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Chicken Ovalbumin Upstream Promoter Transcription Factor, a Transcriptional Activator of HIV-1 Gene Expression in Human Brain Cells*

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Viral infection of the central nervous system by the human immunodeficiency virus type 1 leads to a wide range of neuropathological disorders. However, the molecular mechanisms governing transcription of the human immunodeficiency virus type 1 genome in brain remain unclear. We have recently established that in brain cells, proteins belonging to the steroid/thyroid/ retinoic acid receptor family bind to the −352 to −320 region of the long terminal repeat (LTR). Here, by supershift experiments, we have identified chicken ovalbumin upstream promoter transcription factor (COUP-TF), an orphan member of this nuclear receptor family, as one of the major proteins interacting with this LTR site. Cotransfection studies revealed that COUP-TF is able to dramatically activate LTR-directed gene transcription in human oligodendroglialoma but not in astrocytoma cells. This activation occurs through two mechanisms, depending on the LTR sequence. Moreover, in human cells COUP-TF and dopamine a catecholamine neurotransmitter, enhance LTR-directed transcription by acting on the proximal LTR region. These results reveal the importance of COUP-TF and the dopamine signaling pathway as activators of human immunodeficiency virus type 1 gene expression in brain.

The human central nervous system (CNS) is an important target of the human immunodeficiency virus type 1 (HIV-1). Viral infection of the brain leads to a wide range of neurological complications (1, 2). A number of studies have revealed that together with cells of the monocyte/macrophage lineage, neuronal and glial cell infection underlies neuropathological damage. Virus proteins and nucleic acids have been detected in oligodendrocytes, astrocytes, and neurons (3–5).

HIV-1 gene expression is controlled by a combination of viral and host cell transcription factors interacting with the long terminal repeat (LTR) region (6–8). Besides differences in envelope sequences of the virus that influence the occurrence of nervous system disease (9), the LTR region is also able to contribute to HIV-1 cell type-specific expression. This was demonstrated by transgenic mouse studies which revealed that the LTR of neurotropic HIV-1 strains JR-FL and JR-CSF directs gene expression in CNS neurons (10, 11); in contrast, the lymphotropic LAI strain was unable to direct gene expression in the CNS of transgenic mice (12, 13). These transgenic data strongly suggest the importance of cellular transcription factors present in brain cells, different from those in non neural cells.

Recent studies have focused on transcription factors that regulate HIV-1 expression in brain cells. They have shown that the activity of the HIV promoter is mediated by kB-regulatory sequences of the LTR, through the action of the transcription factor NF-kB, in both neurons (14, 15) and astrocytes (16).

Besides the kB-regulatory element, recent in vitro data have highlighted the importance of an upstream-located regulatory element. The nuclear receptor-responsive element (NRRE) appears to be the point of convergence of a complex network of physiological signals that modulate HIV-1 gene expression. This element contains binding sites for transcription factors belonging to the steroid/thyroid/retinoic acid receptor superfamily as well as for nuclear receptors with unknown ligands, such as Ear-3/COUP-TF (17–19). In non-CNS-derived cells COUP-TF acts as a repressor of the retinoid response (19). In brain cells, however, the action of nuclear receptors remains to be investigated.

We have recently described (20) that the orphan nuclear receptors COUP-TF/Ear3 (21–23) are present in three human brain cell lines that are permissive to HIV-1 infection, oligodendroglialoma TC-620, astrocytoma U373-MG, and neuroblastoma SK-N-MC cells. A number of studies revealed that COUP-TF may play a dual regulatory role and direct either positive (24–26) or negative regulation of eukaryotic gene expression (25, 27–29) depending on the promoter and the cell context. Here we have examined the role of COUP-TF on LTR-directed gene expression in glial and neuronal cells. We have analyzed both the lymphotropic LAI and the neurotropic JR-CSF HIV-1 LTR. Our data demonstrate the importance of COUP-TF as a potent transcriptional activator in oligodendroglialoma cells. They reveal the action of the dopamine transduction pathway, which coupled to COUP-TF contributes to enhancement of HIV-1 gene transcription in neuronal cells.

MATERIALS AND METHODS

Construction of Plasmids—To generate LTR(JR-CSF)-CAT, −283/+20 LTR-CAT, and −159/+20 LTR-CAT, the plasmid pSAFYre containing the JR-CSF LTR (gift of Dr. J. Clements) was digested with, respectively, EcoRV + BglII, Ear1 + BglII, and AvaI + BglII. The LTR inserts were isolated, blunt-ended, and subcloned in the Smal site of pUC19-CAT0. To generate LTR(LAI)-CAT, −283/+80 LTR-CAT, and −159/+80 LTR-CAT, the plasmid pSV1b-CAT containing the LAI LTR (gift of Dr. N. Israel) was digested with, respectively, SmaI, AvaI, Ear1 + HindIII, Ear1 + HindIII, and Ava1 + HindIII. The LTR inserts were blunt-ended and subcloned in the Smal site of pUC19-CAT0. The −68/+29 LTR-
CAT vector was constructed by subcloning in the Smal site of pUC19-CAT the BstNI-BstNI blunt-ended LTR insert. To construct the LTRmut3-CAT vectors, site-directed mutagenesis was performed with the mutant oligonucleotide LTRmut: 5'CCAGGGGTTAAGATCTCAAAGCTTTG3'. To construct the 3Ltk-CAT and 3Lmut/tk-CAT vectors, the 3L and 3Lmut oligonucleotides were, respectively, subcloned in the blunt-ended SaI site of pBLCAT2, containing the herpes simplex virus thymidine kinase promoter in front of the CAT gene.

**Cell Culture, Transfections, and CAT Assays**—Human astrocytoma U373-MG (ATCC HTB17) and neuroblastoma SK-N-MC cells (ATCC HTB10) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 10 mM 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 1% gentamicin. All these cell lines were cultured in the presence of penicillin-streptomycin (100 units/ml). Human oligodendroglioma TC-620 cells (30) were grown in Iscove's medium containing 10% non-heat-inactivated fetal calf serum and 1% gentamicin.

Cells (10⁶) were either transfected by the calcium phosphate precipitation method with 1 µmol of plasmid reporter DNA or cotransfected with reporter DNA (1 µmol) and the COUP-TF expression vector (0.5 µmol), as described previously (25). 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, and CAT assays were performed as described previously (25); reaction mixtures containing 5, 10, or 20 µg of protein were incubated at 37 °C for 1.2 h or 2 h, respectively, oligodendroglioma, astrocytoma, and neuroblastoma cells.

**Electrophoretic Mobility Shift Assays**—Nuclear proteins were extracted from at least 10⁶ cells, as described previously (31), or were prepared from a small number of transfected cells (5 × 10⁶) according to the procedure of Andrews and Fallar (32). Protein-DNA binding reactions were performed with 5 µg of nuclear extracts in a buffer containing 1 ng of 32P-labeled oligonucleotide, 1 µg of poly(dI-dC), 50 ng of sonicated salmon sperm DNA, 10 mM MgCl₂, 25 mM KCl, 1 mM dithiothreitol, 12.5 mM HEPES, pH 7.8, 10% glycerol, 0.05% Nonidet P-40. Mixtures were incubated for 15 min at 4 °C, and protein-DNA complexes were analyzed by electrophoresis on a 6% polyacrylamide gel in 0.25 × Tris-buffered EDTA. For supershift assays, antibodies directed against COUP-TF (gift of Dr. M. J. Tsai, Baylor College of Medicine, Houston, TX) or normal rabbit serum were mixed with nuclear proteins 3 h before the addition of the probe.

**RESULTS AND DISCUSSION**

**COUP-TF Present in Brain Cells Binds to the Nuclear Receptor-responsive Element of the HIV-1 LTR**—To examine the nature of the proteins interacting with the NRRE located between nucleotides −356 and −320 of the LTR, gel mobility supershift assays were performed with nuclear proteins isolated from three human brain cell lines, oligodendroglioma TC-620, astrocytoma U373-MG, and neuroblastoma SK-N-MC cells, using the 3L oligonucleotide corresponding to the NRRE sequence (Fig. 1). We have previously shown that proteins forming complexes C1 to C3 belong to the nuclear receptor family, while proteins forming the less specific complex C4 do not belong to this superfamily (20). Complexes C1 to C4 were detected in all three cell lines; the relative amount of each complex varied slightly from one extraction to the next and also depended on the method of nuclear extract preparation (31, 32). With the rapid extraction method, a nonspecific complex appeared with the mutant oligonucleotide 3Lmut. The results of the supershift experiments indicate that the COUP-TF species do form the majority of the DNA-protein complexes C1, C3, and C3' (Fig. 1). These findings reveal that within human brain cells, COUP-TFs appear as the major nuclear protein species interacting with the nuclear receptor-responsive element.

**Functional Role of COUP-TF on LTR-directed HIV-1 Gene Transcription in Brain Cells**—The three glial and neuronal cell lines were transfected with a LTR-CAT reporter vector, containing the chloramphenicol acetyltransferase (CAT) gene under the control of the LTR region from either the neurotropic JR-CSF or the lymphotropic LAI HIV-1 strain. We used the LTR region from the CNS-derived JR-CSF HIV-1 isolate (33) since, unlike the LTR of the LAI isolate, it was found to direct gene expression in the CNS of transgenic mice (10). The results presented in Fig. 2 demonstrate the cell type-specific activity of COUP-TF. In oligodendroglioma TC-620 cells, LTR-driven CAT expression was dramatically stimulated 9–10-fold. The exceptional magnitude of this stimulation has never been reported for COUP-TF. This interesting result indicates that COUP-TF functions as a potent HIV-1 transcriptional activator in TC-620 cells. In contrast, in astrocytoma and neuronal cells, COUP-TF was unable by itself to affect LTR-driven transcription (Fig. 2).

Dopamine, a catecholamine neurotransmitter, has been described to activate COUP-TF by a phosphorylation-mediated event, resulting from dopamine stimulation of cell surface receptors (34). We therefore investigated the possibility that dopamine might modulate LTR-driven transcription. Treatment of neuronal SK-N-MC cells with 100 µM dopamine resulted in a weak increase in LTR-driven CAT activity over untreated cells. However, overexpression of COUP-TF combined with dopamine treatment elicited a 3-fold stimulation of LTR-directed CAT activity (Fig. 2). Similar results were obtained with the LTR of the LAI and JR-CSF HIV-1 strains. Dopamine treatment of TC-620 and U373-MG cells in similar conditions did not change LTR-directed CAT activity. These findings indicate that specifically in neuronal cells, HIV-1 gene transcription is enhanced by COUP-TF following activation of the dopamine pathway. We found that this activation was reversible by the addition of the selective D1 receptor antagonist SCH23390 and was not affected by sulpiride, a D2 antagonist (results not shown).

To compare the transfection efficiencies of the COUP-TF vector in the different cell lines, we performed gel retardation assays using the 3L probe and nuclear extracts from trans-
fected or dopamine-treated cells (Fig. 3). In the presence of COUP-TF, complexes C3 and C3' were strongly increased in glial but not in neuronal cells. This result indicates that the differences in trans-activation in oligodendrogloma and neuronal cells are due to different transfection efficiencies of the COUP-TF vector. Dopamine treatment increased the binding of COUP-TF to the NRRE site in complexes C3 and C3' formed with glial proteins, suggesting that a direct phosphorylation event of COUP-TF enhances its binding ability (Fig. 3). These data suggest that in TC-620 cells, high COUP-TF overexpression results in high binding and thus, to an optimal level of trans-activation, which is unaffected by dopamine. In contrast, in SK-N-MC cells, a low level of COUP-TF overexpression correlates with a low level of trans-activation, which is unaffected by dopamine. In contrast, in SK-N-MC cells, a low level of COUP-TF overexpression correlates with a low level of trans-activation, which is stimulated, via phosphorylation, by dopamine-induced increased binding. Surprisingly, such an enhanced binding on the NRRE site was not detected in Fig. 3. It therefore appeared essential to examine whether the NRRE element was involved in the COUP-TF- and dopamine-induced transcriptional stimulation.

**Elements of the HIV-1 LTR Involved in COUP-TF-induced Transcriptional Stimulation in TC-620 Cells**—To define the molecular mechanisms that control COUP-TF-induced transcriptional stimulation, we first analyzed the role of the nuclear receptor-responsive element. The NRRE sequence was inactivated within the LTR by site-directed mutagenesis, using the 3Lmut oligonucleotide. We tested by gel shift assays that 3Lmut was unable to bind COUP-TF. Transfection of the mutant constructs 2 and 7 (Fig. 4a) in TC-620 cells resulted in a 3-fold decrease in COUP-TF-induced CAT stimulation (Fig. 4b); this result confirmed the importance of the NRRE sequence. Similarly, the linkage of the NRRE sequence to the heterologous thymidine kinase promoter in the 3Ltk-CAT vector could confer COUP-TF responsiveness. The 3Ltk-CAT vector led to a 3-fold increase in CAT activity, while the basal
activity of the control pBLCAT2 and the mutant 3Lmut/tk-CAT vectors was not modified (results not shown). These results clearly indicate that the 3L (or NRRE) sequence is responsible for mediating, at least in part, the COUP-TF action.

However, the mutation of the NRRE site did not completely abolish the COUP-TF-induced activation. To delineate the LTR sequences responsible for this residual activation, we transfected a series of LTR-CAT vectors containing 5’ deletions of the LTR (Fig. 4a). Surprisingly, the experiments revealed that the −68/+29 LTR region (Fig. 4, constructs 5 and 10) was able to promote a basal level of transcription similar to that of the entire LTR. This interesting finding indicates that in TC-620 cells, only two Sp1 sites and the TATA box region are sufficient to function as a minimal promoter. In the LAI series, the 3-fold residual COUP-TF-induced stimulation was abolished by removal of sequences located between base pairs −159 and −68 (Fig. 4b, constructs 4 and 5). This region contains the binding site of the NF-κB transcription factor (12). These data indicate that the action of COUP-TF on the LAI LTR is mediated mostly through direct interactions with the NRRE site or 5’-end deletion. However our findings differ from those reported with T3R, another member of the nuclear receptor superfamily, interacts with several sites in the proximal promoter spanning the Sp1 and NF-κB elements (35) or overlapping the Sp1 sites within the −74/−50 region (36). However our findings differ from those reported with T3R, since the minimal −68/+29 COUP-TF- and dopamine-responsive region contains only two Sp1 sites and the TATA region. They suggest that the action of COUP-TF combined with dopamine-induced phosphorylation events are likely to be mediated through interactions with components of the basal transcription machinery. In vitro studies have demonstrated interactions between COUP-TF and the basal transcription factor TFII B (37). Further investigations are nevertheless required to establish whether such interactions are relevant to the in vivo situation. Similarly, in vivo and in vitro experiments have demonstrated the ability of several nuclear receptors, including thyroid hormone receptor β (38), vitamin D receptor (39, 40), and retinoid X receptor (41), to interact with TFII B.

In conclusion, our results establish the essential and new role of COUP-TF as a cell type-specific transcriptional activator of LTR-directed HIV-1 gene expression in brain. These data reveal the remarkable diverse mechanisms by which COUP-TF enhances viral gene transcription. This orphan nuclear receptor is able to exert a major effect on distinct HIV-1 strains in different brain cells types. In oligodendrocytes, this transcription factor is able to dramatically stimulate HIV-1 gene tran-

Elements of the HIV-1 LTR Involved in COUP-TF- and Dopamine-induced Stimulation in SK-N-MC Cells—A similar deletion analysis performed in SK-N-MC cells showed that the truncated LTR regions, including the minimal −68/+29 region were responsive to the combined action of COUP-TF and dopamine, since a 2–3-fold stimulation was detected (Fig. 4c). As a control, COUP-TF and dopamine were unable to induce any CAT activity of the pUC-CAT vector (results not shown). These surprising findings indicate that the NRRE sequence is not involved in the transcriptional activation mediated by COUP-TF and dopamine. Recent reports indicated that the thyroid hormone receptor T3R, another member of the nuclear receptor superfamily, interacts with several sites in the proximal promoter spanning the Sp1 and NF-κB elements (35) or overlapping the Sp1 sites within the −74/−50 region (36). However our findings differ from those reported with T3R, since the minimal −68/+29 COUP-TF- and dopamine-responsive region contains only two Sp1 sites and the TATA region. They suggest that the action of COUP-TF combined with dopamine-induced phosphorylation events are likely to be mediated through interactions with components of the basal transcription machinery. In vitro studies have demonstrated interactions between COUP-TF and the basal transcription factor TFII B (37). Further investigations are nevertheless required to establish whether such interactions are relevant to the in vivo situation. Similarly, in vivo and in vitro experiments have demonstrated the ability of several nuclear receptors, including thyroid hormone receptor β (38), vitamin D receptor (39, 40), and retinoid X receptor (41), to interact with TFII B.

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Fig. 4. Analysis of COUP-TF interactions with the LTR derived from the lymphotropic LAI and the neurotropic JR-CSF HIV-1 strains. a, plasmid constructs used in transient expression assays. The LTR region of the LAI and JR-CSF HIV-1 strains was mutated within the NRRE site or 5’-end deleted. b, expression of CAT activity in TC-620 cells transfected with the LTR-CAT constructs, either alone (a) or in the presence of the COUP-TF expression vector (b). CAT activities are expressed relative to that of construct 1 (LAI series) and 6 (JR-CSF series) taken as 1. Numbers in parentheses indicate the fold stimulation induced by COUP-TF. c, expression of CAT activity in SK-N-MC cells, transfected with the LTR-CAT constructs (a) and the COUP-TF expression vector followed by 100 μM dopamine treatment 24 h after transfection (b). CAT activities are expressed as in b.
scription, either by direct interactions with its NRRE target site or, depending on the LTR sequence, by cross-coupling interactions with downstream-located proteins. In neuronal cells, where COUP-TF is inactive by itself, HIV-1 appears to couple COUP-TF and the dopamine signal transduction pathway with the general cellular transcription machinery. Since dopamine antagonists appear able to reverse the dopamine-induced stimulation, they could provide a way of limiting HIV-1 activation in neuronal cells.

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