JOURNAL OF VIROLOGY, Nov. 1995, p. 6634–6642 Vol. 69, No. 11
0022-538X/95/$04.00+0

Interactions of the Transcription Factor AP-1 with the Long Terminal Repeat of Different Human Immunodeficiency Virus Type 1 Strains in Jurkat, Glial, and Neuronal Cells

FRANÇOIS CANONNE-HERGAUX, DOMINIQUE AUNIS, AND EVELYNE SCHAEFFER*

Unité INSERM 338, Centre de Neurochimie, 67084 Strasbourg Cedex, France

Received 8 May 1995/Accepted 31 July 1995

Human immunodeficiency virus type 1 (HIV-1) infection of the neuronal and astroglial cells of the central nervous system has been proposed to contribute to HIV-1-associated dementia. Recently it was shown that differences in the nucleotide sequence of the long terminal repeat (LTR) of different HIV-1 strains govern the tissue-specific pattern of viral expression. The LTR from central nervous system-derived HIV-1 strains JR-FL and JR-CSF directs expression in the neurons of transgenic mice, in contrast with the lymphotropic LAI strain. By in vitro footprinting, gel retardation, and methylation interference experiments, we have studied the interactions of host cell proteins from human neuronal, glial, HeLa, and Jurkat T cells with the LTRs from the neurotropic JR-FL and JR-CSF strains, compared with the LAI strain. Proteins belonging to the nuclear receptor family bind with different affinities to variant −352 to −324 sites. Gel supershift assays with Jun and Fos antibodies showed that the AP-1 transcription factor present in the various cell types was unable to recognize the −352 to −324 and −306 to −285 AP-1 putative binding sites. Interestingly, Jun and Fos components of AP-1 interact with the variant TGGCTCA sequence located in the −247 to −222 region of both neurotropic strains. These interactions were cell type specific, since they were detected only with extracts from glial and HeLa cells and not from neuronal or Jurkat cells. Cotransfection experiments further revealed that the −247 to −222 sequence is able to mediate AP-1-induced transcriptional activation in glial and not neuronal cells.

Direct infection of the neuronal and astroglial cells of the central nervous system (CNS) has been proposed to contribute to human immunodeficiency virus type 1 (HIV-1)-associated dementia (33). A recent study showed that some strains of HIV-1 have a selective advantage for gene expression in the brain. It has been well established that tropism, replication, and cytopathicity are linked to the envelope region of the viral genome (36) and that differences in envelope sequences influence the occurrence of CNS disease (24). Moreover the long terminal repeat (LTR) from two CNS-derived strains of HIV-1, JR-FL and JR-CSF (15), was found to direct gene expression in the CNS neurons of transgenic mice (5). These data indicate that neurons can produce cellular transcription factors that govern expression from the LTR. Indeed, it was shown that neurons contain the transcription apparatus capable of HIV-1 promoter activation. Similar to the situation in T lymphocytes and macrophages, the transcription factor NF-κB was reported to be an activator of HIV-1 transcription in neurons (13, 26).

In contrast, transgenic mice that contained the LTR of HIV-1 LAI did not have any gene expression in the CNS (18, 34). These data indicate that differences in the nucleotide sequences of the LTRs of different strains can alter the binding properties of transcription factors and thus affect the tissue-specific pattern of LTR-directed gene expression. It is well established that HIV gene transcription is directed by specific interactions of viral and host cell transcription factors with different LTR regions (8, 9, 27). Comparison of the sequences in the U3 region of the LTRs of HIV-1 LAI, JR-CSF, and JR-FL showed that the nuclear factor of NF-κB and Sp1 proximal promoter sites were completely conserved. However, there were more than 27 base differences upstream of these sites of both JR-CSF and JR-FL compared with LAI (5).

These data prompted us to investigate whether the nucleotide variations between distinct LTR sequences gave rise to additional or missing binding sites for *trans*-acting factors. Here we describe experiments to characterize the modulatory elements located between nucleotides −480 and −160 in the neurotropic JR-CSF and JR-FL strains, compared with the lymphotropic LAI strain. Using in vitro footprinting and electrophoretic mobility shift assays, we have mapped DNA elements that interact with host nuclear factors present in human neuronal and glial cells, compared with T lymphocytes and HeLa cells. We have established that in glial and neuronal cells, the −352 to −324 sequence is the binding site for proteins belonging to the nuclear receptor family. Moreover, we provide evidence that the AP-1 transcription factor is unable to interact with this −352 to −324 motif, as well as with the −306 to −285 putative AP-1 site. In contrast, in astrocytoma, oligodendroglioma, and HeLa cells, but not in neuronal or Jurkat cells, AP-1 is able to bind to the −247 to −222 site present in the neurotropic HIV-1 isolates. Functional studies further showed that this sequence placed in a reporter vector is able to confer c-*jun* responsiveness in glial but not in neuronal cells. Our data therefore illustrate the differences in the transcriptional utilization of the distal regulatory LTR regions, depending on both the cell type and the viral strain.

MATERIALS AND METHODS

Materials. DNA-modifying enzymes were purchased from Boehringer Mannheim or New England Biolabs. Radioactive compounds were from Amersham.

* Corresponding author. Mailing address: Unité INSERM 338, Centre de Neurochimie, 5, rue Blaise Pascal, 67084 Strasbourg Cedex, France. Phone: (33) 88 45 67 18. Fax: (33) 88 60 08 06.

Tissue culture media and reagents were obtained from GIBCO-BRL. All other reagents were obtained from Sigma.

Cell culture. Human HeLa, astrocytoma U373-MG, and neuroblastoma SK-N-MC cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES). The medium of neuroblastoma SK-N-MC cells was supplemented with nonessential amino acids. Jurkat T cells were grown in RPMI 1640 with 10% fetal calf serum and 10 mM HEPES. All these cell lines were cultured in the presence of penicillin-streptomycin (100 U/ml). Human oligodendroglioma TC-620 cells (gift of B. Zalc, Unité INSERM 134, Paris, France) were grown in Iscove medium containing 10% non-heat-inactivated fetal calf serum and 1% gentamicin. In experiments involving phorbol ester treatment, cells were kept for 24 h without fetal calf serum and treated for 4 to 6 h with 100 ng of tetradecanoyl phorbol acetate (TPA) per ml prior to harvesting.

Transfections and CAT assays. Astrocytoma, oligodendroglioma, and neuroblastoma cells (10^6) were transfected by the calcium phosphate precipitation method with 1 pmol of plasmid reporter DNA and 1.5 μg of either hc-Jun vector (gift of C. Quirin-Stricker, Unité INSERM 184, Strasbourg, France) or the parental expression vector pSG5 (10), as described previously (11). Each transfection was done in duplicate and repeated a minimum of three separate times with at least two different plasmid preparations. Cell extracts were prepared 48 h after transfection. Chloramphenicol acetyltransferase (CAT) assays were performed as described previously (11) with 5, 10, and 20 μg of protein and a 1-, 2-, and 2-h incubation for, respectively, oligodendroglioma, astrocytoma, and neuroblastoma cells.

Preparation of nuclear proteins. Nuclear proteins were extracted from at least 10^8 untreated cells, as previously described (11). Nuclear extracts were prepared from a small number (5×10^6) of TPA-treated cells, according to the procedure of Andrews and Faller (1).

Plasmid constructs. Plasmid pSAFYre, containing the JR-FL LTR (gift of J. Clements, Johns Hopkins University, Baltimore, Md.), was digested with *Kpn*I and *Hin*dIII; the LTR insert was blunt ended and subcloned into the *Kpn*I-*Sma*I sites of pUC19-CAT0. Plasmid pSAFYre, containing the JR-CSF LTR (gift of J. Clements), was digested with *Eco*RV and *Bgl*II; the −418 to +20 LTR insert was blunt ended and subcloned into the *Sma*I site of pUC19-CAT0. Plamid pSV1b-CAT, containing the LAI LTR (gift of N. Israel, Institut Pasteur, Paris, France), was digested with *Bgl*II and *Hin*dIII; the −1489 to +79 LTR insert was blunt ended and subcloned into the *Sma*I site of pUC19-CAT0. To construct the 5N/tk-CAT, 5Nmut/tk-CAT, and 5L/tk-CAT vectors, the 5N, 5Nmut, and 5L oligonucleotides, respectively, (see Fig. 7) were subcloned in the blunt-ended *Sal*I site of pBLCAT2, containing the herpes simplex virus thymidine kinase promoter in front of the CAT gene (20) (gift of C. Kedinger, Unité INSERM 184, Strasbourg, France).

DNase I footprinting. The *Ava*I-*Kpn*I LTR fragment was 3′ end labled on the *Ava*I site by using [α-^{32}P]dCTP and Klenow enzyme. The *Eco*RI-*Ava*I LTR fragment was 3′ end labeled on the *Eco*RI site by using [α-^{32}P]dATP and Klenow enzyme. DNase I digestion assays were carried out with 20 μg of nuclear protein extracts in each sample, as described by Brunel et al. (3).

Electrophoretic mobility shift assay. Protein-DNA binding reactions were performed with 5 or 10 μg of nuclear protein extracts in a buffer containing 1 ng of oligonucleotide labeled with ^{32}P on the 5′ end, 1 μg of the nonspecific competitor poly(dI-dC), 50 ng of sonicated salmon sperm DNA, 10 mM $MgCl_2$, 25 mM KCl, 1 mM dithiothreitol, 12.5 mM HEPES (pH 7.8), 10% glycerol, and 0.05% Nonidet P-40. Mixtures were incubated for 15 min at 4°C, and protein-bound DNA complexes were further analyzed by electrophoresis on a 6% polyacrylamide gel in 0.25× TBE (1× TBE is 89 mM Tris, 89 mM boric acid, and 2 mM EDTA). For competition assays, unlabeled oligonucleotides were added at a 50-fold molar excess at the same time as probes. The sequences of the double-stranded oligonucleotides used as competitors were as follows: PRI, 5′-ACAAA CACGGGAGGTCAAAGATTGCGCCCAG-3′; PRImut, 5′-ACAAACACGG GAGGTTAACTATTGCGCCCAG-3′ (30); and URI, 5′-GTGGAAGGAATC TTGACCTTTCCCA-3′ (29a). The sequences of the other oligonucleotides used as probes or competitors are given in Fig. 2, 6, and 7.

For supershift assays, specific antibodies directed against c-Jun, JunB, and JunD or against c-Fos, FosB, Fra1, and Fra2 (obtained from Santa Cruz Biotechnology) or normal rabbit serum or antibodies directed against chicken ovalbumin upstream promoter transcription factors (COUP-TFs) (gift of M. J. Tsai, Baylor College of Medicine, Houston, Tex.) were mixed with nuclear proteins for 1 h at 4°C prior addition of probes.

Methylation interference. Oligonucleotide 3L was 5′ end labeled, annealed with the nonlabeled complementary oligonucleotide, and partially methylated at guanine residues by using dimethyl sulfate. The methylated probe (10 ng) was incubated with the nuclear protein extracts (20 μg) under the conditions described above for the mobility shift assay. The complexes were separated on a preparative 6% mobility shift gel. The gel was exposed for 2 h; the complexed and free oligonucleotides were excised from the gel, eluted, and ethanol precipitated. The samples were treated with 1 M piperidine and electrophoresed on a urea–25% polyacrylamide sequencing gel.

RESULTS

Interactions of host cell nuclear proteins with the −488 to −159 LTR region from lymphotropic and neurotropic HIV-1 strains. To characterize the host cell nuclear proteins interacting with the −488 to −159 LTR region of the lymphotropic LAI and the neurotropic JR-FL and JR-CSF HIV-1 strains, we performed DNase I footprint analysis. The *Eco*RI-*Ava*I LTR insert of the three LTR-pUC-CAT vectors was 3′ end labeled on each strand, as described in Materials and Methods. Nuclear extracts were isolated from three brain cell lines, astrocytoma U373-MG, oligodendroglioma TC-620, and neuroblastoma SK-N-MC, which were all shown to be permissive for HIV-1 infection. Nuclear extracts were also prepared from Jurkat T cells and epithelial HeLa cells. Figure 1 shows the pattern obtained with the strand labeled on the *Ava*I site with the LAI and JR-FL strains.

With the LAI strain, two adjacent protected regions, A (−385 to −354) and B (−352 to −314), were detected with all five extracts. A DNase I-hypersensitive site is located at the border of region B (Fig. 1A). This pattern has been described with extracts from either activated or nonactivated Jurkat cells (23). Our result confirms that nuclear proteins present in glial and neuronal cells give a pattern of regions A and B similar to that obtained with Jurkat cells. However, with extracts from U373-MG cells, an additional region, C (−314 to −285), was detected. A hypersensitive site located at position −285 was also present with extracts from U373-MG, TC-620, and HeLa cells.

With the JR-FL strain (Fig. 1B), the protection pattern of regions A and C was similar to that obtained with the LAI strain. In contrast, the protection of region B could be detected only with extracts from U373-MG and Jurkat cells. An additional protected region, D, located between positions −245 and −224 was clearly visible with 20 μg of U373-MG proteins (Fig. 1B). This footprint could also be detected with 40 μg of TC-620 and HeLa protein extracts (results not shown). With the JR-CSF strain, the protection pattern of regions A and B was similar to that of the LAI LTR; the protection pattern of region D was similar to that of the JR-FL LTR (results not shown).

Analysis of the −352 to −324 region, region B. Studies concerning nuclear proteins interacting with site B have been reported for Jurkat and HeLa cells. They showed that the −352 to −324 sequence is the binding site for proteins of the COUP-TF family (4, 22, 23). More recently, it was reported that multiple nuclear hormone receptors and orphan receptors have the potential to interact with region B (16). In order to analyze the nuclear proteins present in glial and neuronal cells interacting with site B, we performed DNA mobility shift assays using oligonucleotides 3L, 3Lv, and 3N (Fig. 2). The 3L sequence, which corresponds to region B of the LAI and JR-CSF strains, contains a perfect palindromic GGTCA binding site for transcription factors of the nuclear receptor family (Fig. 2C). The 3L probe gave rise to four DNA-protein complexes, C1 to C4, with extracts from U373-MG and SK-N-MC cells. Three complexes, C1, C3, and C4, were formed with extracts from TC-620 and HeLa cells. Only two complexes, C1 and C4, were formed with Jurkat cell extracts (Fig. 2A). Oligonucleotide 3Lv, which corresponds to region B of the LAI B8 strain (25), contains an AGTCA half site, mutated on one guanine residue. With the 3Lv probe, the formation of complexes C1, C2, and C3 was decreased. The 3N oligonucleotide corresponds to region B of the JR-FL strain and contains two base mutations within the GGTCA site. With the 3N probe, the formation of complexes C1 and C2 was abolished; com-

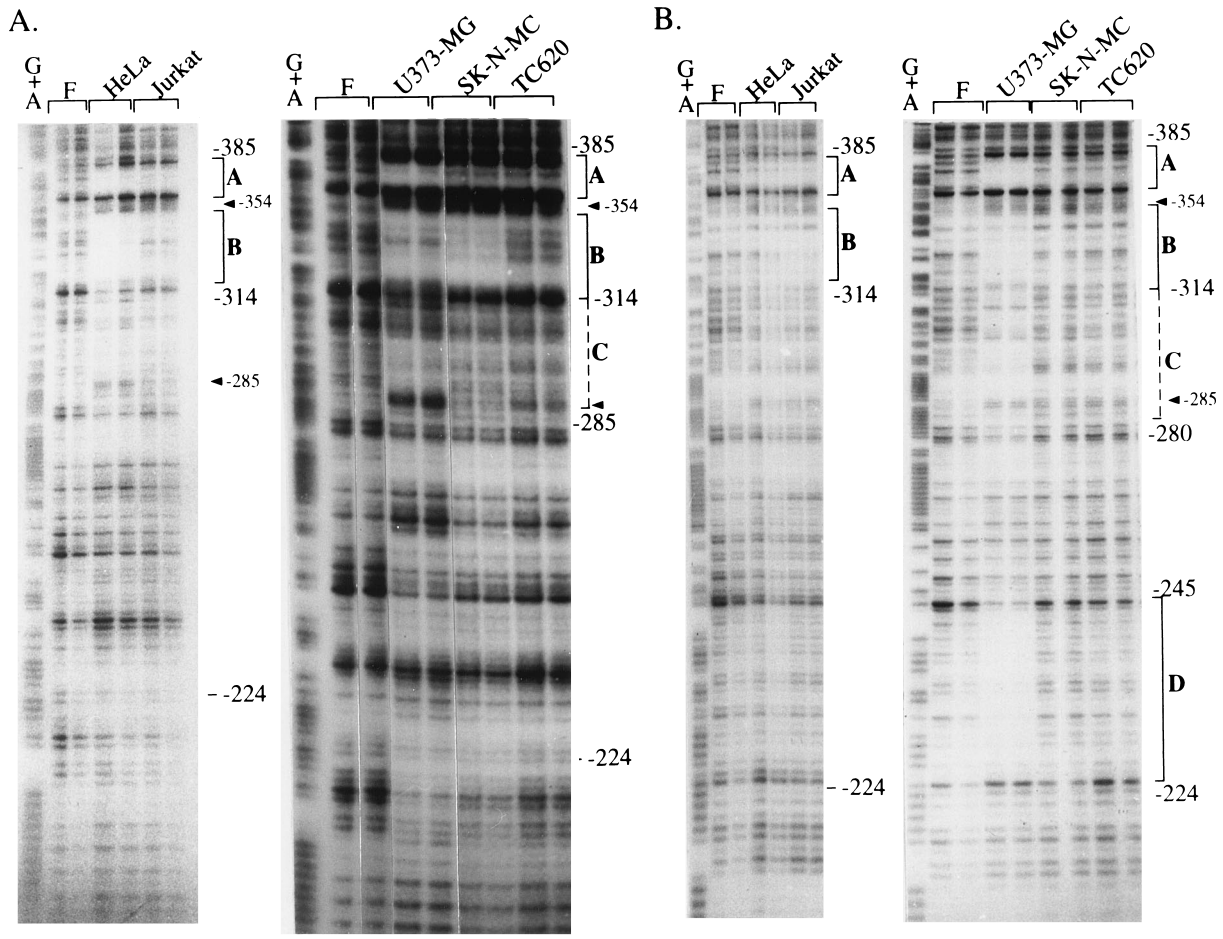

FIG. 1. Interactions of nuclear proteins from different cell types with the −488 to −159 LTR from the HIV-1 lymphotropic LAI (A) and neurotropic JR-FL (B) strains. For footprint analysis, the *Eco*RI-*Ava*I fragment was 3′ end labeled at position −159. Nuclear extracts were prepared from brain cell lines permissive to HIV-1 infection: astrocytoma U373-MG, neuroblastoma SK-N-MC, and oligodendroglioma TC620. Nuclear proteins from HeLa and Jurkat cells were also used. DNase I digestion was performed with 20 μg of nuclear proteins. Lanes G+A, the sequence ladder; Lanes F, assays with DNA without nuclear extracts. The brackets delineate protected sequences. The dashed lines delineate protected sequences detected with U373-MG extracts. Horizontal arrows indicate sites of enhanced DNase I cleavage.

plexes C3 and C4 could be detected with U373-MG extracts, while only complex C4 was detected with all other extracts (Fig. 2A).

To test for the specificity of these interactions and attempt to identify the proteins contributing to binding activity in each complex, competitive binding assays were performed. Figure 2B shows the results of competition experiments performed with extracts from Jurkat and astrocytoma cells, using oligonucleotide 3L as a probe. The formation of complexes C1, C2, and C3 was abolished with a 50-fold molar excess of both the homologous 3L and heterologous PRI oligonucleotides. PRI was previously shown to bind the orphan nuclear receptors hepatocyte nuclear factor 4 and COUP-TF within the transferrin gene promoter (30). In contrast, the mutant oligonucleotides PRImut and URI failed to compete for the formation of complexes C1 to C3. Similar results were obtained with extracts from all other cell types (results not shown). These results suggest that proteins forming complexes C1, C2, and C3 have a DNA binding specificity similar to that of the nuclear receptors. The formation of complex C4 appeared less specific, since it was affected only slightly by the addition of the 3L competitor. In contrast to complexes C1 to C4, reproducibly detected, the fastest-migrating complex observed with U373-MG proteins varied with different extract preparations; it is likely to correspond to a proteolytic degradation of protein forming complex C4, since it has a similar behavior in competition assays and therefore appears nonspecific.

Methylation interference analysis allowed us to confirm that in the three brain cell types, proteins forming complexes C1 and C3 belong to the nuclear receptor family. Nuclear proteins forming the C1 and C3 complexes interacted with the guanine residues of the GGTCA palindromic sequence, as shown with TC-620 cell extracts (Fig. 3). Similar results were obtained with proteins from U373-MG, SK-N-MC, and Jurkat cells (results not shown). This pattern of interference is typical of the pattern described with the nuclear receptor proteins (16). In contrast, proteins forming the C4 complex did not interact with any guanine residue (Fig. 3), further suggesting that these proteins do not belong to the nuclear receptor family.

To further identify the nature of the DNA-binding proteins, shift assays were performed with oligonucleotide 3L in the presence of antibodies raised against the COUP-TF subfamily of factors (35). About 80% of complexes C1, C2, and C3 disappeared, while complex C4 was not affected. In the presence of nonimmune serum, the complexes were unaffected (results not shown). These data further indicate that COUP-TFs form the majority of complexes C1 to C3.

Oligonucleotide 3Lv has been reported to interact, directly

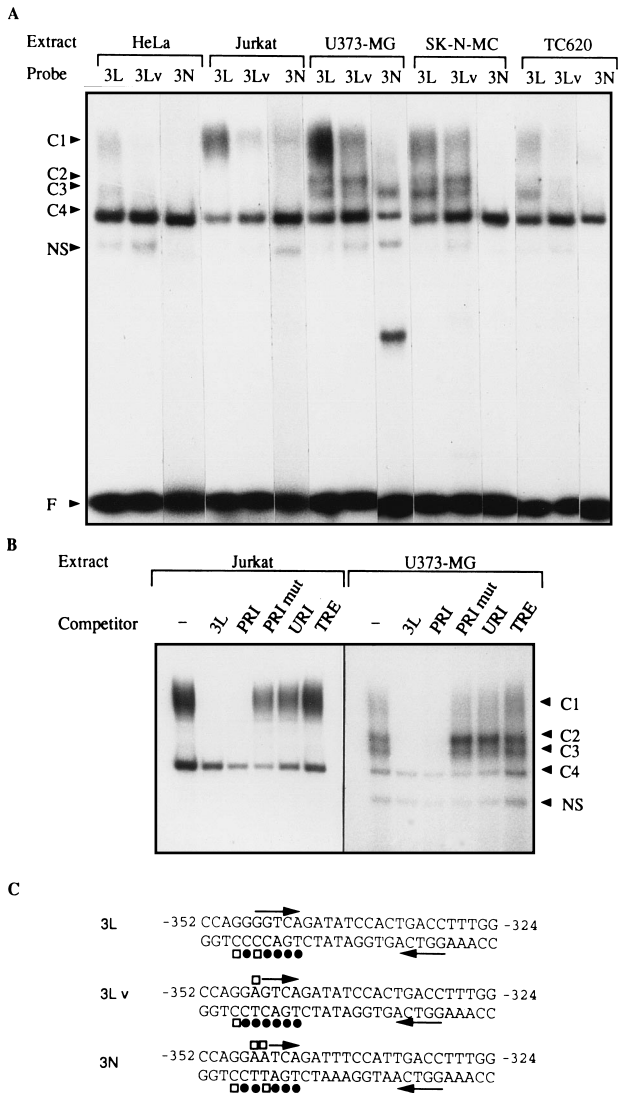

FIG. 2. Analysis of nuclear proteins from distinct cell types binding to the LTR region B of different HIV-1 strains. (A) Gel retardation assays were carried out by incubating 10 μg of nuclear proteins extracted from the different cell types indicated on top with probes 3L, 3Lv, and 3N (sequences in panel C). The specific DNA-protein complexes C1 to C4 and the nonspecific complexes (NS) are indicated. F corresponds to the free probe. (B) Gel retardation assays carried out with nuclear proteins from Jurkat and U373-MG cells and the 3L probe. Competition experiments were performed with a 50-fold molar excess of the unlabeled oligonucleotide indicated on top. The sequences of PRI, PRImut, and URI are presented in Materials and Methods. The sequence of TRE is presented in Fig. 4. Specific complexes C1 to C4 and nonspecific complex (NS) are indicated. (C) Sequences of oligonucleotides 3L, 3Lv, and 3N, corresponding to the LTR region B of HIV-1 LAI, clone BH8, and JR-FL, respectively. Arrows indicate the palindromic consensus binding site for COUP-TF. Closed circles underline the TGACTCA consensus binding site for AP-1. Open squares indicate a mutant nucleotide.

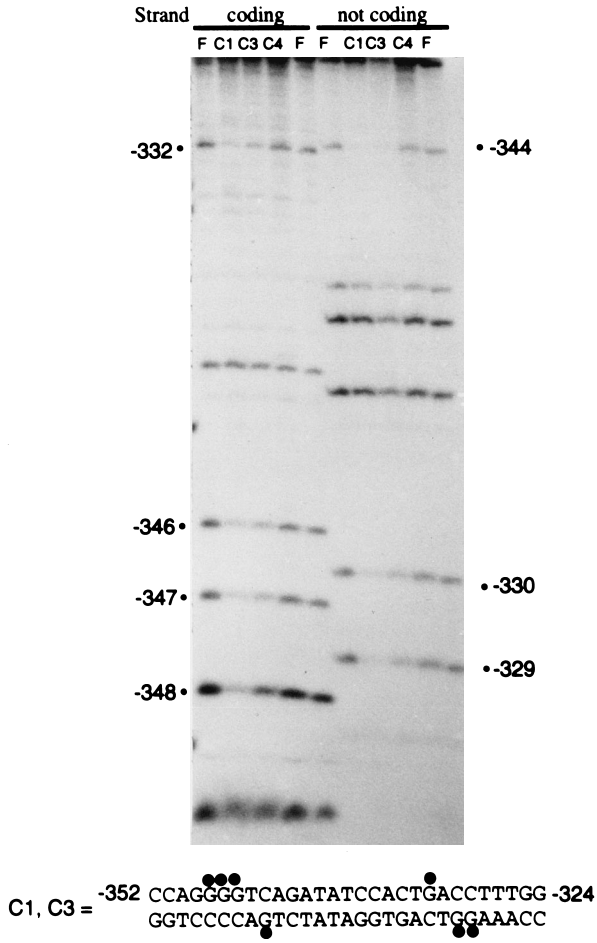

FIG. 3. Methylation interference patterns of C1, C3, and C4 DNA-protein complexes obtained with the 3L probe and TC-620 nuclear proteins. Oligonucleotide 3L was labeled at the 5′ end of one strand. Guanine residues were partially methylated with dimethyl sulfate. The oligonucleotide was used in a gel shift assay with nuclear proteins from TC-620 cells. The DNA-protein complexes C1, C3, and C4 (Fig. 2) were eluted from the polyacrylamide gel and cleaved at methylated guanine residues with piperidine. The protein-bound DNA (lanes C1, C3, and C4) was compared with free DNA (lanes F) on a sequencing gel. Guanine residues that interfere with binding are indicated by closed circles.

or indirectly, with Fos and Fra proteins (7). To investigate a possible association of these proteins with 3Lv, we performed competition experiments with the TRE oligonucleotide containing the consensus AP-1 binding site from the human metallothionein IIA promoter (17). The formation of complexes C1 to C4 was not affected by the addition of a 50-fold molar excess of TRE oligonucleotide (Fig. 2B). Similar results were obtained with extracts from all five cell types. This result already suggests that the AP-1 protein is not present in complexes C1 to C4.

It was necessary to control whether AP-1 was present in the various cell types cultured in the presence of fetal calf serum. Nuclear extracts from all five cell types were used to perform mobility shift experiments, using the TRE probe (Fig. 4A). Two distinct complexes, a and b, were formed and were specifically inhibited with a 50-fold molar excess of unlabeled TRE oligonucleotide. Depending on the cell type, the a complexes were more or less abundant and presented different electrophoretic mobilities. To further identify the proteins contributing to binding activity in each complex, nuclear extracts were incubated with antibodies raised against the DNA-binding domains of c-Jun, JunB, and JunD proteins. This antiserum prevented the formation of the a complexes formed with all cell extracts and had no effect on the b complexes, as shown with extracts from Jurkat and U373-MG cells (Fig. 4B). This confirmed that AP-1 is present, although in different amounts, in the various serum-cultured cells and contributes to the formation of the a complexes.

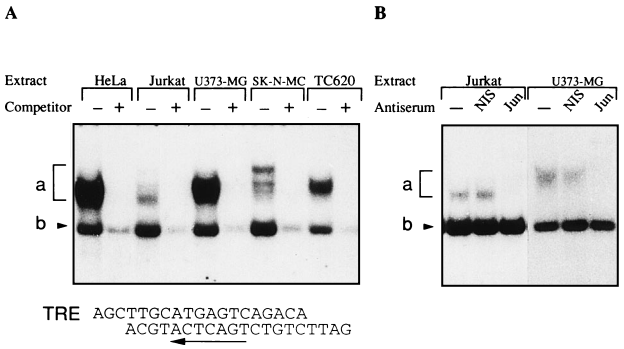

FIG. 4. Cellular distribution of TRE-binding proteins. (A) Gel retardation assays with the TRE probe and 10 μg of nuclear extracts prepared from the different cell lines indicated on top, cultured in the presence of fetal calf serum. Competitions were performed with a 50-fold molar excess of unlabeled TRE oligonucleotide (lanes +). The specific complexes a and b are indicated. (B) Effects of the addition of either immunoglobulin G directed against c-Jun, JunB, and JunD or nonimmune serum (NIS) to the binding reaction performed with 4 μg of nuclear proteins from Jurkat and U373-MG cells.

The phorbol ester TPA has been demonstrated to induce the expression of Fos and Jun proteins, which bind as heterodimers to 5′-TGACTCA-3′ motifs. Since the interaction of Fos and Fra proteins with the 3Lv sequence has been reported with stimulated Jurkat cells, we used nuclear extracts from Jurkat cells treated with TPA for 4 and 6 h. Clear induction of the AP-1 binding activity was detected with the TRE probe. However, with the 3Lv probe, besides complexes C1 and C4, no induction of a new complex could be detected (Fig. 5). Moreover, antibodies directed against either the Jun or the Fos family had no effect on either complex C1 or C4 (results not shown). This result clearly indicates that the AP-1 protein, although present in both serum-cultured and TPA-treated Jurkat cells, is unable to interact with the 3L and 3Lv sequences.

Examination of the 3Lv and 3L sequences revealed that one and two bases, respectively, are mutated within the consensus binding site for AP-1. It has already been reported that a synthetic 5′-TGACTCA-3′ TRE site bearing base substitution mutations failed to bind AP-1 in DNase footprint experiments (17). This prompted us to test whether a 1-mutant-nucleotide

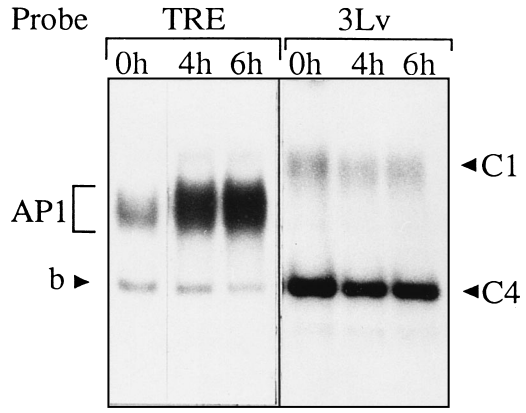

FIG. 5. Analysis of AP-1 binding activity to the TRE and 3Lv oligonucleotides with nuclear extracts from TPA-treated Jurkat cells. Electrophoretic mobility shift assays with nuclear extracts prepared from Jurkat cells cultured for 24 h in the absence of fetal calf serum and further treated with TPA (100 ng/ml) for the indicated time. For each assay, 10 μg of nuclear proteins was incubated with 1 ng of the TRE or 3Lv probe. The TPA-induced AP-1 complex and complexes b, C1, and C4 are indicated.

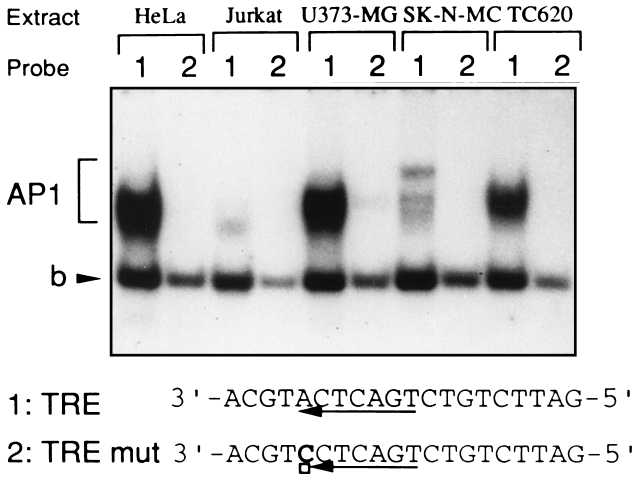

FIG. 6. Comparison of AP-1 binding activity with the TRE and TREmut sequences. (Top) Gel retardation analysis with nuclear proteins extracted from the different cell types indicated above, using the TRE (lanes 1) and the TREmut (lanes 2) probes. The AP-1 complex present with the TRE probe is indicated. (Bottom) Sequences of the TRE and TREmut oligonucleotides. The consensus AP-1 binding site is underlined. The open square indicates a mutant nucleotide.

TRE probe, containing the 5′-TCTGACTCCTG-3′ sequence identical to the sequence present in 3Lv, was able to bind the AP-1 protein. Figure 6 clearly reveals that the TREmut probe totally failed to interact with AP-1 present in all five protein extracts. These results do clearly account for the inability of AP-1 to interact with LTR site B.

Analysis of the −247 to −222 LTR region, region D. To identify the proteins contributing to the interaction with LTR region D, detected with the JR-FL and JR-CSF isolates, the synthetic oligonucleotide 5N (Fig. 7) was used as a probe to perform mobility shift assays. Interestingly, two distinct patterns of DNA-protein complexes, 5a-5b and 5c-5b, were formed with the various nuclear extracts (Fig. 8A). The 5a-5b pattern was detected with proteins from HeLa, U373-MG, and TC-620 cells, while the 5c-5b pattern was formed with proteins from Jurkat and SK-N-MC cells. To examine the specificity of these interactions, competitive binding assays were performed. Competition with unlabeled oligonucleotide 5N specifically eliminated the abundant complex 5a; it also strongly decreased the formation of complex 5b with all five nuclear extracts. The two 5c complexes formed with Jurkat and SK-N-MC proteins appeared less specific than the 5a complexes. Oligonucleotide 5N contains the 5′-TGGCTCA-3′ sequence, which corresponds to an AP-1 binding site mutated on the third nucleotide. This prompted us to perform competition experiments with a 50-fold molar excess of the TRE oligonucleotide. The effect on complexes 5a, 5b, and 5c was similar to that obtained with the homologous oligonucleotide 5N (Fig. 8A). When competition assays were performed with oligonucleotide TREmut or 3L, the formation of the 5a complexes was not affected, while the formation of the 5b complexes was eliminated (results not shown). These results indicate that proteins forming complex 5a have a DNA binding specificity idential to that of AP-1; in contrast, proteins forming complex 5b do not appear to be specific.

To further investigate the presence of AP-1 in the various complexes, supershift experiments were performed with antibodies raised against either the Jun or the Fos family (Fig. 8B). Both anti-Jun and anti-Fos antibodies were able to eliminate the formation of 5a complexes. As a control, all complexes

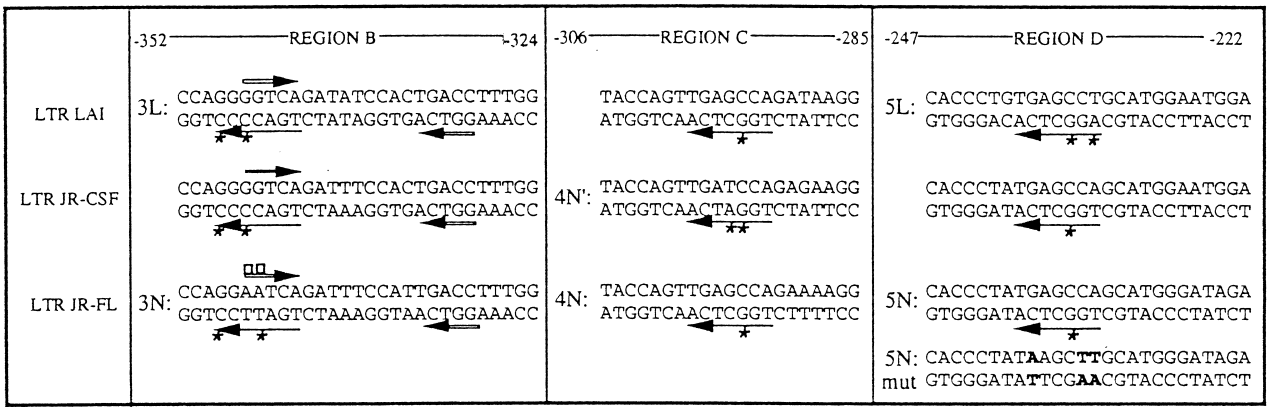

FIG. 7. Sequences of the various oligonucleotides corresponding to regions B, C, and D. The double arrows in region B delineate the palindromic GGTCA sequence; open squares indicate a mutant nucleotide. The simple arrows underline the TGACTCA consensus binding site for AP-1; stars indicate a mutant nucleotide. The boldface type in 5Nmut represents the mutant nucleotides.

were unaffected by the presence of nonimmune serum. In contrast, addition of Jun or Fos antibodies did not affect the formation of 5b complexes as well as of 5c complexes (Fig. 8B).

TPA induction experiments further supported the presence of the TPA-induced AP-1 proteins in 5a complexes. The formation of 5a complexes was clearly enhanced after TPA treatment of HeLa, U373-MG (Fig. 8C), and TC-620 cells. In contrast, the formation of complexes 5b and 5c was not affected following TPA treatment of the cells (Fig. 8C). Taken together, these results indicate that in either serum-cultured or TPA-treated HeLa, U373-MG, and TC-620 cells, AP-1 proteins have the ability to interact with oligonucleotide 5N. Surprisingly, in Jurkat and SK-N-MC cells, Jun and Fos proteins appear unable to bind to oligonucleotide 5N.

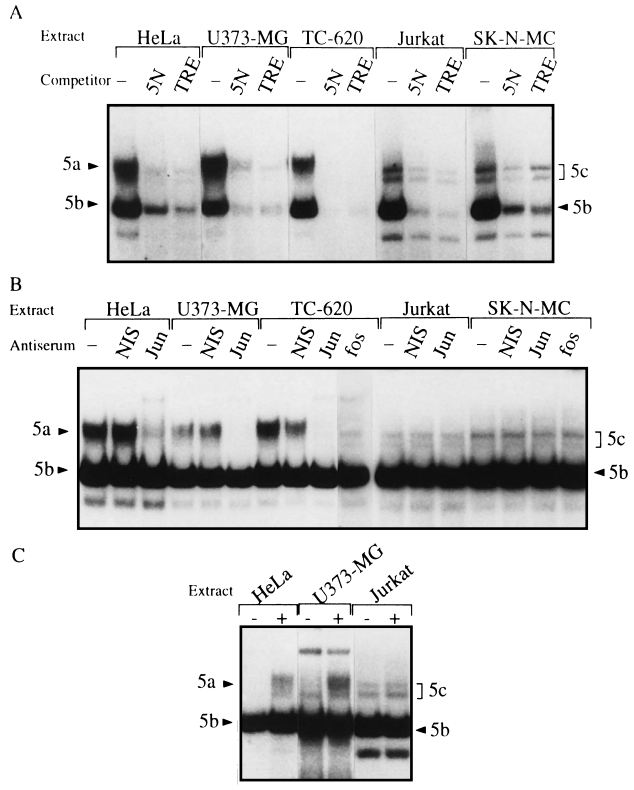

FIG. 8. Interactions of nuclear proteins from different cell types with LTR region D of HIV-1 JR-FL. Gel retardation assays with the 5N oligonucleotide probe (Fig. 7) and 10 μg of nuclear extracts prepared from the cell lines indicated on top. (A) Competition assays performed with a 50-fold molar excess of unlabeled 5N or TRE oligonucleotide. The DNA-protein complexes 5a, 5c, and 5b are indicated. (B) Effects of the addition of nonimmune serum (NIS), or antibodies directed against c-Jun, JunB, and JunD (Jun) or against cFos, FosB, Fra1, and Fra2 (Fos) to the binding reactions. (C) Effects of TPA treatment: the indicated cells were cultured in absence of serum (lanes −) and further treated with 100 ng of TPA per ml for 4 h (lanes +).

Oligonucleotide 5L (Fig. 7) corresponds to region D of the LAI LTR and contains the double-mutation 5′-AGGCTCA-3′ TRE sequence. In gel retardation experiments, no specific complex 5a or 5c could be detected with all five nuclear extracts (results not shown). This results correlates with the absence of protection of region D in footprinting experiments with the LAI LTR. It indicates that the AP-1 factor is unable to interact with region D of HIV-1 LAI.

To evaluate the functional importance of the AP-1 transcription factor on LTR-driven transcription, we performed cotransfection experiments in glial and neuronal cells. In the presence of the human c-Jun expression vector, CAT activity of the LTR(JR-CSF)-CAT reporter vector was stimulated sevenfold in TC-620 cells and was not affected in SK-N-MC cells (results not shown). To test whether this functional effect was mediated by region D, we constructed reporter plasmids containing one copy of either oligonucleotide 5N, 5Nmut, or 5L (Fig. 7) in front of the thymidine kinase promoter, upstream of the CAT gene in pBLCAT2. These constructs and the tk-CAT control vector were transfected in the presence or absence of the c-Jun expression vector in glial and neuronal cells. As shown in Fig. 9, all the reporter vectors showed a low basal transcriptional activity. Upon cotransfection of the c-Jun expression vector, the activity of the control tk-CAT and 5Nmut/tk-CAT vectors increased 1.5-fold in U373-MG cells and 3.5-fold in TC-620 cells (Fig. 9). A similar low increase was observed with the 5L/tk-CAT plasmid (results not shown). Interestingly, with the 5N/tk-CAT vector, overexpression of c-Jun increased CAT activity five- and eightfold in U373-MG and TC-620 cells, respectively. In contrast, in SK-N-MC cells a low two- to threefold Jun-induced activation was observed with all vectors (Fig. 9). These results demonstrate that the 5N sequence is able to mediate c-Jun-induced transcriptional activation in glial and not in neuronal cells.

Analysis of the −306 to −285 LTR region, region C. To examine the proteins interacting with region C of the LAI and

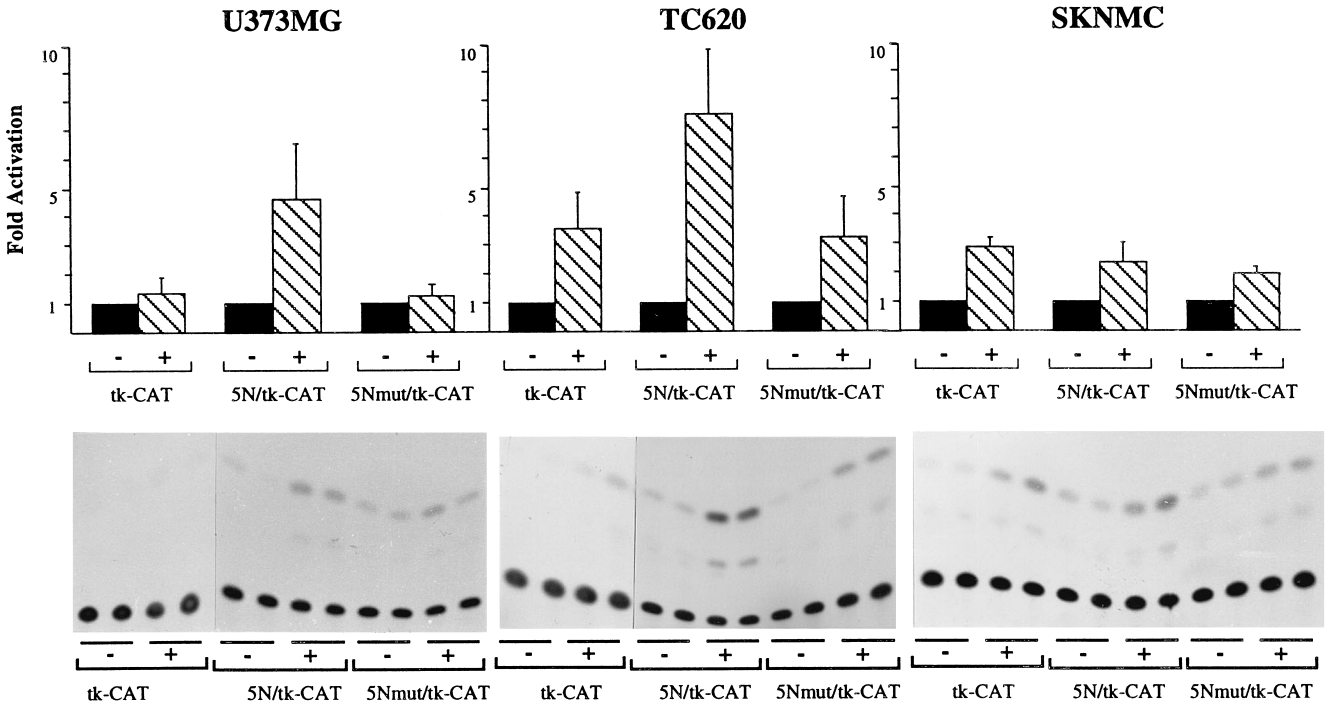

FIG. 9. Functional effect of c-Jun on a tk-CAT reporter plasmid containing region D. Glial (U373-MG and TC-620) and neuronal (SK-N-MC) cells were transfected with 1 pmol of tk-CAT, 5N/tk-CAT, or 5Nmut/tk-CAT vector in the presence of 1.5 μg of either the c-Jun expression vector (lanes +) or the parental pSG5 vector (lanes −). At least three separate transfections were performed in duplicate, with different plasmid preparations. CAT activities were determined as described in Materials and Methods. (Top) Histograms show the fold activation, calculated from the value of the control for each construct (lanes −) set at 1; (bottom) autoradiograms of one typical CAT assay.

JR-FL isolates, mobility shift experiments were performed with oligonucleotide 4N, corresponding to the −306 to −285 sequences of these two isolates (Fig. 7). Sequence inspection revealed that oligonucleotides 4N and 5N share the identical 5′-TGGCTCA-3′ sequence motif shown to behave as a variant AP-1 binding site. Surprisingly, oligonucleotides 4N and 5N did not give rise to the same mobility shift pattern. With all nuclear extracts, oligonucleotide 4N gave a unique complex, 4b, the electrophoretic mobility of which was similar to that of complex 5b (results not shown). No complex containing the Jun and Fos proteins could be detected. This result suggests that flanking sequences surrounding the core AP-1 binding site contribute to the stability of the Jun-Fos complexes, as recently demonstrated (12). It is likely that the flanking sequences in the −306 to −285 sequence do not allow efficient AP-1 binding.

DISCUSSION

This report presents the mapping of the binding sites and the identification of some of the transcription factors interacting with the −480 to −160 modulatory region of the neurotropic JR-FL and JR-CSF strains, compared with the lymphotropic LAI strain of the HIV-1 genome. Although most of the binding sites for nuclear proteins were analyzed in lymphoid cells (for a review, see reference 9), the LTR includes elements that can mediate transcription in many cell types. Dramatic differences were described in the transcriptional control mediated by the LTR between carcinoma, HeLa, and Jurkat cells (37). Few studies have concentrated on the elements of the LTR that elicit expression of the viral genome in the nervous system. Recent reports have shown that astrocytes support TAR-independent activation of HIV-1 transcription by Tat (31) and express a κB-binding activity that differs from prototypical NF-κB (32).

Here we have concentrated our studies on the distal −480 to −160 region and attempted, as a first approach, to define sequence-specific interactions with nuclear proteins from different brain cell types, human astrocytoma, oligodendroglioma, and neuronal cells, compared with HeLa and Jurkat cells. Our footprinting analysis revealed the existence of three or four protein binding sites, depending both on the viral isolate and the cell type. Interactions with site A were detected with the nuclear proteins of all the cell types studied. This interaction has previously been described with nuclear extracts from Jurkat cells (23). This site A-binding protein remains to be characterized. Site B, which spans the −356 to −320 LTR region, corresponds to the recently described nuclear receptor-responsive element. It was shown to contain binding sites for the nuclear orphan receptors COUP-TF, ARP-1, and hepatocyte nuclear factor 4 and the retinoic acid receptors RAR and RXR (16). Cooney et al. (4) demonstrated that in HeLa cells, low- and high-molecular-weight COUP-TF species bind to this site, while in Jurkat cells, the predominant species is the high-molecular-weight COUP-TF. In contrast, Orchard et. al. (22) reported that in Jurkat cells, a novel T-lymphocyte protein distinct from COUP-TF, but with a similar binding specificity, interacts with this site. Our gel retardation, competition, and methylation interference data indicate that the protein species forming complexes C1, C2, and C3 in glial and neuronal cells are also likely to belong to the nuclear receptor family. Our supershift data further indicate that the major part of complexes C1, C2, and C3 is formed by the COUP-TF subfamily of proteins. Our data present evidence for cell-type-specific differences in the amount and nature of the various complexes. In astrocytoma U373-MG cells, three protein species interact

TABLE 1. AP-1 binding activity to LTR regions B, C, and D of three HIV-1 strains

Strain	Binding[a] to region:			
	B	C	D[b]	
			1	2
LAI	−	−	−	−
JR-CSF	−	−	+	−
JR-FL	−	−	+	−

[a] −, no binding; +, Jun-Fos binding activity.
[b] 1, in HeLa, U373-MG, and TC-620 cells; 2, in Jurkat and SK-N-MC cells. Results for regions B and C were the same for all five cell lines.

with site B, the predominant species forming the low-mobility complex C1. In oligodendroglioma TC-620 and neuronal SK-N-MC cells, element B is the binding site for two protein species forming complexes C1 and C3.

Previous studies showed that in Jurkat cells, the T-cell protein interacting with site B acts as a repressor, since mutation of element B led to an increase in LTR-driven CAT activity (23). Cotransfection experiments in choriocarcinoma cells showed that the retinoic acid receptors RXR-α and RAR-α, in the presence of retinoic acid, and the orphan receptors hepatocyte nuclear factor and NGFI-B were able to activate the LTR-driven transcription (16). These observations raise the possibility that the effects of the nuclear receptors LTR-driven transcription depend on the cell type. Therefore, it will be important to correlate the binding of the site B-binding proteins and the functional activity of the LTR in astrocytoma, oligodendroglioma, and neuronal cells. These future investigations should help clarify the mechanisms of HIV-1 gene expression in brain cells.

Moreover, our data demonstrate that the different HIV-1 isolates, LAI and JR-FL, display distinct patterns of nuclear receptor binding to region B. This pattern variability is detected in the five cell types. Similar results were reported in Jurkat cells (4). It is therefore likely that the differences in nuclear receptor interactions among the various HIV-1 strains and in the various cell types contribute to differences in LTR-driven transcriptional activity and consequently in cell-type-specific growth variations of the various isolates. These differences are likely to account for the differential tissue activities of distinct LTRs.

Three putative binding motifs for transcription factor AP-1 have been reported in the −480 to −160 LTR modulatory region (9, 19). However direct interactions of AP-1 with these sites have not yet been demonstrated. AP-1 is composed of several Jun and Fos protein complexes interacting with the TRE core consensus sequence TGACTCA (2). The various members of the Jun family, c-Jun, JunB, and JunD, together with the Fos and Fos-related proteins participate in AP-1 complexes. All three members of the Jun family are present in a variety of tissues and cell lines. It is known that the relative amounts of each Jun species vary markedly in different cell lines (28). These data account for the differences observed in the amount and nature of the AP-1 complexes formed with the TRE probe in the various cell types.

Our studies demonstrate that the Jun and Fos components of AP-1, although present in serum-cultured or TPA-activated cells, do not have the ability to interact directly with site B of the various HIV-1 isolates, LAI, JR-FL, and JR-CSF (Table 1). These results were shown in all five cell types and agree with the results of Orchard et al. (23), who found no evidence for a direct interaction of AP-1 and site B in T cells. Our data suggest that the reported binding of a Fos protein complex to the −357 to −316 sequence of HIV-1 LAI B8 was indirect rather than direct (7). We have further demonstrated that this binding inability results from the mutation of the A residue at position 7 of the TRE consensus sequence TGACTCA. Evidence that this last nucleotide is critical for AP-1 binding supports earlier observations that a TRE site bearing a base substitution mutation on the last nucleotide failed to bind AP-1 (2, 17). Moreover, our studies revealed the inability of the AP-1 factor to interact with the −306 to −285 site C, containing the variant TGGCTCA sequence, identical to that present in site D. This result reveals the importance of the flanking sequences for AP-1 binding, as recently described (12).

Interestingly, our studies revealed the novel interactions of the Jun and Fos components of AP-1 with the variant TGG CTCA sequence present in site D of both neurotropic HIV-1 isolates (Table 1). The absence of AP-1 interaction with the lymphotropic LAI LTR is easily accounted for by the presence of two mutations within the TGACTCA consensus sequence. Moreover, one of the most interesting findings is the cell type specificity of the AP-1 interactions. It appeared that AP-1 was present in different amounts within the nuclear proteins extracted from either serum-treated or TPA-activated cells. However, only AP-1 present in glial and HeLa cells had the ability to interact with site D. This intriguing result suggests that different forms of AP-1 are present in Jurkat and neuronal cells and differ in some way from the forms of AP-1 present in glial and HeLa cells. This raises the possibility for a cell-type-specific control of AP-1 on LTR-directed gene transcription. It is well established that AP-1 activity is regulated by de novo synthesis of Jun and Fos and by posttranslational modifications, including phosphorylation and dephosphorylation (for a review, see reference 29). The control of AP-1 activity may involve transcriptional and posttranslational mechanisms that vary among different cell types (14). Recent data demonstrated that c-*jun* expression is differentially regulated in neurons and glial cells (6). Considerable evidence that Jun and Fos play key roles in many neurophysiological processes has accumulated. A great variety of stimuli such as neurotropic factors or neurotransmitters are able to induce Jun and Fos and to alter the degree of posttranslational modifications of Fos. Various pharmacological, physiological, and electrical stimuli have been shown to elicit c-*fos* expression in neurons of the CNSs of intact animals. In contrast, anesthetics and sedatives are potent inhibitors of *fos* induction (for a review, see reference 21). These various conditions give rise to dynamic alterations in the composition of AP-1. Therefore, each particular situation may influence the composition and the transcriptional activity of AP-1 in the various cell types.

It was recently reported that the potential AP-1 binding site, corresponding to site B of the lymphotropic strains, does not seem to be involved in the transcriptional activation by phorbol myristate acetate in Jurkat cells (19). These results may be easily accounted for, since our data show that the AP-1 factor present in Jurkat cells appears unable to interact with all the potential AP-1 sites. Our transient expression data in brain-derived cell lines reveal the functional importance of c-Jun on JR-CSF LTR-driven transcription. We demonstrate that the binding of c-Jun to site D of the neurotropic LTRs in a reporter vector is able to specifically stimulate transcription in glial and not in neuronal cells. The role of the interaction of the various components of AP-1 with the JR-FL and JR-CSF LTRs will be the subject of our future investigations. Further characterization of the functional contribution of the various combinations of proteins on HIV gene transcription, and especially the role of AP-1, is necessary to provide new insights

into the complex molecular mechanisms that control viral gene expression in brain cells.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Recherche Médicale (U338) and the Agence Nationale des Recherches sur le SIDA.

We thank N. Israël for the gift of the vector containing the LAI LTR, J. Clements for the gift of the vectors containing the JR-FL and JR-CSF LTRs, C. Kedinger for providing the pBLCAT2 plasmid, C. Quirin-Stricker for providing the pSG5 and hc-Jun vectors, and M. J. Tsai for the gift of COUP-TF antibodies.

REFERENCES

1. **Andrews, N. C., and D. V. Faller.** 1991. A rapid micropreparation for extraction of DNA-binding proteins from limiting number of mammalian cells. Nucleic Acids Res. **19:**2499.
2. **Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin.** 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell **49:**729–739.
3. **Brunel, F., A. Ochoa, E. Schaeffer, F. Boissier, Y. Guillou, S. Cereghini, G. N. Cohen, and M. M. Zakin.** 1988. Interactions of DNA-binding proteins with the 5′ region of the human transferrin gene. J. Biol. Chem. **263:**10180–10185.
4. **Cooney, A. J., S. Y. Tsai, B. W. O'Malley, and M.-J. Tsai.** 1991. Chicken ovalbumin upstream promoter transcription factor binds to a negative regulatory region in the human immunodeficiency virus type 1 long terminal repeat. J. Virol. **65:**2853–2860.
5. **Corboy, J. R., J. M. Busy, M. C. Zink, and J. E. Clements.** 1992. Expression directed from HIV long terminal repeat in the central nervous system of transgenic mice. Science **258:**1804–1808.
6. **De Felipe, C., and S. P. Hunt.** 1994. The differential control of c-Jun expression in regenerating sensory neurons and their associated glial cells. J. Neurosci. **14:**2911–2923.
7. **Franza, B. R., Jr., F. J. Rauscher III, S. F. Josephs, and T. Curran.** 1988. The fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. Science **239:**1150–1153.
8. **Garcia, J. A., F. K. Wu, R. Mitsuyasu, and R. B. Gaynor.** 1987. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. EMBO J. **6:**3761–3770.
9. **Gaynor, R.** 1992. Cellular transcription factors involved in the regulation of HIV-1 gene expression. AIDS **6:**347–363.
10. **Green, S., I. Issemann, and E. Sheer.** 1988. A versatile *in vivo* and *in vitro* expression vector for protein engineering. Nucleic Acids Res. **16:**369.
11. **Guillou, F., M. M. Zakin, D. Part, F. Boissier, and E. Schaeffer.** 1991. Sertoli cell-specific expression of the human transferrin gene. J. Biol. Chem. **266:** 9876–9884.
12. **Hadman, M., M. Loo, and T. J. Bos.** 1993. In vivo viral and cellular Jun complexes exhibit differential interaction with a number of in vitro generated ′AP-1- and CREB-like′ target sequences. Oncogene **8:**1895–1903.
13. **Kaltschmidt, C., B. Kaltschmidt, H. Neumann, H. Wekerle, and P. A. Baeuerle.** 1994. Constitutive NF-κB activity in neurons. Mol. Cell. Biol. **14:**3981–3992.
14. **Karin, M., and T. Smeal.** 1992. Control of transcription factors by signal transduction pathways: the beginning of the end. Trends Biochem. Sci. **17:**418–422.
15. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236:**819–822.
16. **Ladias, J. A. A.** 1994. Convergence of multiple nuclear receptor signaling pathways onto the long terminal repeat of human immunodeficiency virus-1. J. Biol. Chem. **26:**5944–5951.
17. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell **49:**741–752.
18. **Leonard, J. M., J. W. Abramczuk, D. S. Pezen, R. Rutledge, J. H. Belcher, F. Hakim, G. Shearer, L. Lamperth, W. Travis, T. Fredrickson, A. L. Notkins, and M. A. Martin.** 1988. Development of disease and virus recovery in transgenic mice containing HIV proviral DNA. Science **242:**1665–1670.
19. **Li, Y., G. Mak, and B. R. Franza, Jr.** 1994. In vitro study of functional involvement of Sp1, NF-κB/Rel, and AP1 in phorbol 12-myristate 13-acetate-mediated HIV-1 long terminal repeat activation. J. Biol. Chem. **269:** 30616–30619.
20. **Luckow, B., and G. Schütz.** 1987. CAT constructions with multiple unique site restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. **15:**5490.
21. **Morgan, J. I., and T. Curran.** 1989. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci. **12:**459–462.
22. **Orchard, K., G. Lang, M. Collins, and D. Latchman.** 1992. Characterization of a novel T lymphocyte protein which binds to a site related to steroid/thyroid hormone receptor response elements in the negative regulatory sequence of the human immunodeficiency virus long terminal repeat. Nucleic Acids Res. **20:**5429–5434.
23. **Orchard, K., N. Perkins, C. Chapman, J. Harris, V. Emery, G. Goodwin, D. Latchman, and M. Collins.** 1990. A novel T-cell protein which recognizes a palindromic sequence in the negative regulatory element of the human immunodeficiency virus long terminal repeat. J. Virol. **64:**3234–3239.
24. **Power, C., J. C. McArthur, R. T. Johnson, D. E. Griffin, J. D. Glass, S. Perryman, and B. Chesebro.** 1994. Demented and nondemented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. J. Virol. **68:**4643–4649.
25. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313:**277–284.
26. **Rattner, A., M. Korner, M. D. Walker, and Y. Citri.** 1993. NF-κB activates the HIV promoter in neurons. EMBO J. **12:**4261–4267.
27. **Reddy, E. P., and P. Dasgupta.** 1992. Regulation of HIV-1 gene expression by cellular transcription factors. Pathobiology **60:**219–224.
28. **Ryder, K., A. Lanahan, E. Perez-Albuerne, and D. Nathans.** 1989. JunD: a third member of the Jun gene family. Proc. Natl. Acad. Sci. USA **86:**1500–1503.
29. **Sassone-Corsi, P.** 1994. Goals for signal transduction pathways: linking up with transcriptional regulation. EMBO J. **13:**4717–4728.

29a. **Sawaya, B. E., D. Aunis, and E. Schaeffer.** Distinct positive and negative regulatory elements control neuronal and hepatic transcription of the human transferrin gene. J. Neurosci. Res., in press.

30. **Schaeffer, E., F. Guillou, D. Part, and M. M. Zakin.** 1993. A different combination of transcription factors modulates the expression of the human transferrin promoter in liver and in Sertoli cells. J. Biol. Chem. **268:**23399–23408.
31. **Taylor, J. P., R. J. Pomerantz, O. Bagasra, M. Chowdhury, J. Rappaport, K. Khalili, and S. Amini.** 1992. TAR-independent transactivation by Tat in cells derived from the CNS: a novel mechanism of HIV-1 gene regulation. EMBO J. **11:**3395–3403.
32. **Taylor, J. P., R. J. Pomerantz, G. V. Raj, F. Kashanchi, J. N. Brady, S. Amini, and K. Khalili.** 1994. Central nervous system-derived cells express a κB-binding activity that enhances human immunodeficiency virus type 1 transcription in vitro and facilitates TAR-independent transactivation by Tat. J. Virol. **68:**3971–3981.
33. **Tornatore, C., K. Meyers, W. Atwood, K. Conant, and E. Major.** 1994. Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. J. Virol. **68:**93–102.
34. **Vogel, J., S. H. Hinrich, R. K. Reynolds, P. A. Luciw, and G. Jay.** 1988. The HIV tat gene induced dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature (London) **335:**606–611.
35. **Wang, L. H., S. Y. Tsai, R. G. Cook, W. G. Beattie, M. J. Tsai, and B. W. O'Malley.** 1989. COUP transcription factor is a member of the steroid receptor superfamily. Nature (London) **340:**163–166.
36. **York-Higgins, D., C. Cheng-Mayer, D. Bauer, J. A. Levy, and D. Dina.** 1990. Human immunodeficiency virus type 1 cellular host range, replication, and cytopathicity are linked to the envelope region of the viral genome. J. Virol. **64:**4016–4020.
37. **Zeichner, S. L., G. Hirka, P. W. Andrews, and J. C. Alwine.** 1992. Differentiation-dependent human immunodeficiency virus long terminal repeat regulatory elements active in human teratocarcinoma cells. J. Virol. **66:**2268–2273.