The Nuclear Receptor Chicken Ovalbumin Upstream Promoter Transcription Factor Interacts with HIV-1 Tat and Stimulates Viral Replication in Human Microglial Cells*

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Human immunodeficiency virus type 1 (HIV-1) infects the central nervous system and plays a direct role in the pathogenesis of AIDS dementia. However, the molecular mechanisms underlying HIV-1 expression in the central nervous system are poorly understood. We have recently reported that the nuclear receptor chicken ovalbumin upstream promoter transcription factor (COUP-TF), an orphan member of the nuclear receptor superfamily, is an activator of HIV-1 gene transcription. Here, our results show that COUP-TF stimulates HIV-1 transcription in primary cultured human microglial cells, the primary target for HIV-1 infection in brain. Run-on assays indicated that COUP-TF acts on the initiation step of transcription. Results from reverse transcription-polymerase chain reaction and immunocytochemistry analysis further revealed the importance of this factor by demonstrating that overexpression of COUP-TF leads to initiation of viral replication in primary HIV-infected human microglia. In addition, COUP-TF is able to physically interact and cooperate with the viral transactivator Tat. The combination of COUP-TF and Tat leads to NF-κB- and Sp1-independent enhanced transcriptional stimulation. *In vitro* binding studies showed that COUP-TF interacts with Tat through amino acids within the N-terminal DNA-binding domain of COUP-TF. Amino acids 48–72 in the basic and C-terminal regions of Tat are required for the binding of Tat to COUP-TF. These results suggest that COUP-TF is an essential transcription factor involved in HIV-1 expression in microglia and reveal a novel interplay of Tat and COUP-TF during regulation of viral expression.

Human immunodeficiency virus type 1 (HIV-1)[1] infects the central nervous system (CNS) and leads to a wide range of neurological complications (1, 2). Although astrocytes, oligodendrocytes, and perhaps neurons are capable of harboring a restricted infection with HIV-1 (3–5), the CNS-resident macrophages or microglial cells (6) are the primary target of HIV-1 infection in brain (7–10). HIV-1 infection is established in the CNS by viruses present early in infection (11). Macrophage-tropic isolates infect microglial cells more efficiently than do lymphotropic isolates (12). We have therefore performed our studies with both a macrophage-tropic and a lymphotropic strain.

The regulation of HIV-1 gene expression is governed by interactions of viral and host cell transcription factors with the long terminal repeat (LTR) region (for review, see Refs. 13 and 14). Recent studies have focused on cellular factors that regulate HIV-1 expression in CNS cells (15–18). Our previous studies have highlighted the importance of members of the steroid/thyroid/retinoid receptor family in the regulation of HIV-1 gene transcription in brain cells. We have shown that the orphan nuclear receptor COUP-TF/Ear3 (19) function as a potent HIV-1 transcriptional activator in different human brain cell lines. Whereas in oligodendrocytes, COUP-TF acts by direct interaction with the nuclear receptor-responsive element located in the −352/−320 LTR region (17), in microglial cells, COUP-TF enhances transcription via the minimal −68/+29 LTR region by direct association with the Sp1 protein (18).

These transcription factors activate LTR-driven transcription in the immediate-early phase of HIV infection, in which viral transcription proceeds at a low rate through dependence solely on cellular transcription factors. This leads to the early phase, in which a dramatic increase in transcription results from the accumulation of the primary transactive viral protein Tat (14, 20). A number of studies have shown that Tat increases the rate of transcription initiation, elongation and translation of TAR-containing mRNAs. The ability of Tat to function as a transcriptional activator is mediated by the interaction with a number of different cellular proteins (for review, see Ref. 20). The general transcription factors TBP/TFIID (21) and TFIIH (22) and the elongation factors TAK/PTEFb (23, 24) and cyclin T (25) associate with Tat. In addition, transcriptional activation by Tat requires the concerted action of Tat and factors binding to the Sp1 and κB LTR region. The importance of the Sp1 factor for Tat-mediated transactivation is well established (26, 27); a direct interaction occurs between Sp1 and amino acids 30–72 of Tat during transactivation (26, 28, 29). Specific NF-κB subunits also act in concert with Tat to enhance LTR-driven transcription (30, 31). Moreover, the thyroid hormone receptor T3Rα, a member of the nuclear receptor superfamily, was found to interact with Tat through amino acids located in the DNA-binding domain of T3Rα and through amino acids 49–72 in the basic and C-terminal region of Tat

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[1] The abbreviations used are: HIV-1, human immunodeficiency virus type 1; CNS, central nervous system; LTR, long terminal repeat; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CAT, chloramphenicol acetyltransferase; PIPES, 1,4-piperazinediethanesulfonic acid.

(32). These data explain why mutations within the LTR sequence decrease or abolish Tat-mediated activation (33, 34). Interestingly, TAR-independent Tat transactivation has also been described, in the presence of an intact NF-κB element (35, 36).

In this report, we have investigated the functional effect of the nuclear receptor COUP-TF on HIV-1 gene expression in primary cultured human microglial cells. Our results reveal the importance of COUP-TF as an activator of HIV-1 gene transcription and replication in primary microglia. In addition, we provide evidence for a novel functional and physical interaction between COUP-TF and Tat. This interaction leads to enhanced synergistic NF-κB and Sp1-independent transactivation of the LTR. Our data suggest a model of an alternative pathway for Tat transactivation in microglia that is independent of both NF-κB and Sp1, through direct interactions of Tat with the nuclear receptor COUP-TF.

MATERIALS AND METHODS

Plasmids—LTR-chloramphenicol acetyltransferase (CAT) vectors were described previously (16–18). LTR-CAT Sp1mut and LTR-CAT ΔκB/Sp1 and pCMVp53 were a gift from Dr. B. E. Sawaya (Philadelphia, PA). The GST-COUP-1 and GST-COUP-3 vectors were described (18). To construct T7-Tat, the Tat fragment was excised from pCMV-Tat vector (gift of Dr. N. Israel, Institut Pasteur, Paris France) and subcloned into the pCDNA3 vector (Invitrogen). To construct T7-COUP-TF, the plasmid RSV-COUP-TF (gift of Dr. M. J. Tsai, Houston, TX) was digested with *Eco*RI and the COUP-TF insert was subcloned into the *Eco*RI site of pCDNA3. To construct pCMV-Tat 72R P18IS, the Tat 72R P18IS sequence (from Dr. A. Rice, AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health) was excised from GST-Tat 72R P18IS vector and subcloned into the pCDNA3 vector. To construct the −40/+80-SV40 TATA-CAT vector, the −40/+80 HIV-1 LTR containing the HIV-1 LAI TATA box (TATAAGC) was mutated by PCR into the SV40 virus TATA box (TATTTAT) and subcloned into the pMOSBlue T-vector (Amersham Pharmacia Biotech). The 1.6-kilobase region containing the CAT reporter gene and polyadenylation signals was subcloned in the *Hin*dIII site in front of the modified −40/+80 LTR.

Cell Culture, Transfections, and CAT Assays—Two types of microglial cell cultures were tested: primary cells and a cell line. Primary human fetal microglial cells were prepared as described previously (37). They were plated in 6-cm-diameter dishes and were transfected either by the calcium phosphate precipitation method or with the Escort reagent (Sigma) following the manufacturer's instructions. The human microglial cell line (38) was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 10 mM HEPES in the presence of penicillin-streptomycin (100 units/ml) and was transfected by the calcium phosphate coprecipitation method.

Cells were either transfected with 1 pmol of plasmid reporter DNA or cotransfected with 1 pmol of reporter DNA and 0.5 pmol of the indicated expression vector: RSV-COUP-TF (gift of Dr. M. J. Tsai, Houston, TX), CMV-p53 (gift of Dr. B. E. Sawaya, Philadelphia, PA), pEF-NF-IL6 (gift of Dr. S. Akira, Hyogo, Japan), or CMV-Sp1 (gift of Dr. R. Tjian, Berkeley, CA). Each transfection was done in duplicate and repeated a minimum of three separate times with two different plasmid preparations. CAT assays were done using standard techniques (16).

Infection of Cells and RT-PCR Analysis—Primary cells (3–4 × 10^5) were incubated with HIV-1 LAI or 95.33 (titer of 15–20 ng/ml of p24) for 18 h at 37 °C and then washed four times to remove residual virus. When indicated, cells were transfected 18 h postinfection. At 24 and 48 h posttransfection, total RNA was prepared with the RNable kit (Eurobio), and gp120 mRNA detection was performed by RT-PCR analysis, using gp120 5′ biotinylated primer, TAGGACGAGCAATGTATGCC, (0.25 pmol) and 3′ primer, TATATAATTCACTTCTCCAATTGTCCCTCAT (0.25 pmol). PCR was performed in a 50-μl PCR mixture containing 10 nmol of each dNTP, 0.25 pmol of each primer, and 1.25 units of *Taq* DNA polymerase (AmpliTaq gold, Perkin Elmer). PCR products were visualized after ethidium bromide staining and agarose gel electrophoresis.

Immunocytochemistry—Primary cells incubated with HIV-1 (LAI) and transfected as described above were tested by immunochemistry after 24 h posttransfection and fixation in acetone for 8 min. Primary antibodies were mouse IgG1 directed against HIV-1 p24 and HIV-1 Tat (1:200, AIDS Research and Reference Reagent Program, Rockville, MD). The immunostaining was revealed by secondary peroxidase-conjugated antibodies and visualized with diaminobenzidine (0.6 mg/ml, DAKO) and 0.01% hydrogen peroxide.

GST Pull-down Assays—GST and GST fusion proteins were expressed in *Escherichia coli* BL-21(DE3). GST-Tat expression vectors were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS (NIAID, National Institutes of Health) from Dr. A. Rice (23, 39). Overnight cultures of bacteria that were newly transformed with the plasmids were diluted with 20 volumes of medium, cultured for several hours to an absorbance at 600 nm of 0.6 and induced with 0.4 mM isopropyl β-D-thiogalactopyranoside at 37 °C for 3 h. Bacteria from 125 ml of culture were harvested and resuspended in 1.5 ml of NETN (20 mM Tris, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10 μg of leupeptin per ml, 10 μg of pepstatin per ml, 10 μg of aprotinin per ml, 1 mM phenylmethylsulfonyl fluoride). The lysates were sonicated and after centrifugation, the supernatants were mixed with glutathione-Sepharose 8A beads (40 μl, Pharmacia) at 4 °C overnight in NETN buffer. The ^35S-labeled input Tat or COUP-TF protein was prepared by *in vitro* translation using the TNT T7 system (Promega) according to the manufacturer's suggestions. The coated beads (40 μl) were washed with NETN and further incubated for 2 h at 4 °C with 15 μl of the total *in vitro* translated protein reaction mixture in a final volume of 300 μl of binding buffer (50 mM Tris-HCl, pH 7.6, 50 mM NaCl, 0.02% Tween 20, 0.02% bovine serum albumin) containing antiproteases as in NETN. After extensive washing with washing buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.02% Tween 20) containing the antiproteases, the bound proteins were dissociated by boiling for 3 min in Laemmli sample buffer and subjected to SDS-polyacrylamide gel electrophoresis.

Immunoprecipitations and Western Blot Analysis—Lysates were prepared from cells plated in a 10-cm dish, as described (18). Cell lysates were resuspended in 400 μl of TNE (50 mM Tris, pH 8.0, 1% Nonidet, 2 mM EDTA, and a mixture of protease inhibitors), and protein A-agarose beads (20 μl), and gently shaken for 1 h at 4 °C. The suspension was briefly centrifuged, and the supernatant was mixed with 3 μl of monoclonal antibodies against HIV-1 (BH10) Tat (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health, from Drs. K. Krohn and V. Ovod (40). After overnight incubation at 4 °C, protein A-agarose (30 μl) was added and mixed for 2 h. After extensive washing of the beads with TNE, 15 μl of beads were mixed with 5 μl of Buffer Z (20 mM HEPES, pH 7.9, 1 mM MgCl_2, 60 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol) and processed for Western blotting, with COUP-TF antibodies (a gift from Dr. K. Karathanasis; Philadelphia, PA).

Run-on Analysis—Microglial cells were harvested, suspended in lysis Buffer A (0.3 M sucrose, 60 mM KCl, 15 mM NaCl, 15 mM Tris, pH 7.5, 0.5 mM spermidine, 0.15 mM spermine, 2 mM EDTA, 0.5 mM EGTA, 14 mM β-mercaptoethanol), and spun down at 1500 × g for 5 min. The pellet was resuspended in 3 ml of Buffer B (Buffer A plus 0.1% Triton X), poured gently on 4 ml of Buffer C (Buffer A with 0.7 M sucrose), and spun down at 2500 × g for 10 min. The permeabilized nuclei were resuspended in storage Buffer D (50 mM HEPES, pH 8, 1 mM MgCl_2, 0.5 mM dithiothreitol, 1 mg/ml bovine serum albumin, and 25% glycerol) and stored at −80 °C.

1 μg of CAT (LTR-CAT *Hin*dIII-excised total CAT gene), 1 μg of CAT I (−40/+79 LTR-CAT *Alw*NI/*Hin*dIII fragment), and 1 μg of CAT II (+79/+229 *Hin*dIII/*Pvu*II CAT fragment) were excised from the LTR-CAT vector, single-stranded and applied onto a nitrocellulose filter preincubated in 2 M ammonium acetate. After 2 h in a vacuum oven at 80 °C, filters were washed with 1× SSC, 0.1% SDS and prehybridized for 1 h in reaction buffer (50 mM HEPES, pH 8, 1 mM MgCl_2, 2 mM MnCl_2, 4 mM dithiothreitol, 200 mM KCl, 300 mM NH_4Cl) containing 20 μg/ml tRNA. Filters were boiled for 1 min and cooled in ice-cold water before use or were stored in a plastic bag at 4 °C.

The elongation reaction was carried out with 50 μl of isolated and permeabilized nuclei for 20 min at 26 °C in reaction buffer supplemented with 1 μg of LTR-CAT vector, 200 μCi of [^32P]UTP, 0.5 mM of other NTP, and 30 units of RNasin inhibitor (New England Biolabs). The mRNA transcripts were phenol extracted and precipitated successively with 10% (w/w) trichloroacetic acid and ethanol. The pellet was dissolved in 10 mM Tris, pH 7.5, supplemented with 1% SDS, applied to the previously prepared nitrocellulose filters in hybridization buffer (0.5 mM NaCl, 50 mM PIPES, pH 7, 33% formamide, 0.4% SDS, 2 mM EDTA, and 20 μg/ml tRNA), and incubated at 45 °C for 72 h. Filters were washed twice with 2× SSC, 0.1% SDS at room temperature for 30 min and twice with 0.1% SSC, 0.1% SDS at 55 °C for 15 min. Filters were exposed overnight on a BIOMAX MR film (Eastman Kodak Co.).
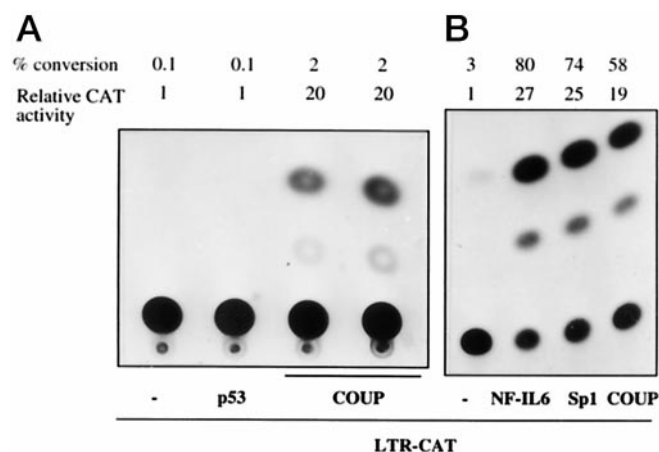
FIG. 1. **COUP-TF stimulates HIV-1 LTR-driven transcription in primary cultured human microglial cells.** Transfection experiments were performed in primary microglia with the HIV-1 LTR-CAT vector in the absence or presence of a vector expressing COUP-TF, p53, NF-IL6, or Sp1, as indicated. One typical CAT assay is shown after transfection by the calcium phosphate coprecipitation method (*A*) and with the Escort reagent (Sigma) (*B*). At the *top* of the autoradiograms are indicated the percentage of conversion into the acetylated forms of chloramphenicol and the CAT activities expressed relative to the value obtained with the LTR-CAT reporter vector alone.

RESULTS

COUP-TF Stimulates HIV-1 Transcription in Primary Human Microglial Cells—We have previously shown that the transcription factor COUP-TF stimulates HIV-1 LTR-driven transcription in a microglial cell line (18). Because we also detected endogenous COUP-TF in primary cultures of microglial cells (results not shown), it appeared essential to examine how COUP-TF regulates HIV-1 gene transcription and viral replication in these cells. Transfection experiments were performed with a LTR-CAT reporter vector in primary cultures of microglial cells containing the CAT gene under the control of the HIV-1 LTR region. When cells were cultured in the absence of cytokine stimulation and transfected with the calcium coprecipitation method, the basal transcriptional activity was barely detectable (Fig. 1*A*). This is consistent with our recent results, which showed that HIV-1 expression in cultured microglial cells is dependent on prestimulation with proinflammatory cytokines (37). Upon cotransfection with the COUP-TF expression vector, LTR-driven CAT expression was induced 20-fold. As a control, overexpression of the tumor suppressor p53, which is known to repress HIV-1 transcription (41), did not induce any CAT activity (Fig. 1*A*). When cells were transfected with a cationic liposome (Fig. 1*B*), the transfection efficiency was better. The basal transcription activity remained quite low. Interestingly, the level of induction following COUP-TF overexpression was 19-fold, similar to the one obtained in Fig. 1*A*. Moreover, upon overexpression of the positive transactivators Sp1 and NF-IL6, CAT activity was induced 25- and 27-fold. These results confirm that, similar to the situation observed in brain cell lines, COUP-TF, Sp1, and NF-IL6 function as HIV-1 transcriptional activators in primary microglia.

COUP-TF Stimulates the Initiation Step of HIV-1 Gene Transcription—To investigate at which step COUP-TF enhances HIV-1 gene expression, we performed run-on assays using isolated nuclei from microglial cells that had been transfected with COUP-TF or left untransfected (Fig. 2). In the absence of COUP-TF overexpression, transcription in the CAT reporter gene was low; overexpression of COUP-TF led to a large increase in the transcription rate (Fig. 2*A*), confirming its transcriptional stimulatory activity. To test whether COUP-TF acted on the initiation or the elongation step of transcription,
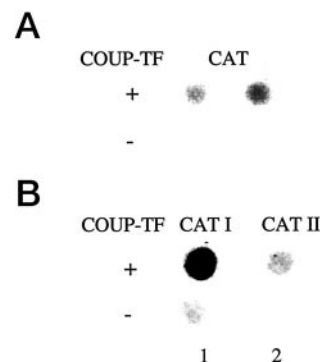
FIG. 2. **Nuclear run-on analysis.** Microglial cells were transfected with COUP-TF expression vector (+) or left untransfected (−). Nuclei were permeabilized and subjected to a 15-min *in vitro* transcription assay. *A*, two aliquots of 1 μg of the CAT gene (excised from LTR-CAT vector) were spotted onto nitrocellulose papers and used in hybridization assays as described under "Materials and Methods." *B*, aliquots of 1 μg of CAT I (−40/+79 LTR-CAT *AlwNI/Hin*dIII DNA fragment) and CAT II (+79/+229 *Hin*dIII/*Pvu*II LTR-CAT DNA fragment) were spotted onto nitrocellulose paper as indicated under "Materials and Methods" and used in hybridization assays.
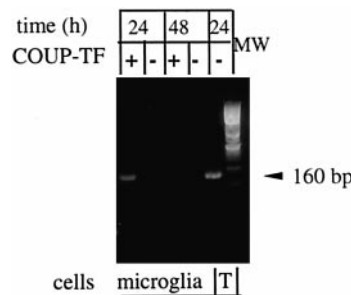
FIG. 3. **COUP-TF stimulates HIV-1 mRNA production in primary cultured human microglial cells.** Cells (4 × 10⁵) were infected with HIV-1(LAI) and after 18 h were transfected with the COUP-TF vector or left untransfected. Expression of HIV-1 gp120 mRNA was measured 24 and 48 h posttransfection by RT-PCR analysis. PCR products were visualized by gel electrophoresis. HIV-1 gp120 mRNA was detected as a 160-base pair fragment (160 base pairs). mRNA from infected T cells was used as a control.

run-on assays were performed with CAT I (−40/+79 LTR-CAT *AlwNI/Hin*dIII fragment) and CAT II (+79/+229 *Hin*dIII/*Pvu*II CAT fragment). COUP-TF increased the amount of small mRNAs because the amount of transcripts of CAT I was higher than that of CAT II (Fig. 2*B*). This result indicates that COUP-TF acts on the initiation step of transcription.

COUP-TF Stimulates Replication in Primary Human Microglial Cells—To investigate the effect of COUP-TF on viral replication itself, we devised a novel experimental procedure. Primary cultures of microglial cells were first infected with either the lymphotropic LAI or the macrophage-tropic 95.33 HIV-1 strain (37). After 18 h, cells were either treated with the transfection reagent alone or transfected with the COUP-TF vector. Expression of the viral gp120 mRNA after 24 and 48 h was monitored by RT-PCR analysis. Results clearly showed that overexpression of COUP-TF induces gp120 expression, peaking at 24 h, similar to the expression obtained in T lymphocytes (Fig. 3). As a control, transfection with a vector expressing the tumor suppressor p53, known to repress LTR-driven transcription (42) did not induce any gp120 expression (results not shown).

In addition, we performed immunocytochemistry experiments with primary cultured microglial cells infected with HIV-1 and transfected with the COUP-TF vector or left untransfected. The percentage of transfected cells with the Escort

FIG. 4. **COUP-TF stimulates HIV-1 replication in primary cultured human microglial cells.** Immunostaining by anti-p24 HIV-1 antigen of microglial cells infected with HIV-1 (LAI) and either left untransfected (*A*) or transfected with COUP-TF (*B*). Photographs at a low magnification (× 100) show the staining (*brown*) of a cluster of transfected cells, whereas control infected cells remained unstained. At a higher magnification (× 400), aspect of infected and transfected cells stained either with anti-p24 HIV-1 antigen (*C*) or with anti-Tat HIV-1 antigen (*D*).
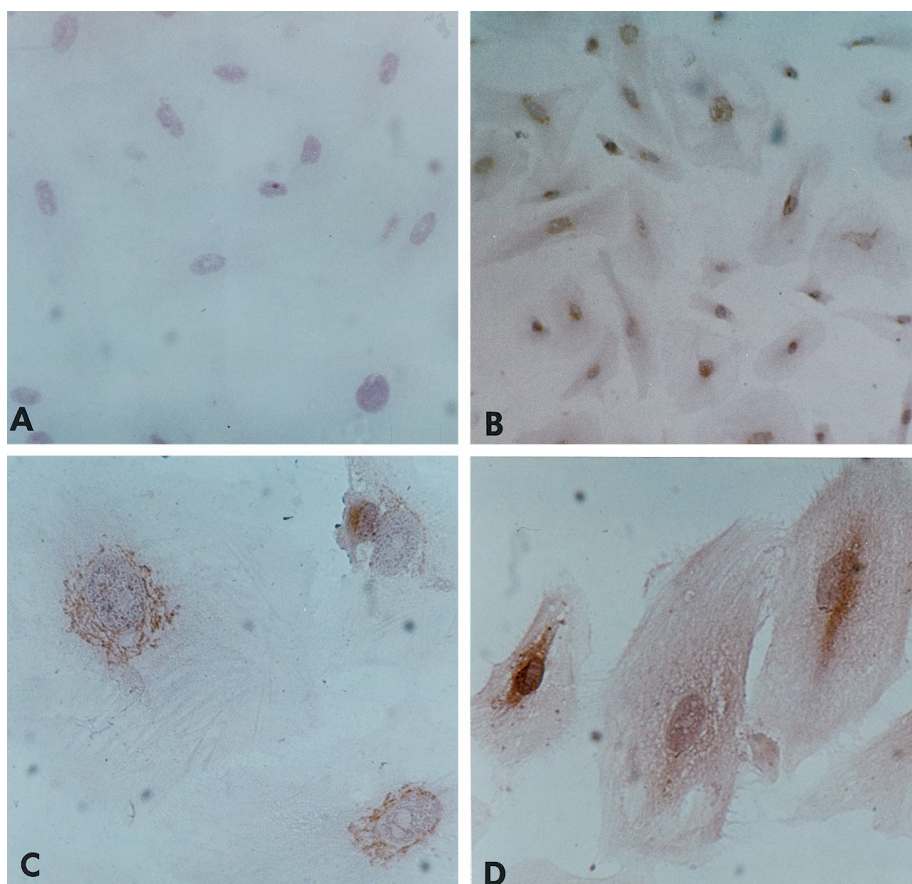
reagent, determined by transfection of the green fluorescent protein, was between 5 and 10% (results not shown). Results showed that cells transfected with COUP-TF expressed HIV-1 antigen proteins p24 (Fig. 4, *B* and *C*) and Tat (Fig. 4*D*). As a control, cells that were infected, but not transfected with COUP-TF, did not express any HIV-1 antigen (Fig. 4*A*). In Fig. 4, *B–D,* we have selected an area of transfected cells, because cells expressing viral antigens were found mostly in clusters, scattered on the preparation. Taken together, these findings demonstrate that, consistent with the effect on transcription, overexpression of COUP-TF is able to initiate viral replication. Interestingly, this effect was observed with both a lymphotropic strain of HIV-1 that otherwise replicates very poorly in microglia and a macrophage-tropic strain.

Effect of COUP-TF on HIV-1 Expression in the Presence of Viral Tat—It is well established that in the early phase of HIV infection, a dramatic increase in transcription results from the accumulation of the viral protein Tat (14, 20). We therefore investigated how COUP-TF affects LTR-driven transcription in the presence of Tat. Because our transcription results obtained in primary cells could be compared with those obtained with the microglial cell line (38), we used the cell line to perform a series of experiments that necessitated a large number of cells. Microglial cells were transfected with various LTR-CAT reporter vectors, presented in Fig. 5, either alone or in the presence of vectors expressing COUP-TF, Tat, or a combination of both vectors.

With LTR-CAT containing the full-length −489/+80 LTR region, COUP-TF alone activated transcription 4-fold. Tat alone or in combination with COUP-TF activated transcription 30-fold (results not shown). As shown previously, the truncated −68/+80 region, containing two Sp1 sites and the TATA box, was still able to mediate COUP-TF responsiveness (Fig. 5, *lane 2*), as a result of the direct interaction of COUP-TF with the

Sp1 protein (18). With this −68/+80 LTR-CAT vector, Tat alone or in combination with COUP-TF stimulated CAT activity 35-fold (Fig. 5, *lanes 3* and *4*).

Deletion of the TAR region, the binding site of Tat (43) in −68/+29LTR-CAT, did not significantly affect COUP-TF-mediated stimulation (Fig. 5, *lane 6*), and as expected, it abolished Tat-induced activation (*lanes 7* and *8*). Deletion or mutation of the Sp1 sites in the truncated −40/+80 LTR, in −283/+80 LTRSp1mut, or in −283/+80ΔκB/Sp1 led to the abolition of COUP-TF-induced activation (Fig. 5, *lanes 10, 14,* and *18*) because Sp1 is required for COUP-TF transactivation in microglial cells (18); similarly, Tat-induced activation was abolished (*lanes 11, 15,* and *19*), because Sp1 is required for efficient Tat transactivation (26, 28). Surprisingly, overexpression of the combination of Tat and COUP-TF resulted in a 8–14-fold transcriptional stimulation (*lanes 12, 16,* and *20*).

To determine whether this COUP-TF/Tat cooperation requires the cognate HIV-1 TATAA region, we constructed a vector containing the −40/+80 LTR region in which the HIV-1 TATA sequence (TATAAGC) was replaced with the SV40 TATA box (TATTTAT). Transfection experiments performed with the −40/+80-SV40-TATA-CAT vector showed that COUP-TF or Tat overexpression was unable to stimulate transcription, similar to the results obtained with the nonmutated vector. However, overexpression of both COUP-TF and Tat in the presence of the mutated vector did not lead to any increase in CAT activity, indicating that COUP-TF is able to discriminate between different versions of TATAAs (result not shown).

These findings reveal a functional synergism between COUP-TF and Tat, able to transactivate the LTR in the absence of NF-κB and Sp1 sites. These results further suggest that Tat and COUP-TF are able to interact, directly or indirectly.

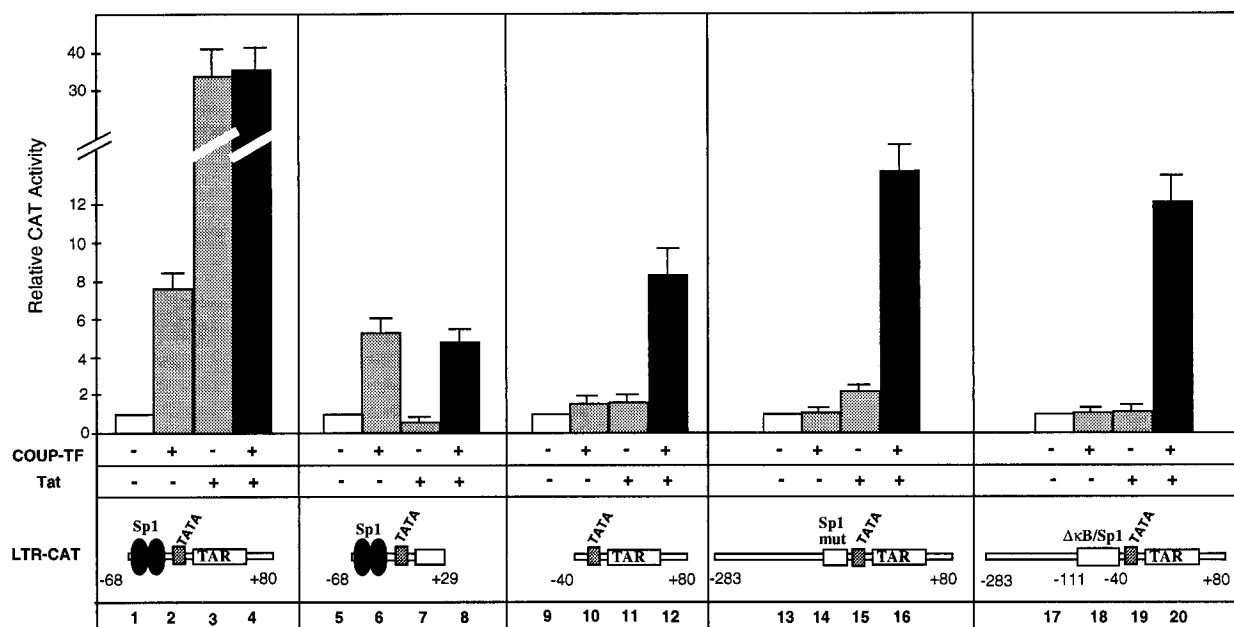*Domains of COUP-TF and Tat Involved in the Synergistic*

FIG. 5. **Tat and COUP-TF synergistically activate HIV-1 gene transcription directed from a NF-κB and Sp1-deleted or mutated LTR region.** Microglial cells were cotransfected with the indicated LTR-CAT expression vectors (1 pmol) in the presence or absence of vectors expressing COUP-TF (2 μg) and Tat (10 ng), as indicated. Histograms represent CAT activities expressed relative to the value obtained with the corresponding LTR-CAT reporter vector. Values correspond to an average of at least three independent experiments done in duplicate.

Transcriptional Activation—To investigate which region of COUP-TF was involved in the synergistic effect with Tat, we used the pRSV-COUPdel148 vector expressing a C-terminal truncated form of COUP-TF. Transfection experiments were performed with −68/+80 LTR-CAT and pRSV-COUPdel148 in the presence or absence of CMV-Tat (Fig. 6A). The truncated COUP-TF was unable to stimulate CAT activity (*lane 2*), because we have shown previously that the C-terminal domain of COUP-TF is essential for its transcriptional activity (18). Interestingly, in the presence of Tat, overexpression of the truncated COUP-TF led to a 50% inhibition of Tat-mediated activation (lane 4). This result reveals the dominant negative role of the C-terminal truncated protein on Tat activity as well as the importance of the C-terminal region for the synergistic effect with Tat.

To examine the role of a transactivation negative form of Tat on COUP-TF activity, we performed cotransfection experiments using the −68/+80 LTR-CAT and pCMV-Tat 72RP18IS, expressing a transactivation negative Tat protein, in the presence or absence of RSV-COUP-TF (Fig. 6B). As expected, the transactivation negative Tat mutant was unable to stimulate CAT activity (*lane 3*). Interestingly, overexpression of this Tat mutant led to a 50% inhibition of COUP-TF-mediated transcriptional stimulation (*lane 4*). This result suggests that the mutated form of Tat is able to inhibit the binding of COUP-TF to Sp1 proteins either by masking the DBD domain of COUP-TF or by competing for interactions with Sp1.

COUP-TF and Tat Interact in Vitro and in Cells—To decipher the mechanism whereby COUP-TF and Tat mediate a Sp1- and κB-independent transcriptional synergistic activation, we examined whether COUP-TF and Tat interact *in vitro*. We performed GST pull-down experiments with GST-COUP fusion proteins and ^{35}S-labeled Tat. Results showed that ^{35}S-labeled Tat (Fig. 7B, *lane 1*) associates with the GST-COUP-1 fusion protein (*lane 3*). Under the experimental conditions, about 1–2% of the total added Tat bound to GST-COUP. This association was specific, because Tat bound to GST-COUP-1, but not to the control GST protein alone (Fig. 7B, *lane 2*). To localize the domain of COUP-TF that mediates interaction with

Tat, we used the GST-COUP-3 expression vector encoding residues 49 to 148 of COUP-TF. The truncated GST-COUP-3 was still able to mediate association with Tat (Fig. 7A, *lane 4*), indicating that the N-terminal part of COUP-TF containing the DNA-binding domain (Fig. 7A) is sufficient for interaction with Tat *in vitro*.

The Tat protein is composed of distinct structural domains, presented in Fig. 7A. We examined which domain of Tat associates with COUP-TF, by using various GST-Tat mutants. Fig. 7C shows that the GST fusion proteins containing 86R Tat (*lane 3*), the truncated 72R Tat (*lane 4*), and the mutant 72RP18IS Tat containing exon 1 and an insertion between amino acids 18 and 19 (*lane 5*) were all able to interact with COUP-TF; in contrast, GST fusion proteins Tat 48Δ and 48ΔP18IS, truncated after amino acid 48, were unable to bind COUP-TF (*lanes 6* and *7*), similar to the GST control (*lane 2*). About 1–2% of the total added COUP bound to GST-Tat. These results indicate that *in vitro* association of Tat with COUP-TF does not depend on exon 2 and requires the Tat region located between amino acids 48 and 72. This region is formed by the basic domain, at amino acids 48–58, which is known to mediate specific binding to TAR RNA, and the C-terminal domain, at amino acids 58–72.

We next investigated whether COUP-TF and Tat were able to associate within cells. Microglial cells were transfected with the CMV-Tat expression vector (Fig. 7D, *lane 3*) or left untransfected (*lane 2*). Nuclear extracts were used for co-immunoprecipitation experiments. The ability of monoclonal anti-Tat antibodies to coimmunoprecipitate the COUP-TF protein was visualized by Western blotting with COUP-TF antibodies (Fig. 7D). These data confirm the existence of a direct association between Tat and COUP-TF within cells.

DISCUSSION

In the present work, we have established the importance of the nuclear receptor COUP-TF as an activator of HIV-1 gene transcription and replication in primary microglia, the main target of HIV-1 in brain. Overexpression of COUP-TF is able to stimulate viral expression of not only the macrophage-tropic
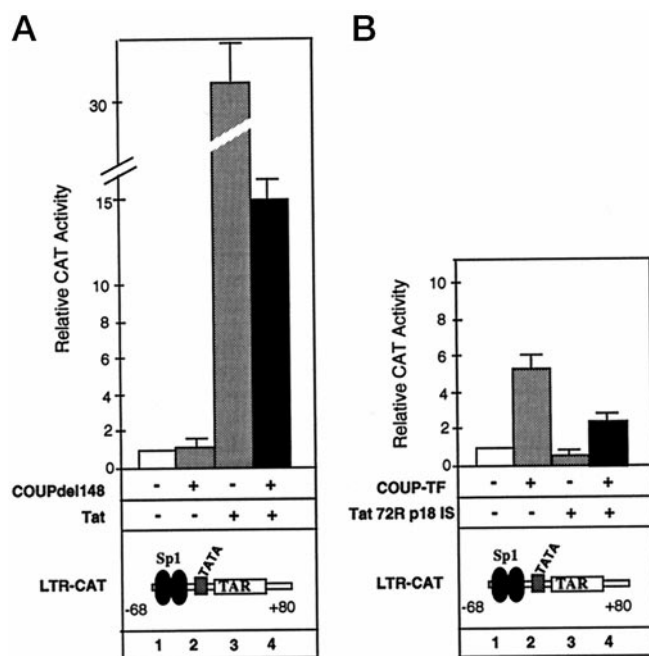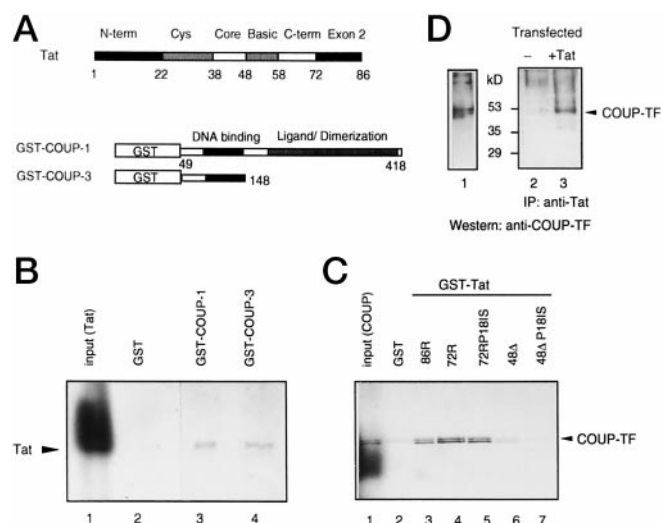
FIG. 6. **Domains of COUP-TF and Tat involved in the synergistic transcriptional activation.** *A,* microglial cells were transfected with 1 pmol of −68/+80 LTR-CAT vector and 0.5 pmol of pRSV-COUP-del148 (*lane 2*) in the presence (*lane 4*) or absence (*lane 3*) of 10 ng of CMV-Tat. *B,* cells were transfected with 0.5 pmol of pCMV-Tat 72RP18IS vector, expressing a transactivation-negative Tat protein, in the presence (*lane 4*) or absence (*lane 3*) of 0.5 pmol of RSV-COUP-TF. Histograms represent CAT activities expressed relative to the value obtained with the corresponding −68/+80 LTR-CAT reporter vector. Values correspond to an average of at least three independent experiments done in duplicate.

FIG. 7. **The viral protein Tat and the nuclear receptor COUP-TF interact *in vitro* and *in vivo*.** *A,* schematic representation of the domains present in the Tat protein and in the GST-COUP-TF constructs. *B,* GST-COUP-1 and -3 fusion proteins interact with *in vitro* translated ^{35}S-Tat in GST pull-down assays. ^{35}S-Tat (1 μl) (*lane 1*) was translated in wheat germ lysate using the TNT kit (Promega) and incubated with bacterially expressed GST (5 μg) (*lane 2*) or GST-COUP-1 and -3 (3 μg) (*lanes 3* and *4*) immobilized on glutathione-Sepharose beads. After extensive washing, the bound proteins were eluted and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. *Lane 1,* input Tat (1 μl); *lanes 2–4*, GST and GST-COUP incubated with ^{35}S-Tat (15 μl). *C,* interactions of *in vitro* translated ^{35}S-COUP-TF with different GST-Tat mutants in GST pull-down assays. ^{35}S-COUP-TF (0.1 μl) (*lane 1*) was incubated with GST (5 μg) (*lane 2*) or GST-Tat proteins (3 μg) (*lanes 3–7*) immobilized on glutathione-Sepharose beads. After washing, the bound proteins were eluted and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. *Lane 1,* input COUP-TF (0.1 μl); *lanes 2–7*, GST and GST-Tat mutants incubated with ^{35}S-COUP-TF (15 μl). *D,* co-immunoprecipitation of Tat and COUP-TF. Nuclear extracts from microglial cells, previously transfected or not with CMV-Tat, were immunoprecipitated with monoclonal anti-Tat antibodies. Nuclear protein extracts (10 μg) (*lane 1*) and immunoprecipitates from 50 μg of nuclear proteins (*lanes 2* and *3*) were analyzed for the presence of COUP-TF by Western blot analysis.

95.33 strain but also of the lymphotropic LAI HIV-1 strain, previously described to poorly replicate in microglia (44). Our findings highlight the unexpected role of COUP-TF, which appears to possess a transactive ability similar to that of the transcription factors Sp1 and NF-IL6. These nuclear factors are essential for inducing HIV-1 gene transcription in the immediate-early phase of HIV infection, when viral transcription proceeds at a low rate prior to the participation of viral activating proteins. Our run-on data show that COUP-TF stimulates transcription by acting at the level of initiation.

The action of these cellular proteins leads to the early phase of HIV infection and to the production of the viral protein Tat (14, 20). By a series of *in vitro* and *in vivo* experiments, we have demonstrated the existence of a novel physical and functional interaction between the transcription factor COUP-TF and the viral transactivator Tat. *In vitro* GST pull-down experiments indicate that the N-terminal part of COUP-TF containing the DNA-binding domain is sufficient for interaction with Tat. *In vitro* association of Tat with COUP-TF does not depend on exon 2 and requires the Tat region located between amino acids 48 and 72 (formed by the basic domain, which mediates specific binding to TAR RNA) and the C-terminal domain (amino acids 58–72). Interestingly, the regions involved in the protein-protein interaction are similar to those described for the thyroid hormone receptor, another member of the nuclear receptor superfamily. T3Rα also interacts with Tat through amino acids present in the DNA-binding domain of the receptor; the basic and C-terminal regions of Tat are involved in the binding to T3Rα (32). In addition, our data obtained with microglial cells transfected with a Tat expression vector confirm that Tat and COUP-TF are able to interact directly within cells. Recent data showed that Sp1 binds Tat amino acids 20–55 (45). When these data are considered together, one could suspect that a ternary

complex of COUP-TF, Sp1, and Tat represents the functionally important molecule. However, we show here that a synergistic effect between COUP-TF and Tat is observed only in the absence of Sp1 sites, whereas the Sp1 and COUP-TF proteins lead to a synergistic transcriptional increase in the presence of Sp1 sites (18). This suggests that COUP-TF interacts preferentially with either Sp1 bound to its DNA site or to Tat bound to the TAR element. Moreover, Tat and Sp1 both interact with the same N-terminal region of COUP-TF, which further strengthens the idea that the binary complexes COUP-TF/Sp1 and COUP-TF/Tat are functionally important.

A number of reports have demonstrated that the Sp1 and κB sequences are required for TAR-dependent transactivation by Tat (26, 28–31, 33). Here, our studies with various LTR mutants reveal that an interaction between COUP-TF and Tat can lead to NF-κB- and Sp1-independent enhanced transactivation of the HIV-1 LTR, in the presence of an intact TAR element. Previous reports have shown that replication of HIV-1 of which the Sp1 sites had been deleted was efficient in MT4 lymphocytes and in activated peripheral blood lymphocytes and appeared to require NF-κB as a substitute for Sp1 (46). Here, our data suggest an alternative regulatory pathway for Tat transactivation in microglia that is independent of both NF-κB and Sp1, through direct interactions of Tat with the nuclear receptor COUP-TF.

The following model may account for the interaction of the

nuclear receptor COUP-TF and viral Tat in regulating the HIV-1 LTR. In the presence of Sp1 sites, the DNA-binding domain of COUP-TF associates with the Sp1 protein bound to its DNA binding site (18). In such a situation, COUP-TF is unable to interact with Tat and thus is unable to synergize with Tat. In contrast, in the absence of Sp1 sites, COUP-TF appears to be able to recruit Tat and to substitute for the Sp1 protein. However, this effect is TAR-dependent and requires binding of Tat to TAR. As shown here, the DNA-binding domain of COUP-TF binds to Tat, whereas the C terminus of COUP-TF interacts with the general transcription factor TFIIB, as described previously (47). These interactions may thus anchor Tat to the transcriptional apparatus. However, this recruitment is insufficient for Tat activity as it does not permit a TAR independent transactivation. It is clear that the precise molecular mechanisms need to be further investigated.

The nuclear receptor COUP-TF is known to be ubiquitously expressed, including in T lymphocytes, the main target of HIV-1 in the immune system. Similar to its action in microglia, COUP-TF functions as a transcriptional activator in lymphocytes, by acting through the Sp1 element of the HIV-1 LTR (48). Because our data in CNS cells revealed the unexpected role of COUP-TF in the presence of Tat, it will be interesting in further studies to examine the role of COUP-TF in the regulation of various HIV-1 strains in cells of the immune system.

REFERENCES

1. Fauci, A. S. (1988) *Science* **239,** 617–622
2. Portegies, P., and Brew, B. J. (1991) *AIDS* **5,** Suppl. 2, S211–S217
3. Albright, A. V., Strizki, J., Harouse, J. M., Lavi, E., O'Connor, M., and Gonzalez, S. F. (1996) *Virology* **217,** 211–219
4. Hatch, W. C., Pousada, E., Losev, L., Rashbaum, W. K., and Lyman, W. D. (1994) *AIDS Res. Hum. Retroviruses* **10,** 1597–1607
5. Nuovo, G. J., Gallery, F., MacConnell, P., and Braun, A. (1994) *Am. J. Pathol.* **144,** 659–666
6. Perry, V. H., Lawson, L. J., and Reid, D. M. (1994) *J. Leukocyte Biol.* **56,** 399–406
7. Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W., and Oldstone, M. B. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 7089–7093
8. Price, R. W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. C., and Cleary, P. (1988) *Science* **239,** 586–592
9. Watkins, B. A., Dorn, H. H., Kelly, W. B., Armstrong, R. C., Potts, B. J., Michaels, F., Kufta, C. V., and Dubois, D. M. (1990) *Science* **249,** 549–553
10. Peudenier, S., Hery, C., Montagnier, L., and Tardieu, M. (1991) *Ann. Neurol.* **29,** 152–161
11. Strizki, J. M., Albright, A. V., Sheng, H., O'Connor, M., Perrin, L., and Gonzalez-Scarano, F. (1996) *J. Virol.* **70,** 7654–7662
12. He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., Busciglio, J., Yang, X., Hofmann, W., Newman, W., Mackay, C. R., Sodroski, J., and Gabuzda, D. (1997) *Nature* **385,** 645–649
13. Garcia, J. A., and Gaynor, R. B. (1994) *Prog. Nucleic Acids Res. Mol. Biol.* **49,** 157–196
14. Kingsman, S. M., and Kingsman, A. J. (1996) *Eur. J. Biochem.* **240,** 491–507
15. Taylor, J. P., and Khalili, K. (1994) *Adv. Neuroimmunol.* **4,** 291–303
16. Sawaya, B. E., Rohr, O., Aunis, D., and Schaeffer, E. (1996) *J. Biol. Chem.* **271,** 22895–22900
17. Sawaya, B. E., Rohr, O., Aunis, D., and Schaeffer, E. (1996) *J. Biol. Chem.* **271,** 23572–23576
18. Rohr, O., Aunis, D., and Schaeffer, E. (1997) *J. Biol. Chem.* **272,** 31149–31155
19. Wang, L. H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M. J., and O'Malley, B. W. (1989) *Nature* **340,** 163–166
20. Gaynor, R. B. (1995) *Curr. Top. Microbiol. Immunol.* **193,** 51–77
21. Kashanchi, F., Khleif, S. N., Duvall, J. F., Sadaie, M. R., Radonovich, M. F., Cho, M., Martin, M. A., Chen, S. Y., Weinmann, R., and Brady, J. N. (1996) *J. Virol.* **70,** 5503–5510
22. Parada, C. A., and Roeder, R. G. (1996) *Nature* **384,** 375–378
23. Herrmann, C. H., and Rice, A. P. (1995) *J. Virol.* **69,** 1612–1620
24. Mancebo, H. S., Lee, G., Flygare, J., Tomassini, J., Luu, P., Zhu, Y., Peng, J., Blau, C., Hazuda, D., Price, D., and Flores, O. (1997) *Genes Dev.* **11,** 2633–2644
25. Wei, P., Garber, M. E., Fang, S. M., Fischer, W. H., and Jones, K. A. (1998) *Cell* **92,** 451–462
26. Sune, C., and Garcia-Blanco, M. A. (1995) *J. Virol.* **69,** 6572–6576
27. Jones, K. A., Kadonaga, J. T., Luciw, P. A., and Tjian, R. (1986) *Science* **232,** 755–759
28. Kamine, J., and Chinnadurai, G. (1992) *J. Virol.* **66,** 3932–3936
29. Jeang, K. T., Chun, R., Lin, N. H., Gatignol, A., Glabe, C. G., and Fan, H. (1993) *J. Virol.* **67,** 6224–6233
30. Demarchi, F., d'Adda di Fagagna, F., Falaschi, A., and Giacca, M. (1996) *J. Virol.* **70,** 4427–4437
31. Liu, J., Perkins, N. D., Schmid, R. M., and Nabel, G. J. (1992) *J. Virol.* **66,** 3883–3887
32. DesaiYajnik, V., Hadzic, E., Modlinger, P., Malhotra, S., Gechlik, G., and Samuels, H. H. (1995) *J. Virol.* **69,** 5103–5112
33. Alcami, J., Lain de Lera, T., Folgueira, L., Pedraza, M. A., Jacque, J. M., Bachelerie, F., Noriega, A. R., Hay, R. T., Harrich, D., Gaynor, R. B., et al. (1995) *EMBO J.* **14,** 1552–1560
34. Berkhout, B., Gatignol, A., Rabson, A. B., and Jeang, K. T. (1990) *Cell* **62,** 757–767
35. Harrich, D., Garcia, J., Mitsuyasu, R., and Gaynor, R. (1990) *EMBO J.* **9,** 4417–4423
36. Taylor, J. P., Pomerantz, R., Bagasra, O., Chowdhury, M., Rappaport, J., Khalili, K., and Amini, S. (1992) *EMBO J.* **11,** 3395–3403
37. Janabi, N., Di Stefano, M., Wallon, C., Hery, C., Chiodi, F., and Tardieu, M. (1998) *Glia* **23,** 304–315
38. Janabi, N., Peudenier, S., Heron, B., Ng, K. H., and Tardieu, M. (1995) *Neurosci. Lett.* **195,** 105–108
39. Rhim, H., Echetebu, C. O., Herrmann, C. H., and Rice, A. P. (1994) *J. Acquired Immune Defic. Syndr.* **7,** 1116–1121
40. Campioni, D., Corallini, A., Zauli, G., Possati, L., Altavilla, G., and Barbanti, B. G. (1995) *AIDS Res. Hum. Retroviruses* **11,** 1039–1048
41. Duan, L., Ozaki, I., Oakes, J. W., Taylor, J. P., Khalili, K., and Pomerantz, R. J. (1994) *J. Virol.* **68,** 4302–4313
42. Subler, M. A., Martin, D. W., and Deb, S. (1992) *J. Virol.* **66,** 4757–4762
43. Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Heaphy, S., Karn, J., Lowe, A. D., Singh, M., Skinner, M. A., and Valerio, R. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 6925–6929
44. Ghorpade, A., Nukuna, A., Che, M., Haggerty, S., Persidsky, Y., Carter, E., Carhart, L., Shafer, L., and Gendelman, H. E. (1998) *J. Virol.* **72,** 3340–3350
45. Chun, R. F., Semmes, O. J., Neuveut, C., and Jeang, K. T. (1998) *J. Virol.* **72,** 2615–2629
46. Parrott, C., Seidner, T., Duh, E., Leonard, J., Theodore, T. S., Buckler, W. A., Martin, M. A., and Rabson, A. B. (1991) *J. Virol.* **65,** 1414–1419
47. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M.-J., and O'Malley, B. W. (1992) *J. Biol. Chem.* **267,** 17617–17623
48. Rohr, O., Schwartz, C., Aunis, D., and Schaeffer, E. (1999) *J. Cell. Biochem.* **75,** 404–413

**The Nuclear Receptor Chicken Ovalbumin
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Cells**

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