleotides 282 to 9010 are shown. This cDNA, which contains the complete coding sequence of the PRDII-BF1 protein, was cloned into a eucaryotic expression vector downstream of the RSV promoter. (B) A portion of the PRDII-BF1 cDNA extending from nucleotides 5737 to 7006 was cloned downstream of the RSV promoter in an antisense orientation. (C) Portions of PRDII-BF1 containing binding domain 1 (nucleotides 1348 to 1972) or binding domain 2 (nucleotides 6454 to 7540) were cloned into pGEX-2T for expression as GST fusion proteins. (D) The PRDII-BF1 cDNA was cloned downstream of the T7 promoter and the 5′ untranslated region of encephalomyocarditis virus (EMC) in the expression vector pTM1.

acetate (TPA) (50 μg/μl) and phytohemagglutinin (PHA) (4 μg/μl) added 8 h posttransfection. Cells were harvested at 36 h posttransfection, and protein determinations were quantitated by the method of Bradford (8a). Equal quantities of protein were used in CAT assays as described previously (16). All transfections and CAT assays were repeated between three to six times with equivalent results.

RESULTS

Structure of the PRDII-BF1 gene. The PRDII-BF1 cDNA is capable of encoding a 300-kDa protein. The restriction sites used for the construction of a eucaryotic vector for PRDII-BF1 expression (Fig. 1A) and an antisense construct (Fig. 1B) are indicated. Gel retardation assays with the HIV-1 LTR enhancer element were performed with GST fusion proteins containing portions of the PRDII-BF1 cDNA encoding either binding domain 1 or 2 (Fig. 1C). A pTM1 expression vector for in vitro translation and vaccinia virus expression of the PRDII-BF1 protein is also shown (Fig. 1D).

PRDII-BF1 binds specifically to NF-κB motifs in the HIV-1 LTR. Previous data indicated that bacterially produced PRDII-BF1 protein was capable of binding to NF-κB sequences (5, 10, 30). We first determined whether bacterially synthesized GST fusion proteins, containing either the first or the second zinc finger DNA binding domain of PRDII-BF1, bound to the NF-κB motifs in the HIV-1 LTR. Gel retardation analysis was performed with oligonucleotides encompassing the HIV-1 LTR enhancer. Protein containing the first binding domain of PRDII-BF1 bound to the HIV enhancer sequences (Fig. 2A, lane 2). This binding was inhibited by an excess of wild-type enhancer oligonucleotide competitor (Fig. 2A, lanes 3 to 5) but not by oligonucleotides containing mutated NF-κB sequences (Fig. 2A, lanes 7 to 9). Similar gel retardation analysis was performed with the second binding domain of PRDII-BF1. This protein also bound specifically to the HIV-1 enhancer sequences (Fig. 2B, lane 2) and was competed for by wild-type (Fig. 2B, lanes 3 to 5) but not mutated (Fig. 2B, lanes 7 to 9) oligonucleotides. These results indicated that proteins containing either of the two PRDII-BF1 binding domains could specifically interact with HIV-1 LTR NF-κB binding sequences.

PRDII-BF1 also binds to the TAR DNA element. To further elucidate the binding of PRDII-BF1 to NF-κB motifs in the HIV-1 LTR, DNase I footprinting was performed on a region of the HIV-1 LTR extending from −179 to +80. GST fusion proteins containing PRDII-BF1 binding domain 1, binding domain 2, or GST protein alone were used in these experiments. The quantities and integrity of these proteins were similar.

DNase I footprinting of the HIV-1 LTR on the coding strand revealed that both PRDII-BF1 binding domains 1 and 2 resulted in protection over the enhancer region extending from −108 to −83 (Fig. 3A, lanes 4 to 9). The region of protection was more extensive with the second PRDII-BF1 binding domain (Fig. 3A, lanes 7 to 9). This binding was specific in that GST protein alone did not result in similar protection patterns (Fig. 3A, lanes 1 to 3). Protection of the TAR DNA element was difficult to interpret because of the location of this region

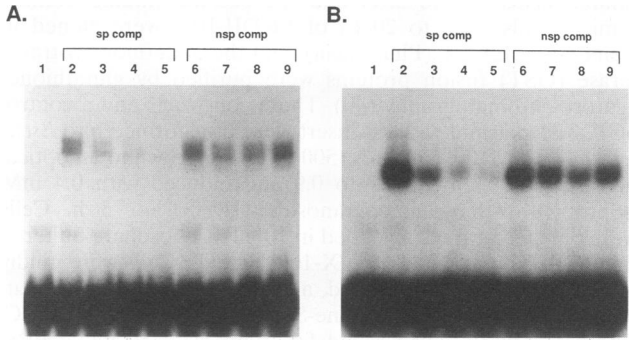

FIG. 2. PRDII-BF1 binds to the HIV-1 enhancer element. PRDII-BF1 binding domain 1 (A) or binding domain 2 (B) was expressed as a GST fusion protein in bacteria and purified by glutathione-agarose chromatography. Gel retardation analysis was performed with oligonucleotides corresponding to a region of the HIV-1 LTR extending from −114 to −72. Samples include 100 ng of either GST protein (lane 1), binding domain 1 or binding domain 2 in the absence of competitor (lanes 2 and 6), 10-, 50-, or 200-fold molar excess of wild-type oligonucleotides (sp comp) (lanes 3 to 5), or similar amounts of mutated oligonucleotides (nsp comp) (lanes 7 to 9).

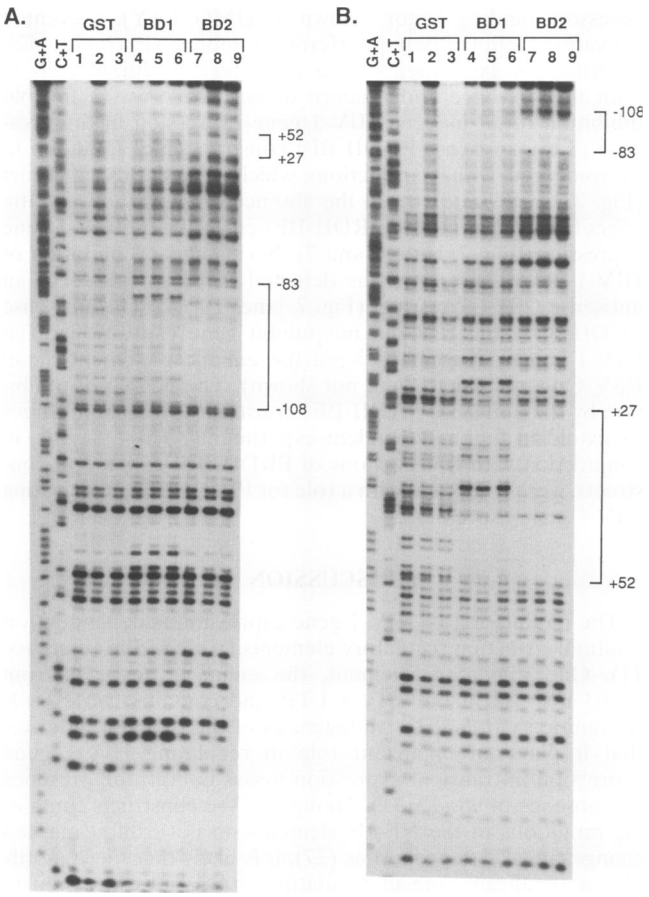

FIG. 3. DNase I footprinting of the HIV-1 LTR with PRDII-BF1. Purified GST fusion protein containing either PRDII-BF1 binding domain 1 or domain 2 was used in DNase I footprinting assays with either the HIV-1 LTR coding (A) or noncoding (B) strand. Either 0, 30, or 60 μg of GST (lanes 1 to 3) or similar amounts of either PRDII-BF1 binding domain 1 (lanes 4 to 6) or binding domain 2 (lanes 7 to 9) were added to these reactions. G+A and C+T are Maxam-Gilbert sequencing reactions. The brackets indicate the regions of the HIV-1 LTR which are protected in the footprinting reactions.

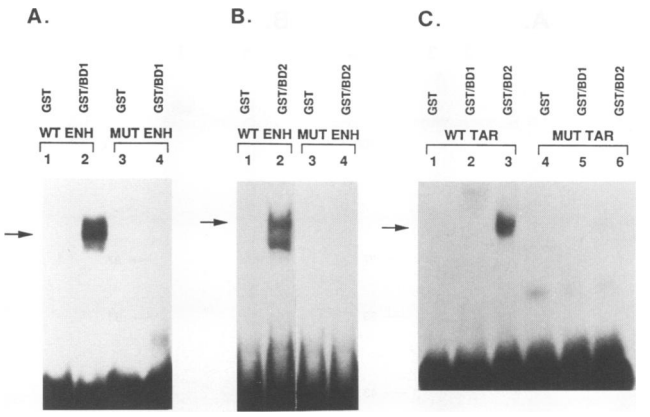

FIG. 4. PRDII-BF1 binds to the HIV enhancer and TAR DNA. Gel retardation was performed with oligonucleotides corresponding to wild-type (WT) and mutated (MUT) HIV-1 LTR enhancer (ENH) elements (A and B) or the TAR DNA elements (C). GST protein alone was added to the gel retardations in lanes 1 and 3 of panel A, lanes 1 and 3 of panel B, and lanes 1 and 4 of panel C. PRDII-BF1 binding domain 1 was added to lanes 2 and 4 of panel A and lanes 2 and 5 of panel C. PRDII-BF1 binding domain 2 was added to lanes 2 and 4 of panel B and lanes 3 and 5 of panel C.

near the top of the gel. DNase I footprinting was also performed on the noncoding strand (Fig. 3B). Again, both PRDII-BF1 binding domains resulted in protection over the enhancer sequences (Fig. 3B, lanes 4 to 9). In addition, binding domain 1 also bound to a portion of the TAR region extending from +40 to +52 (Fig. 3B, lanes 4 to 6). Binding domain 2 bound to a more extensive region of TAR extending from +27 to +52 (Fig. 4B, lanes 7 to 9). This included a portion of TAR DNA from +30 to +35 that contained sequences which formed TAR RNA loop sequences. These sequences have been demonstrated to be critical for *tat* activation (see reference 11 for a review).

These results were confirmed by gel retardation analysis using PRDII-BF1 binding domains 1 and 2, with both enhancer and TAR element probes (Fig. 4). Again, both binding domains 1 and 2 yielded specific gel-retarded species with the HIV-1 enhancer element (Fig. 4A and B, lanes 2). However, gel retardation analysis using TAR DNA sequences extending from +10 to +52 indicated that binding domain 2 (Fig. 4C, lane 3), but not binding domain 1 (Fig. 4C, lane 2), yielded a specific gel-retarded species. Mutation of the sequences between +31 and +34 eliminated the binding of this species (Fig. 4C, lane 6). The sequence GGGAGCTCTCTGG, which encompasses the TAR loop region, has homology with the NF-κB consensus sequence GGGGACTTTCC (39). However, neither binding domain 1 nor binding domain 2 was capable of binding to TAR RNA in gel retardation assays (data not shown). These results demonstrate that PRDII-BF1 bound to NF-κB and a related motif located in the TAR DNA element.

PRDII-BF1 is expressed both in vitro and in vivo. Before transfection experiments were performed to determine whether PRDII-BF1 regulated the level of HIV-1 LTR gene expression, it was critical to demonstrate that full-length PRDII-BF1 protein could be synthesized. The PRDII-BF1 cDNA was cloned downstream of the T7 promoter in the pTM1 expression vector. Protein was expressed following in vitro transcription and translation using reticulocyte lysate in the presence of [^{35}S]methionine (9). Immunoprecipitation with a rabbit polyclonal antibody directed against the second binding domain of PRDII-BF1 followed by polyacrylamide gel electrophoresis and autoradiography resulted in the presence of a 300-kDa species (Fig. 5A, lane 3). This species was not detected when immunoprecipitation with antisera was performed against another transcription factor, CREB (Fig. 5A, lane 2), or in the presence of unprogrammed reticulocyte lysate (Fig. 5A, lane 1). These results indicated that the full-length PRDII-BF1 protein could be synthesized in vitro.

To determine whether the PRDII-BF1 protein could be expressed in vivo, the pTM1 vector containing the PRDII-BF1 cDNA was transfected into HeLa cells. One set of these cells was infected with a recombinant vaccinia virus which contained the T7 polymerase gene, while another set was not infected with this virus (9). A third set of cells was infected with recombinant vaccinia virus but was not transfected with the pTM1 expression vector. At 36 h postinfection, whole-cell lysates were prepared and Western blot analysis was performed with a rabbit polyclonal antiserum directed against the second binding domain of PRDII-BF1 (Fig. 5B). This antibody reacted with a 300-kDa species in extract prepared from HeLa cells transfected with the pTM1-PRDII-BF1 construct and the

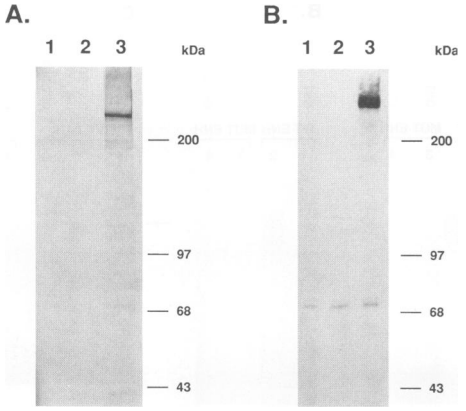

FIG. 5. Expression of the PRDII-BF1 protein. (A) The PRDII-BF1 cDNA was cloned in the pTM1 vector downstream of the T7 promoter, in vitro transcribed and in vitro translated in the presence of [^{35}S]methionine, and then immunoprecipitated. Gel electrophoresis and autoradiography were then performed. Samples include reticulocyte lysate with no added RNA (lane 1), PRDII-BF1 immunoprecipitated with a rabbit polyclonal CREB antiserum (lane 2), or PRDII-BF1 immunoprecipitated with a rabbit polyclonal antiserum directed against PRDII-BF1 binding domain 2 (lane 3). (B) The PRDII-BF1 cDNA in pTM1 was transfected into HeLa cells in both the presence and absence of a recombinant vaccinia virus which expressed the T7 polymerase gene. Whole-cell lysates were prepared and subjected to polyacrylamide gel electrophoresis, and Western blot analysis was performed with an antiserum directed against the second binding domain of PRDII-BF1. Samples include lysates from HeLa cells infected with the recombinant vaccinia virus (lane 1), transfected with pTM1-PRDII-BF1 alone (lane 2), or transfected with pTM1-PRDII-BF1 and infected with recombinant vaccinia virus expressing T7 polymerase (lane 3).

recombinant vaccinia virus (Fig. 5B, lane 3) but not in extract prepared from cells lacking either pTM1 (Fig. 5B, lane 1) or the recombinant vaccinia virus (Fig. 5B, lane 2). These results indicated that the full-length PRDII-BF1 protein could be produced both in vitro and in vivo.

PRDII-BF1 is an activator of HIV-1 gene expression. The PRDII-BF1 cDNA was cloned downstream of the RSV promoter in the expression vector pDP18. Cotransfection experiments were performed to determine whether PRDII-BF1 was capable of altering HIV-1 LTR CAT gene expression. Transfections in both the presence and absence of the *tat* gene were performed in both unstimulated and TPA- and PHA-treated Jurkat cells. When increasing quantities of a PRDII-BF1 expression vector were transfected with the HIV-1 LTR CAT construct, activation of HIV-1 gene expression was found (Fig. 6A). The level of activation varied from 4- to 10-fold in both the presence and absence of the *tat* gene in three independent experiments (Fig. 6A). Extracts were diluted (Fig. 6B) to better demonstrate the effect of PRDII-BF1 on activating HIV-1 LTR CAT gene expression in the presence of *tat*. This activation was dependent on the presence of the HIV-1 enhancer element, since constructs with enhancer deletions or substitutions were not activated by PRDII-BF1 (Fig. 6C). Mutation of the HIV-1 LTR TAR loop sequences between nucleotides +31 and +34 reduced activation by PRDII-BF1 in the presence but not in the absence of *tat* (Fig. 6D).

Next, we performed experiments to determine whether inhibition of endogenous levels of PRDII-BF1 altered HIV-1 gene expression. Similar types of experiments demonstrated that antisense constructs that inhibited both NF-κB and an accessory binding factor, known as HMG 1 (Y), prevented activation from the beta interferon promoter NF-κB site (42). Transfection experiments were performed in Jurkat cells in both the presence and absence of *tat*. There was a fivefold inhibition of *tat*-induced HIV-1 gene expression in the presence of the antisense PRDII-BF1 construct (Fig. 7, lanes 5 to 8) compared with transfections which lacked this construct (Fig. 7, lanes 9 and 10). In the absence of *tat*, there was little effect from the antisense PRDII-BF1 construct on HIV-1 gene expression (Fig. 7, lanes 5 and 7). No significant inhibition of HIV-1 gene expression was detected in the presence of an antisense CREB construct (Fig. 7, lanes 1 to 4). The antisense PRDII-BF1 construct did not inhibit gene expression of an HIV-1 construct which deleted the enhancer element or an RSV CAT construct (data not shown). The degree of inhibition by the antisense PRDII-BF1 construct varied from three- to sixfold in four independent experiments. These results, in conjunction with transfections of PRDII-BF1 expression constructs, were consistent with a role for PRDII-BF1 in activating HIV-1 gene expression.

DISCUSSION

The modulation of HIV-1 gene expression is dependent on multiple *cis*-acting regulatory elements (reviewed in reference 11). One regulatory element, the enhancer, extends from −103 to −82 in the HIV-1 LTR and contains two NF-κB recognition motifs (31). Mutagenesis of this element indicates that it plays an important role in regulating HIV-1 gene expression in transient expression assays in both the presence and absence of *tat* (12, 31). Though HIV-1 constructs containing mutations in the NF-κB elements do not exhibit marked changes in growth properties (27), it is likely that these motifs play a significant role in regulating viral gene expression in response to mitogens or cytokines (19, 31). Thus, a study of cellular factors that bind to the enhancer element is important for understanding HIV-1 gene expression.

A family of Rel-related cellular proteins, including p49, p50, p65, and p80 (4, 15, 23, 24, 28, 33, 35, 37), is likely important in regulating HIV-1 gene expression (22, 24, 37). A number of these proteins are bound to cytoplasmic inhibitory proteins known as IκB which regulate the nuclear levels of these factors (2, 3, 8, 19, 20, 33, 43, 47). For example, changes in the cellular signal transduction pathway mediated by alterations in protein kinase C or the generation of free oxygen radicals result in the phosphorylation of IκB-β, thus releasing cytoplasm-bound p65, which is then translocated to the nucleus. The activation of T lymphocytes by agents such as phorbol esters and PHA can also result in increased levels of NF-κB proteins by increasing the synthesis of RNA from NF-κB genes (28). Thus, members of the NF-κB family may be regulated by altering either their mRNA synthesis or their nuclear localization. The ability of different members of the NF-κB family to homodimerize or heterodimerize results in binding and subsequent activation of gene expression (1, 24, 25, 43).

Several different transcription factors are able to bind to NF-κB motifs. A constitutive nuclear transcription factor, known as PRDII-BF1 or MBP-1, contains two widely spaced zinc finger DNA binding domains, each of which can bind to the NF-κB and related binding motifs (5, 10, 30). Proteins with homology to PRDII-BF1 have been identified in both the mouse and rat and bind to NF-κB sites in gel retardation assays (32, 34). The level of PRDII-BF1 mRNA is induced by activation of T lymphocytes (5). However, no data on the function of the PRDII-BF1 protein have previously been presented.

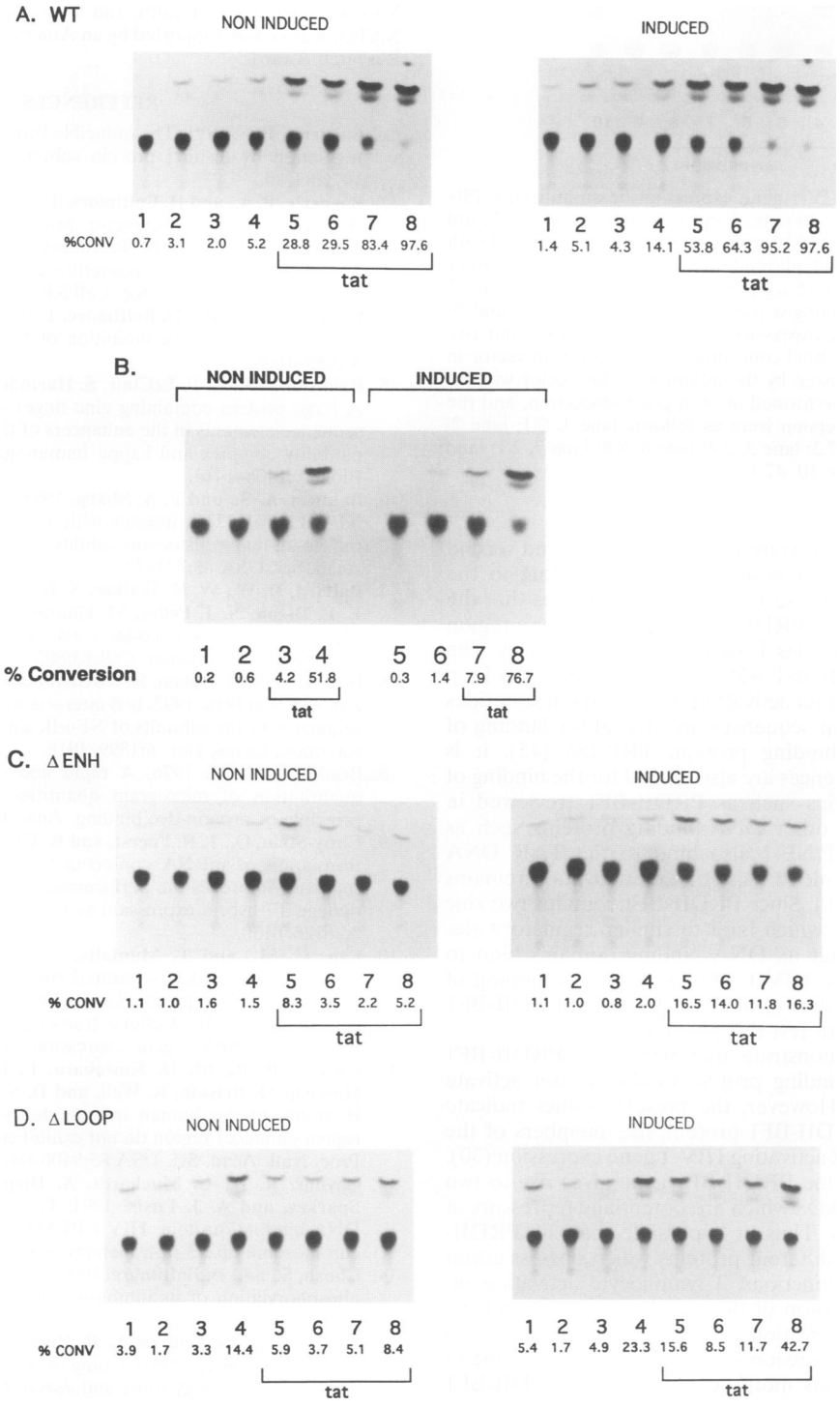

FIG. 6. PRDII-BF1 activates HIV-1 LTR gene expression. Jurkat cells, either untreated (left) or treated with TPA and PHA (right), were electroporated with 2 μg of either wild-type (WT) HIV-1 LTR CAT plasmid (A and B), a similar HIV-1 LTR CAT plasmid which deleted the enhancer region (ΔENH) (C), or a wild-type HIV-1 LTR CAT plasmid with substitution of nucleotides +31 to +34 in the TAR element (D). Electroporations were performed in both the absence (lanes 1 to 4) and presence (lanes 5 to 8) of 1 μg of *tat*. The amount of PRDII-BF1 expression vector included in each electroporation was as follows: 0 (lanes 1 and 5), 0.3 μg (lanes 2 and 6), 1.0 μg (lanes 3 and 7), or 3.0 μg (lanes 4 and 8). In panel B, extracts used in lanes 1, 4, 5, and 8 of panel A from both noninduced and induced Jurkat cells and now labeled lanes 1 to 8 were diluted fivefold, and the CAT assays were repeated. CAT assays were performed on each sample at 36 h posttransfection, and the percentage of CAT conversion (%CONV) is indicated.

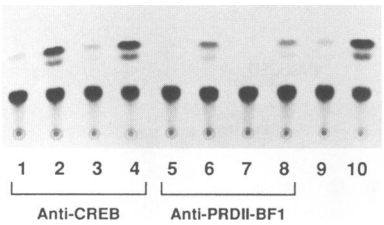

FIG. 7. Inhibition of HIV-1 gene expression by an antisense PRDII-BF1 construct. Jurkat cells in both the absence (lane 1, 3, 5, 7, and 9) and presence (2, 4, 6, 8, and 10) of 1 μg of *tat* were transfected with 5 μg of an HIV-1 LTR CAT plasmid. Antisense constructs, to either the CREB coding sequence (5 μg [lanes 1 and 2] and 10 μg [lanes 3 and 4]), the PRDII-BF1 coding sequence (5 μg [lanes 5 and 6]) and 10 μg [lanes 7 and 8]), or the expression vector alone (lanes 9 and 10), were also transfected. The final concentration of expression vector in each transfection was adjusted by the addition of this vector without insert. CAT assays were performed at 36 h posttransfection, and the percentages of CAT conversion were as follows: lane 1, 2.1; lane 2, 21.3; lane 3, 4.4; lane 4, 37.2; lane 5, 2.4; lane 6, 8.6; lane 7, 2.1; lane 8, 5.4; lane 9, 3.2; and lane 10, 47.4.

In this study, we demonstrate that both the first and second PRDII-BF1 binding domains are capable of binding to the HIV-1 NF-κB motifs. DNase I footprinting indicates that the second binding domain of PRDII-BF1 also binds over a region of TAR DNA which encodes TAR RNA loop sequences. The sequences between +30 and +35 which encompass the loop region are important for *tat* activation. Though the nucleotides which comprise the loop sequences are critical for binding of the TAR RNA loop-binding protein, TRP-185 (45), it is possible that these sequences are also critical for the binding of DNA regulatory proteins such as PRDII-BF1 (reviewed in reference 11). Several other DNA-binding proteins such as UBP-1/LBP-1 and CTF/NF-1 also bind to the TAR DNA element, though their role in basal and *tat* activation remains to be determined (21, 46). Since PRDII-BF1 contains two zinc finger binding domains which bind to similar regulatory elements, it is possible that its DNA binding domains bind to different portions of the HIV-1 LTR, resulting in looping of the DNA. Further studies with the full-length PRDII-BF1 protein are under way to test this possibility.

Previous studies demonstrate that fusions of PRDII-BF1 with the yeast DNA-binding protein GAL4 do not activate gene expression (30). However, the present studies indicate that the full-length PRDII-BF1 protein, like members of the Rel family, is capable of activating HIV-1 gene expression (30). Alternative splicing of the PRDII-BF1 gene gives rise to two proteins of 200 and 68 kDa which are potentially repressors of HIV-1 gene expression. Thus, it is possible that the PRDII-BF1 gene gives rise to different proteins which possess either activator or repressor functions. T-lymphocyte activation increases the gene expression of both PRDII-BF1 (5) and the Rel proteins (20). In addition, during the T-cell activation process, HIV-1 gene expression is increased via the binding of cellular factors to NF-κB motifs (31). Whether PRDII-BF1 and the Rel proteins influence each other's ability to regulate HIV-1 gene expression remains to be determined. The importance of these proteins in influencing HIV-1 gene expression will require further studies on their relative levels and activities in both uninfected and HIV-1-infected cells.

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