COUP-TF and Sp1 Interact and Cooperate in the Transcriptional Activation of the Human Immunodeficiency Virus Type 1 Long Terminal Repeat in Human Microglial Cells

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We have recently reported that chicken ovalbumin upstream promoter transcription factor (COUP-TF) activates human immunodeficiency virus type 1 (HIV-1) gene transcription in glial and neuronal cells. Here, we have examined the role of COUP-TF in microglial cells, the major target cells for HIV-1 infection in brain. We show that COUP-TF activates gene expression from both the lymphotropic LAI and the macrophage-tropic JR-FL HIV-1 strains. Although COUP-TF binds to the −352/−320 nuclear receptor responsive element of the long terminal repeat, it functions as a transcriptional activator by acting on the −68/+29 minimal promoter. This region is a direct target of transcription factors Sp1 and Sp3. We report the discovery and features of a physical and functional interplay between COUP-TF and Sp1. Our cotransfection experiments provide evidence for a functional synergism between Sp1 and COUP-TF leading to enhanced transcriptional activity of the HIV-1 long terminal repeat through the Sp1 element. In contrast, Sp3 functions as a repressor of Sp1- or COUP-TF-induced activation. We further demonstrate that COUP-TF and Sp1 are capable of physically interacting, via the DNA-binding domain of COUP-TF, in vitro and in the cell. These findings reveal how the novel interplay of Sp1 and COUP-TF families of transcription factors regulate HIV-1 gene expression.

Human immunodeficiency virus type 1 (HIV-1) infects the central nervous system (CNS) and plays a direct role in the pathogenesis of AIDS dementia (1, 2), but how the infection leads to brain damage has been poorly understood. The CNS resident macrophages or microglial cells (3) are the primary target of HIV-1 infection in brain (4–7). Glial and neuronal cells are capable of harboring a restricted infection with HIV-1 (8–10). 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 T cell tropic isolates and predominate early in the infection (12). Therefore we have performed our studies with the macrophage-tropic JR-FL strain, compared with the T-tropic LAI strain.

HIV-1 gene expression is controlled by a combination of viral and host cell transcription factors interacting with the long terminal repeat (LTR) region (for review, see Refs. 13 and 14). Recent studies have focused on transcription factors that regulate HIV-1 expression in brain cells. Analysis of HIV-1 directed gene expression in transgenic mice derived from CNS-isolated HIV-1 strains (15), suggests that HIV-1 replication in the CNS uses transcription factors different from those in non-neural tissues (16–18). Transcriptional activity of the HIV-1 promoter is mediated by κB regulatory sequences of the LTR, through the action of the transcription factor NF-κB, both in neurons (19, 20) and in astrocytes (21). Besides the κB regulatory element, our recent data have highlighted the importance of the nuclear receptor-responsive element (NRRE), which appears to be the point of convergence of a network of physiological signals which modulate HIV-1 gene expression in brain cells. We have described that the orphan nuclear receptors COUP-TF/Ear3 (22–24) are present in three human brain cell lines, oligodendroglioma TC-620, astrocytoma U373-MG, and neuroblastoma SK-N-MC cells. Our data have also demonstrated the importance of COUP-TF as a potent transcriptional activator in oligodendroglioma cells, of both the lymphotropic LAI and the neurotropic JR-CSF HIV-1 LTR. They revealed the action of the dopamine transduction pathway, which coupled to COUP-TF, contributes to enhance HIV-1 gene transcription in neuronal cells (25). Our findings have further established the importance of the retinoic acid signaling pathway in HIV-1 gene transcription in glial and neuronal cells (26). Except for retinoid receptors, the role of transcription factors belonging to the steroid/thyroid/retinoid receptor superfamily has not yet been described in microglial cells.

In this report, we have investigated the functional effect and the molecular mechanisms by which the orphan nuclear receptor COUP-TF regulates HIV-1 gene expression in a human microglial cell line (27). Our findings reveal the importance of COUP-TF as an activator of LTR-driven transcription of both the T cell line-tropic LAI and the macrophage-tropic JR-FL HIV-1 strains. We provide evidence for a novel physiological and functional interaction between the DNA-binding domain of COUP-TF and the transcription factor Sp1, leading to enhanced transactivation of the HIV-1 LTR. In addition, our data reveal that the COUP-TF- and Sp1-induced stimulation is repressed by the transcription factor Sp3. These data describe novel molecular mechanisms which govern the network of interactions between the HIV-1 LTR, the transcriptional activators COUP-TF and Sp1, and the repressor Sp3, in the regulation of HIV-1 gene expression.
Materials and Methods

Plasmids Constructs—The LTR/JR-FLI-CAT and LTR/LAI-CAT vectors were described previously (25, 26, 28). The –68/+80 and –40/+80 LTR-CAT vectors were constructed by subcloning into the Smal site of pcUC19-CAT0 the HaeIII-HindIII and DdeI-HindIII blunt-ended LTR insert, respectively, isolated from –489/+80 LTR/LAI-CAT.

To construct the GST-COUP-1 vector, the plasmid RSV-COUP-TF (gift of Dr. M. J. Tsai, Houston, TX) was digested with SmaI and EcoRI and the COUP-TF insert was subcloned in-frame with the GST-encoding sequences into the Smal and EcoRI sites of pGEX2T. To construct GST-COUP-2 and GST-COUP-3, the SmaI-SauI and SmaI-XmnI COUP-TF fragments were isolated from the RSV-COUP vector, blunt-ended, and inserted into the SmaI site of pGEX2T. The Sp1 cDNA was excised with XbaI and EcoRI from the pEVR2-Sp1 vector (gift of Dr. G. Suske, Marburg, Germany) and religated into the XhoI and EcoRI sites of pBluescript KS+ . To construct pRSV-COUP-TF, COUPdel1308, and COUPdel1148, the EcoRI-EcoRI, EcoRI-SauI, and EcoRI-XmnI fragments were isolated from RSV-COUP-TF, blunt-ended, and inserted in the blunt-ended NotI site of pReScR (Invitrogen).

Cell Culture, Transfections, and CAT Assays—Human microglial cells (27) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 10% HEPES in the presence of penicillin-streptomycin (100 units/ml). Cells (106) were transfected by the calcium phosphate precipitation method as described previously (29) with either 1 pmol of plasmid reporter DNA or cotransfected with reporter DNA (1 pmol) and the indicated expression vector: RSV-COUP-TF (0.2 pmol; gift of Dr. M. J. Tsai, Houston, TX), CMV-Sp1 (0.2 pmol; gift of Dr. R. Tjian, Berkeley, CA), pReCMV-Sp1 (0.2 pmol, gift of Dr. G. Suske, Marburg, Germany). 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. CAT assays were performed as described previously (29). Reaction mixtures containing 15 μg of protein were incubated at 37 °C for 2 h.

Electrophoretic Mobility Shift Assays—EMSAs were performed with nuclear proteins as described previously (25). Pure Sp1 protein (2 footprint units, Promega) was used in the presence of 3 μg of bovine serum albumin. Mixtures were incubated for 15 min at 4 °C and protein-DNA complexes were analyzed by electrophoresis on a 4% or 6% polyacrylamide gel in 0.25% TBE. For supershift assays, antibodies directed against COUP-TF (gift of Dr. M. J. Tsai) or against Sp1 or Sp3 (Santa Cruz Biotechnology) or against T3R (gift of Dr. P. Chambon, Strasbourg, France) or normal rabbit serum, were mixed with nuclear proteins for 4 h at 4 °C prior addition of the probe.

Glutathione S-Transferase Fusion Protein Interaction Assay—GST and GST fusion proteins were expressed in Escherichia coli BL-21(DE3). Overnight cultures of bacteria that were newly transformed with the plasmids were diluted with 20 volumes of medium, cultured for several hours to an optical density at 600 nm of 0.6, and induced with 0.4 mM isopropyl-p-galactoside at 37 °C for 4 h. Bacteria from 125 ml of culture were harvested and resuspended in 1.5 ml of binding buffer (50 mM Tris, pH 8.0, 1% NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10 μg of leupeptin/ml, 10 μg of pepstatin/ml, 10 μg of aprotinin/ml, 1 mM phenylmethylsulfonyl fluoride). The lysates were sonicated and after centrifugation, the supernatants were mixed with glutathione-Sepharose 4B beads (40 μl, Pharmacia) at 4 °C overnight in NETN buffer. The 32P-labeled input Sp1 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-Cl, 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-Cl, pH 7.6, 150 mM NaCl, 0.02% Tween 20) containing the antiproteases, the bound proteins were dissociated by boiling for 5 min in Laemmli sample buffer and subjected to SDS-PAGE.

Association of Proteins in Vitro—GST and GST fusion proteins were expressed from a 2 ml bacterial culture and mixed with glutathione-Sepharose 4B beads (Pharmacia) at 4 °C overnight in NETN buffer. The coated beads (30 μl) were washed with NETN and further incubated for 2 h at 4 °C with pure Sp1 (2 footprint units, Promega) in 300 μl of binding buffer. After extensive washing with washing buffer, the bound proteins were dissociated with 20 mM glutathione, 50 mM Tris, pH 8 (15 μl), and subjected to EMSA in the presence of labeled Sp1 oligonucleotide.

Immunoprecipitations—Protein extracts were prepared according to two distinct methods: cell lysates were prepared from cells plated in a 10-cm dish according to the reported procedure (30) or nuclear proteins were isolated as described previously (25). Cell lysates or nuclear proteins were resuspended in 400 μl of TNE (50 mM Tris, pH 8.0, 1% Nonidet, 2 mM EDTA, and a mixture of protease inhibitors), mixed with 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 anti-Sp1 antibodies or preimmune serum. With the 3L oligonucleotide probe, the suspension was mixed with 3 μl of anti-Sp1 antibodies or preimmune serum. 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 MgCl2, 60 mM KCl, 0.5 mM EDTA, 1 mM diethiothreitol, 10% glycerol) and either subjected to EMSA in the presence of the 3L oligonucleotide probe, or processed for Western blotting with COUP-TF antibodies, as described previously (25).

Results

COUP-TF Present in Microglial Cells Binds to the Nuclear Receptor Responsive Element of the LTR of HIV-1 LAI and Not HIV-1 JR-FL—We have previously shown that within different brain cell lines (oligodendroglialoma, astrocytoma, and neuroblastoma) COUP-TFs are the major protein species which interact with the NRRE located in the –352/–320 modulatory region of the LTR (25) (Fig. 1). To examine whether COUP-TFs are also present in human microglial cells and interact with the NRRE, gel mobility supershift assays were performed with nuclear proteins isolated from microglial cells, in the presence of COUP-TF antibodies. With the 3L oligonucleotide probe, corresponding to the NRRE sequence present in the lymphotropic HIV-1 LAI isolate, the results show that the COUP-TFs...
species do form the majority of the DNA-protein complexes C1, C3, and C3’ (Fig. 2, lanes 3–5). This confirms that within human microglial cells, like in other brain cells, COUP-TFs are the major nuclear proteins interacting with the NRRE. When we used as a probe oligonucleotide 3N corresponding to the NRRE site present in the LTR of the CNS-derived macrophage-tropic HIV-1 JR-FL, the formation of complexes C1, C3, and C3’ was prevented (lane 2). This clearly indicates that COUP-TFs present in nuclear extracts are unable to interact with the mutant NRRE sequence present in the JR-FL LTR.

**COUP-TF Stimulates LTR-directed HIV-1 Gene Transcription in Microglial Cells via the Proximal LTR Region**—To examine the role of COUP-TF on HIV-1 gene transcription in microglial cells, transfection experiments were performed with a LTR-CAT reporter vector, containing the CAT gene under the control of the HIV-1 LTR region. With construct 1 containing the LTR of the HIV-1 LAI strain (Fig. 3), COUP-TF acts as a transcriptional activator of the HIV-1 genome, since in transfection experiments, LTR-driven CAT expression was stimulated 4.8-fold. It was interesting to compare this result with that of construct 2 containing the LTR region from the HIV-1 JR-FL, since as shown above (Fig. 2), the NRRE site of the JR-FL LTR contains mutations which prevent the binding of COUP-TF. Surprisingly, CAT expression was still stimulated 3.8-fold, indicating that the mutation of the NRRE does not prevent COUP-TF-induced activation.

The LTR sequences responsible for the COUP-TF-induced activation were delineated by transfecting a series of LTR-CAT vectors containing 5′-deletions of the LTR region (Fig. 3). Although the basal level of transcription dropped to 10% with −89/+80 LTR-CAT and −68/+60 LTR-CAT, compared with the full-length vector, COUP-TF was still able to exert a respective 6.1- and 6.8-fold transcriptional stimulation. The COUP-TF-induced activation was abolished only by the removal of the two binding sites of the Sp1 transcription factor between position −68 and −40. Moreover, the −68/+29 LTR region was still able to mediate a 5.6-fold COUP-TF-induced stimulation, allowing us to localize the COUP-TF response element within the minimal −68/+29 region. This result indicates that the action of COUP-TF on the LTR is not mediated via the NRRE site, but rather via the minimal −68/+29 LTR site. It further suggests that COUP-TF needs the presence of the two Sp1 elements located within the −68−40 region and is unable to function via interactions with the TATA element alone.

To test whether COUP-TF could activate any promoter composed of Sp1 sites and a TATA element, we performed transfection experiments with the simian virus 40 (SV40) early promoter formed by six Sp1 sites and a TATA box. In microglial cells, COUP-TF activated the SV40 promoter only 2-fold. In contrast, in HeLa cells, COUP-TF was able to stimulate transcription about 10-fold (results not shown). These findings suggest that the action of COUP-TF, via Sp1 sites, is dependent both on the promoter context and the cell type.

**COUP-TF Does Not Bind Directly to the T3R Element of the HIV-1 LTR**—It has been reported that the thyroid hormone receptor T3R, another member of the nuclear receptor superfamily interacts directly with the T3R element (T3RE) overlapping the Sp1 sites within the −74−50 HIV-1 LTR (31). Therefore the possibility existed that COUP-TF might also bind directly to this region. To test this hypothesis, we carried out gel retardation experiments, using as a probe oligonucleotide T3RE containing the three Sp1-binding sites (Fig. 4A). With nuclear proteins isolated from microglial cells, four DNA-pro-
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Interestingly, overexpression of both COUP-TF (0.2 pmol) and Sp1 (0.5 pmol) led to a 13-fold increase in CAT activity. This indicates that both proteins are able to function in a synergistic manner. Sp3 has been described as a bifunctional transcription regulator of eukaryotic gene expression (34) and as a repressor of HIV-1 gene transcription in HeLa and Drosophila cells (32). In microglial cells, Sp3 functions also as a potent repressor of LTR-directed HIV-1 gene transcription, since the remaining CAT activity was 30% with 0.2 or 0.5 pmol of expression vector. Moreover overexpression of Sp3 resulted in a 2-fold inhibition of both the Sp1- or COUP-TF-mediated stimulation (Fig. 5).

It was of interest to examine the effect of the activators COUP-TF and Sp1 on the transcriptional activity mediated by the −68/+80 proximal LTR region. In this region, the LTR sequence of the LAI and JR-FL isolates are similar. Interestingly, the proximal promoter mediated a 6.0-fold COUP-TF-induced stimulation, compared with the lower 3.8-fold stimulation mediated by the entire LTR region. Similarly, Sp1 transactivated the proximal promoter 4.2- and 14-fold, after transfection of 0.2 and 0.5 pmol of Sp1 vector, respectively. Moreover, the combined action of COUP-TF (0.2 pmol) and Sp1 (0.2 or 0.5 pmol) resulted in a synergistic effect, since transcription was stimulated 15- and 27-fold, respectively.

The COUP-TF protein can be divided into distinct functional domains, such as a N-terminal DNA-binding domain and a C-terminal dimerization domain. To identify which part of the COUP-TF protein is involved in transactivation, we cotransfected LTR(JR-FL)-CAT with vectors expressing 3′-deletion mutants of COUP-TF (Fig. 6). Results demonstrate that the C-terminal region is required for transactivation; construct pRSV-COUPdel148 containing only the DNA-binding domain was unable to significantly activate the HIV-1 promoter.

COUP-TF and Sp1 Are Able to Interact in Vitro and in the Cell—To decipher the mechanism whereby COUP-TF and Sp1 mediate a transcriptional synergistic activation, we first tested whether COUP-TF and Sp1 interact in vitro. We generated a GST-COUP fusion protein (Fig. 7A) and analyzed the ability of in vitro translated Sp1 in the presence of [35S]methionine to interact with GST-COUP-1. SDS-PAGE analysis of proteins retained by glutathione-Sepharose shows that 35S-labeled Sp1 associates with GST-COUP-1 (Fig. 7B, lane 4). This association is specific, since Sp1 was bound to GST-COUP-1 but not to GST alone (lane 3). Three labeled bands are detected on SDS-PAGE, which correspond to the three bands of approximately 100, 55, and 40 kDa, detected with the input protein on Western blots (lanes 1).

To localize the domain of COUP-TF that mediates interaction with Sp1, we constructed GST-COUP expression vectors containing serial 3′ deletions of COUP-TF (Fig. 7A). Results from GST pull down experiments show that GST-COUP-2 and even GST-COUP-3 encoding residues 49 to 148 of COUP-TF were still able to mediate association with Sp1 (lanes 5 and 6), confirming that the N-terminal part of COUP-TF containing the DNA-binding domain is sufficient for interaction with Sp1 in vitro.

Figure 5. Regulation of HIV-1 gene transcription by COUP-TF, Sp1, and Sp3 in human microglial cells. Transient expression experiments were performed by cotransfecting the HIV-1(JR-FL)-CAT or −68/+80 LTR-CAT reporter vectors (1 pmol) with vectors expressing COUP-TF (0.2 pmol), Sp1 (0.5 pmol), or Sp3 (0.5 pmol) as indicated. Histograms show the CAT activities expressed relative to the value obtained with the LTR-CAT reporter vector. Values correspond to an average of at least three independent experiments done in duplicate. The standard deviation did not exceed 20%.

Figure 6. Transcriptional activity of COUP-TF deletion mutants. Microglial cells were co-transfected with LTR(JR-FL)-CAT and vectors expressing deletion mutants of COUP-TF. Cell extracts were prepared 48 h after transfection and CAT activities were determined. Activities are expressed as fold stimulation over the activity of LTR-CAT cotransfected with the parental pRSV vector.
We further investigated the interactions between COUP-TF, Sp1, and the Sp1 DNA-binding site. Pure Sp1 protein was incubated with glutathione-Sepharose beads retaining bacterially expressed GST-COUP protein. After extensive washings, the bound proteins were eluted and analyzed by gel shift assays with the Sp1 oligonucleotide probe (Fig. 7C). Addition of pure Sp1 protein in the binding reaction led to the formation of a retarded complex (lane 1). As expected GST-COUP fusion proteins alone were unable to bind to the Sp1 probe (lanes 6–8).

Interestingly, addition in the binding reaction of GST-COUP-1 preincubated with Sp1 resulted in a supershift of the Sp1-DNA complex (lane 2). This result demonstrates that COUP-TF is able to physically associate with Sp1 bound to its DNA-binding site. Both truncated GST-COUP-2 and GST-COUP-3 proteins were still able to supershift the Sp1-DNA complex (lanes 3 and 4), indicating that the N-terminal sequences containing the DNA-binding domain of COUP-TF are sufficient for binding with Sp1. As a control, a barely detectable band was formed when Sp1 was incubated with GST (lane 5), showing the specificity of the interaction between GST-COUP and Sp1.

These in vitro results were confirmed in vivo by immunoprecipitation experiments with extracts from microglial cells (Fig. 8). The presence of COUP-TFs species was detected in gel shift assays with the 3L probe (Fig. 8A). Antibodies directed against Sp1 (lanes 4 and 7), but not non-immune serum (lane 6), were able to immunoprecipitate endogeneous COUP-TF species. As with the Sp1 oligonucleotide probe (Fig. 7C). Addition of pure Sp1 protein in the binding reaction led to the formation of a retarded complex (lane 1). As expected GST-COUP fusion proteins alone were unable to bind to the Sp1 probe (lanes 6–8). Interestingly, addition in the binding reaction of GST-COUP-1 preincubated with Sp1 resulted in a supershift of the Sp1-DNA complex (lane 2). This result demonstrates that COUP-TF is able to physically associate with Sp1 bound to its DNA-binding site. Both truncated GST-COUP-2 and GST-COUP-3 proteins were still able to supershift the Sp1-DNA complex (lanes 3 and 4), indicating that the N-terminal sequences containing the DNA-binding domain of COUP-TF are sufficient for binding with Sp1. As a control, a barely detectable band was formed when Sp1 was incubated with GST (lane 5), showing the specificity of the interaction between GST-COUP and Sp1.

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a control, in the absence of antisera, no complex was detected (lane 3). The amount of immunoprecipitated proteins was dependent on the method used for protein extraction, as described under “Materials and Methods”: when we subjected cell lysates to immunoprecipitation, complex C1 was predominant (lane 4); when we used nuclear protein extracts, complexes C1, C3, C3', and C4 were detected (lane 7). As expected, complexes C1, C3, and C3' were specifically competed with an excess of 3L competitor (lane 5) and their formation was prevented in the presence of COUP-TF antibodies (lane 8). Surprisingly, protein forming complex C4 was also partially immunoprecipitated, with anti-Sp1, but not with nonimmune serum, which may suggest an interaction of this protein with either Sp1 or COUP-TF. In addition, the ability of anti-Sp1 antibodies to coimmunoprecipitate the COUP-TF protein was visualized by Western blotting with COUP-TF antibodies (Fig. 8B).

DISCUSSION

In this report we have investigated the regulation of HIV-1 gene transcription in human microglial cells, which represent the primary target of HIV-1 infection in the central nervous system. Since we have previously shown that the orphan nuclear receptor COUP-TF is expressed in brain cells and leads to a transcriptional stimulation of the HIV-1 genome in oligodendroglioma and neuronal cells (25), we have focused our studies on the regulation of HIV-1 gene transcription by COUP-TF in microglial cells. It is now well established that COUP-TF is able to exert both positive and negative effects on gene expression, depending upon the promoter and the cell contexts (29, 35–38). We show here that COUP-TF proteins are present in human microglial cells and interact directly with the NRRE, spanning the −356/−320 LTR region of the lymphotropic HIV-1 LAI isolate, but are unable to bind to the mutant NRRE present in the macrophage-tropic HIV-1 JR-FL isolate. Interestingly, our transient expression data reveal that COUP-TF/Ear3 is able to activate HIV-1 LTR-driven transcription in microglial cells, independently of the NRRE sequence. This shows that COUP-TF is able to transactivate the LTR from the lymphotropic LAI and the macrophage-tropic JR-FL isolates. We have already reported that distinct cell-type specific and sequence-dependent mechanisms govern COUP-TF-induced stimulation. In oligodendrocytes this transcription factor stimulates HIV-1 gene transcription, either by direct interactions with its NRRE target site or also, depending on the LTR sequence, by cross-coupling interactions with downstream-located proteins. In neuronal SK-N-MC cells, the transcriptional stimulation induced by COUP-TF in the presence of dopamine is mediated by the minimal −68/+29 LTR region (25). Similarly the NRRE site in the JR-CSF LTR is not indispensable for the stimulation induced by the retinoic acid receptor, since the retinoid action appears to be mediated by downstream-located elements, such as the −247/−222 AP-1 region and, to a lesser extent, the NF-xB region (26).

We show here that the minimal −68/+29 LTR region, containing two Sp1-binding sites, is sufficient for COUP-TF-mediated stimulation. Our cotransfection and in vitro experiments demonstrate the ability of COUP-TF to transactivate the LTR indirectly via the Sp1 sites, by direct interaction with the Sp1 protein. We present evidence for a direct association between the N-terminal part of COUP-TF, containing the DNA-binding domain, and Sp1. Our data show that COUP-TF can interact with Sp1 both in vitro and in microglial cells. This association leads to a functional cooperation between the two proteins, which is detected with the full-length LTR as well as the −68/+80 LTR region. Moreover we show that the C-terminal domain of COUP-TF is involved in the transcriptional activation.

Previous studies have already described that the action of COUP-TFs can be mediated not only by direct interaction with their DNA-binding site, but also by protein-protein interaction. COUP-TF/Ear3 and ARP-1/COUP-TFIi are able to directly target components of the basal transcription machinery, such as the basal transcription factor TFIIB (39, 40). TFIIB recognizes and associates with COUP-TF and two other members of the steroid hormone receptor family, via their activation domain (39). In contrast, our findings reveal that COUP-TF associates with Sp1 via its N-terminal part, containing the DNA-binding domain. A direct interaction between COUP-TF and the transcription factors Oct1 and Oct2 has also been demonstrated in the regulation of the vHNF1 promoter (30).

It has been well established that the Sp1 transcription factor (41) plays an essential role in the regulation of basal transcription as well as in Tat-mediated transactivation of the HIV-1 LTR (42–44). Recent reports describe that Sp1 is critical for in vivo transcriptional regulation of HIV, through its interaction with other DNA-binding proteins. A cooperative interaction between Sp1 and NF-xB, bound to the two adjacent binding sites, is required for optimal HIV-1 enhancer activation and inducible HIV-1 gene expression (32, 45, 46). A physical interaction between Sp1 and the p53 tumor-suppressor gene has been described in the tumor necrosis factor-induced transcriptional activation of the HIV-1 LTR (47). Moreover in vitro and in vivo data suggest a direct interaction between Sp1 and Tat during transactivation (48, 49).

Here our findings reveal a novel direct association between two zinc finger proteins, Sp1 and COUP-TF, which leads to enhanced transcriptional activation of the HIV-1 genome. These data suggest a more general model for gene activation by the orphan receptor COUP-TF or steroid/thyroid/retinoid receptors, in the presence of Sp1-binding sites. As shown here, Sp1 bound to its DNA-binding site is able to associate directly with the N-terminal DNA-binding domain of the nuclear receptor. It is well known that the adjacent TATA element binds the general transcription factor TFIIID, which directly interacts with TFIIB, which itself is able to bind COUP-TF. In such a situation, the nuclear receptor may function as an adaptor protein, bringing together Sp1 and TFIIB, thereby enhancing communication between the general transcription machinery and Sp1. Alternatively, COUP-TF may bring together Sp1 and a cell type-specific nuclear factor, leading to various levels of activation induced by COUP-TF in different cell types. The precise mechanisms which account for our observations in the case of HIV-1 and SV40 gene transcription need to be further investigated.

The Sp1 multigene family contains three closely related members Sp1, Sp3, and Sp4 which recognize the GC box and the GT motif (33). While Sp1 and Sp4 function as transcriptional activators, Sp3 is a bifunctional transcription regulator, whose activity is dependent upon the promoter and the cellular contexts (33, 34). Recent reports have established that different members of the Sp1 family exert opposite transcriptional regulation of the HIV LTR. While Sp1 and Sp4 stimulate transcription of the LTR, Sp3 markedly represses the HIV promoter activity in HeLa and Drosophila SL2 cells. Sp3-dependent repression is dependent on the presence of the DNA-binding domain, indicating that repression occurs through interference with Sp1 binding to the GC motifs (32). Our data confirm that Sp1 and Sp3 are present in microglial cells, and exert an antagonist action on LTR-driven transcription. Moreover they show that Sp3 is able to mediate transcriptional repression not only of the Sp1 action, but also of the COUP-TF action exerted via Sp1.

Only a limited number of studies have been devoted to HIV-1
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gene transcription in macrophages and microglial cells. A differen-
tial role of LTR elements for the regulation of basal and Tat-
terminated expression of HIV-1 has been reported in stim-
ulated and unstimulated primary human macrophages (50).
Our findings establish the essential role of the orphan nuclear
receptor COUP-TF as a transcriptional activator of LTR-di-
rected HIV-1 gene expression in microglial cells. They further
reveal a novel mechanism of HIV-1 gene transactivation by
direct association of COUP-TF with Sp1, thus enhancing tran-
scription via the minimal LTR region. Our present and previ-
ous studies (25) reveal the remarkable diverse mechanisms by
which COUP-TF regulates gene transcription of distinct HIV-1
strains and in different brain cells.

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