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Hormone Receptor Regulation of the Human Immunodeficiency Virus Type 1 and Type 2 Long Terminal Repeats

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Abstract
Both host cell and viral transcription factors regulate the long terminal repeat (LTR) of human immuno-deficiency virus (HIV) activity and viral replication. Using transient transfection, ligand-activated thyroid hormone and 9-*cis*-retinoic acid receptors (T_3R and RXR) were found to stimulate HIV-1 and HIV-2 LTR activities. They also stimulated HIV-1 viral production. Drosophila SL2 cells that lack Sp1 and T_3R were used to study HIV-1 and HIV-2 LTR activities. Both activities were stimulated by cotransfection of SP1 (120- and 180-fold, respectively); HIV LTR activities were also stimulated ~5-fold by ligand-activated T_3R, ~10-fold by ligand-activated RXR and 20- to 30-fold by both receptors and their cognate ligands. $T_3R \cdot RXR$ heterodimers bound to NF-κB and Sp1 response elements in both HIV LTRs having highest affinity for the HIV-1 NF-κB region. When U937 monocytic cells were cotransfected with HIV-1 viral DNA and T_3R, RXR and retinoic acid receptor (RAR) expression plasmids, hormonal treatment increased viral replication up to 5-fold. Hormonal signals thus have the potential to regulate HIV transcription and viral production.

Studies of the long terminal repeat (LTR) of human immunodeficiency virus (HIV) have revealed a large number of *cis*-acting regulatory elements. Transcription factors from host cells and those encoded by the viral genome have been demonstrated to regulate LTR activity through interaction with these elements [4, 32]. Sp1 and NF-κB are two cellular *trans*-activators that are important in HIV gene expression. In HIV type 1 and type 2 (HIV-1 and HIV-2) LTRs, three tandem Sp1 binding sites have been identified immediately upstream of the TATA box [8, 25]. Sp1 binds to these GC-rich sequences and activates RNA synthesis in reconstituted in vitro transcription systems [11]. Adjacent to the Sp1 sites are two copies of an NF-κB binding site on the HIV-1 LTR [22, 25] and one functional NF-κB site adjacent to a nonfunctional NF-κB site (designated as K) on the HIV-2 LTR [22]. NF-κB increases LTR activity in response to several stimuli including phorbol 12-myristate 13-acetate [25, 29] and TNF-α [6]. Recently, the NF-κB and Sp1 motifs in the proximal region of HIV-1 LTR have been demonstrated to function as thyroid hormone (TH) response elements (TRE) [5]. Mutations in either NF-κB site resulted in only

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monomeric chicken TH receptor (T_3R) binding and markedly reduced TH-dependent promoter activity suggesting that active dimeric receptors bound to half-sites located in each NF-κB site with an 8–10 bp spacing [5]. In the absence of NF-κB sites the Sp1 sites did not function as a TRE; however, with cotransfection of *tat* the Sp1 sites became functional TREs.

Receptors for TH, retinoic acid (RAR), vitamin D (VDR), and certain medium and long-chain fatty acids (PPAR) preferentially interact with their cognate DNA response elements as heterodimers with the 9-*cis* RA receptor (RXR) [13, 15, 36, 37]. Preference for binding to direct repeats of the consensus PuGGTCA sequence results from interactions between the heterodimeric partners that position the RXR partner over the upstream half of the direct repeat [14, 25, 31]. TH binding to the T_3R partner regulates expression, either positively or negatively, from TREs [10, 13]. 9 *cis*-RA regulates expression from homodimeric RXR response elements but ligand effects on heterodimeric response elements are variable [17, 20].

Although closely related, HIV-1 and HIV-2 differ considerably in clinical and biological behavior [reviewed in ref. 21]. To compare the effects of both ligand-activated T_3R and RXR on the LTRs of HIV-1 and HIV-2, expression vectors were transfected into *Drosophila* Schneider line 2 (SL2) cells that are deficient in Sp1, T_3R and RXR [3] and effects on HIV-1 and HIV-2 LTR-luciferase reporter gene expression were measured. In vitro binding of human T_3R and $T_3R \cdot RXR$ heterodimers to the NF-κB and Sp1 motifs of HIV-2 LTR were measured using electrophoretic mobility shift assays (EMSA). Results of these experiments indicate that T_3R activates both HIV-1 and HIV-2 LTR activities through NF-κB and Sp1 motifs. The monocytic cell line U937 was used in transient assays to study the effects of TH and RA on virus replication. Cells cotransfected with virus DNA and plasmids expressing T_3R, RAR and RXR produced significantly more virus upon stimulation with cognate hormones providing a correlation with effects on HIV-LTR activities in model systems.

Materials and Methods

Reporter Plasmids

The LTR of the human immunodeficiency virus type 1 and type 2 was excised by *Hind*III from parental plasmids (pC15CAT [1] and HIV-2_{KR} LTR-CAT [L. Luznik and F. Wong-Staal, unpubl.] and inserted into the luciferase vector pGL2-basic (Promega, Madison, Wisc., USA) to generate the reporter plasmids HIV-1 LTR-LUC and HIV-2 LTR-LUC. Orientation was verified by dideoxynucleotide sequencing. HIV-2 LTR-LUC was digested with *Bgl*II and *Pst*I to remove sequences upstream of −112 bp and re-ligated to generate HIV-2 LTRΔBP-LUC.

Expression Plasmids

pPacSp1 and pPacU+Nde1 plasmids were a generous gift from Drs. Erica Pascal and Robert Tjian (University of California, Berkeley, Calif., USA) [3]. pPacT_3R, pPacT_3RΔDBD, pPacT_3RS, pPacT_3RΔC, and pPacRXR have been described previously [34]. T_3RΔ DBD lacks amino acids 100–171 and is defective in DNA binding. T_3RS has a Thr deletion at codon 332, which destroys ligand-binding activity [23]. T_3RΔC terminates at the codon for amino acid 335 and deletes 121 carboxyl-terminal amino acids, resulting in a truncated form of T_3R defective in both ligand-binding and dimerization. For expression in U937 cells, hormone receptor expression was driven by the RSV promoter [7, 14, 20]. pPacβgal [20] was a generous gift from Dr. Ron Evans (The Salk Institute for Biological Studies, La Jolla, Calif., USA). The plasmid containing the full-length HIV-1 sequence was derived from the HIV-1 HXB-2 clone.

Transfection, Assays of Enzyme Activities and Virus Production

SL2 cells were maintained in Schneider's *Drosophila* medium (GIBCO BRL, Gaithersburg, Md., USA) supplemented with 10% heat-inactivated (56 °C, 30 min) fetal calf serum. Cells were seeded overnight before transfection at a density of 5×10^5 cells per well of 12-well dishes with 1 ml of medium. Transfections were carried out as described [34]. Plasmids were suspended in 0.25 *M* $CaCl_2$ and an equal volume of 2 × HEBS buffer (42 m*M* HEPES, pH 7.05, 274 m*M* NaCl, 9.4 m*M* KCl, 1.44 m*M* Na_2HPO_4, and 0.2% dextrose) was added dropwise. The mixture was incubated at room temperature for 30 min before adding it to the cells. Transfected cells were harvested 48 h later.

Luciferase activity was measured as described by deWet et al. [33]. Aliquots of cell extracts in 100 m*M* potassium phosphate (pH 7.8) and 1 m*M* DTT were added to an assay reaction containing 100 m*M* potassium phosphate (pH 7.8), 5 m*M* ATP and 15 m*M* $MgSO_4$ in a volume of 350 µl. Reactions were initiated by injection of 100 µl of 1 m*M* luciferin and light readings were integrated over 10 s on a Monolight 2001 luminometer (Analytical Luminescence Laboratory, Inc., San Diego, Calif., USA).

β-Galactosidase activity was measured as described by Norton and Coffin [26]. Aliquots of cell extract were added into 500 µl of assay buffer (50 m*M* Tris·HCl, pH 7.6, 100 m*M* NaCl, 100 m*M* $MgCl_2$) with 0.75 mg/ml of *o*-nitrophenyl β-*D*-galactopyranoside. Reactions were incubated at 37 °C and stopped by adding 500 µl of 1 *M* Na_2CO_3 when the solution turned yellow. β-Galactosidase activities were determined by OD readings taken at 414 nm.

For transfection of an infectious clone of HIV-1, U937 cells maintained in RPMI medium (GIBCO) supplemented with 10% fetal calf serum were used. Cells were transfected with the infectious clone HXB-2 and different receptors, using the DOTAP transfection method (Boehringer-Mannheim). U937 cells (1.2×10^6) were transfected with 2.5 µg of plasmid containing the HIV-1 HXB-2 clone and 2.5 µg of the expression plasmids pRSVT_3R, pRSVRAR or pRSVRXR. Control cells were transfected with HIV-1 HXB-2 plasmid alone. The total amount of DNA in each transfection was equalized with nonspecific DNA (pUC-19). Where indicated, transfected cells were treated with 150 n*M* L-T_3 and/or 150 n*M* all-*trans*-RA or 9-*cis*-RA

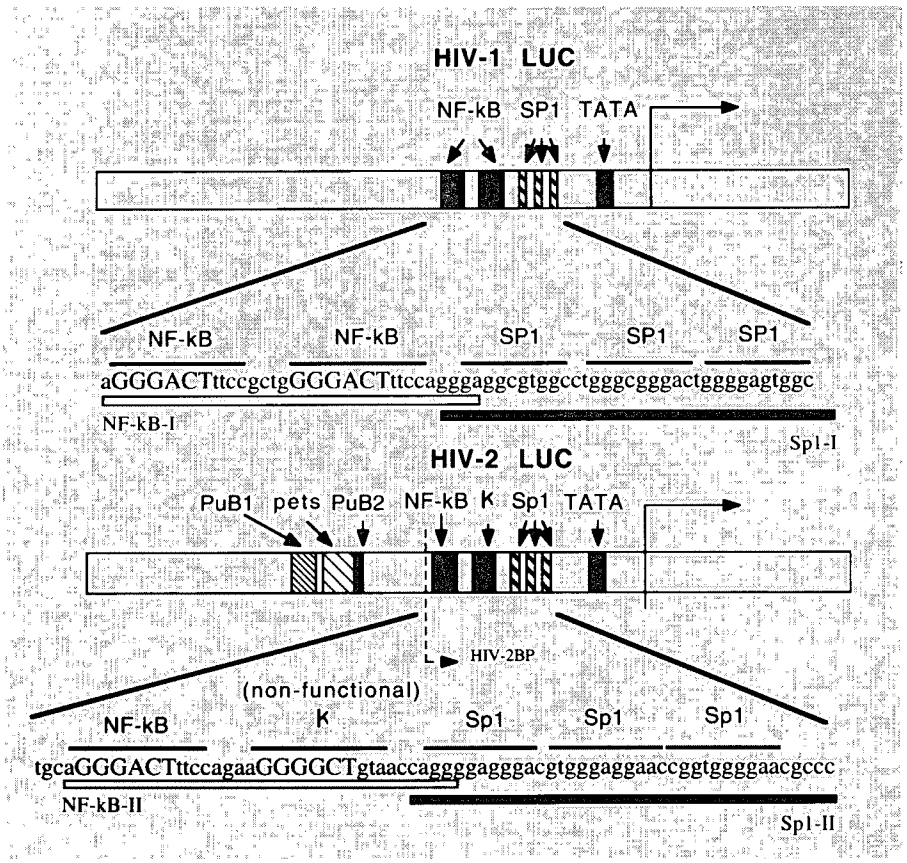


Fig. 1. Schematic representation of HIV-1 and HIV-2 LTR organization. The identified functional NF-κB sites, nonfunctional K site and Sp1 sites are marked by overbars. Identified *pets* and purine box (PUB) binding sites in HIV-2 are also noted. Oligonucleotide sequences for probes NF-κB and Sp1 are indicated by open and filled lower bars, respectively. The deduced direct-repeat TREs in NF-κB_I and NF-κB_{II} are shown in bold letters.

and maintained in RPMI medium containing 10% stripped serum. Supernatants were collected 72 h following transfection and viral production was measured using the antigen capture assay (Coulter, Hialeah, Fla., USA).

Electrophoretic Mobility Shift Assays

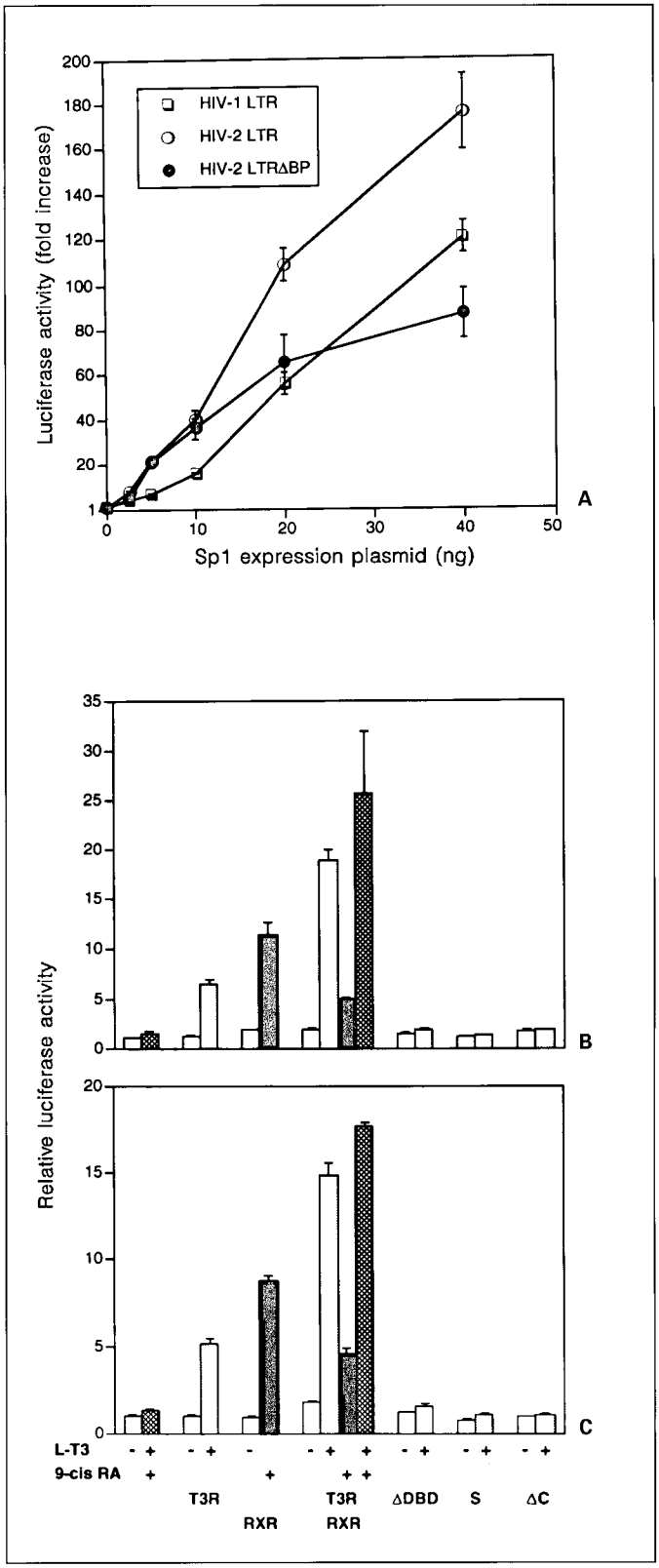
Double-stranded oligonucleotides complementary to the NF-κB and Sp1 regions of HIV-1 and HIV-2 LTRs were synthesized with an Applied Biosystems 380B synthesizer and annealed. Sequences of wild-type HIV NF-κB and Sp1 motifs are shown in figure 1. To verify specificity of $T_3R \cdot RXR$ binding, mutant competitor oligonucleotides were used. The sense strand mutant oligonucleotide corresponding to the HIV-2 NF-κB site has the sequence 5′ AGCTATCTACTTTCCAGAAGTCTCTGTAACCAGGG3′ and the mutant oligonucleotide corresponding to the sense strand of the HIV-2 Sp1 site has the sequence 5′GATCAGATACGTGATAGGAACCGGTGATCAACGGAT3′. Oligonucleotides for the NF-κB sites are flanked by *Hind*III cohesive ends. ^{32}P-end-labeled oligonucleotide (3×10^4 cpm) was incubated with the indicated hormone receptors and competitor DNAs in a 20-μl reaction at room temperature for 15 min, then on ice for 15 min. When antisera were used, the receptors and antisera were incubated at room temperature for 15 min prior to the above incubation procedure. The binding reaction mixture contained 10 m*M* Tris·HCl, pH 8.0, 40 m*M* KCl, 2% Ficoll, 0.1% NP-40, 1 m*M* DTT, 6% glycerol, 5 μg BSA, and 0.1 μg poly (dI-dC). After incubation, samples were loaded onto a 6% nondenaturing polyacrylamide gel (acrylamide:bisacrylamide = 79:1) with 0.5 × TBE which had been first electrophoresed at 4 °C for 1 h. Electrophoresis was carried out at 4 °C at 200 V. The gel was dried and autoradiographed at −80 °C with Amersham Hyperfilm-MP and an intensifying screen.

Human $T_3R\beta$ (T_3R) was expressed in *Escherichia coli* under control of the T7 promoter in the pET-3A vector as a GST-fusion protein [34], purified by glutathionine elution followed by Mono Q chromatography and was kindly provided by Dr. Richard Kurten. Human RXRα (hRXRα) was expressed and purified as described [34]. $hT_3R\beta$ and hRXRα antisera were obtained by injecting rabbits with purified hT_3R and hRXRα. Protein concentrations were determined by the method of Bradford [2].

Results

Regulation of HIV-1 and HIV-2 LTR Activities in SL2 Cells

To study regulation of HIV-LTR activities, SL2 cells that lack Sp1, T_3R and RXR were utilized [3]. This was desirable because RXR forms active heterodimers with a number of receptors in this gene family [13, 15, 36, 37].



The *Drosophila* homolog of RXR, ultraspiracle [27] does not respond to 9-*cis*-RA [35]. Figure 2A shows that increasing amounts of Sp1 expression plasmid progressively increased LTR-driven luciferase expression. The greater increase in HIV-2 LTR activity suggests that the Sp1 motifs in the HIV-2 LTR exert a stronger effect. Removal of sequences upstream of −112 bp in the HIV-2 LTR (HIV-2 LTRΔBP) did not decrease Sp1 activation at low concentrations of Sp1, but impaired Sp1 activation to ~50% at higher concentration of Sp1, suggesting that additional upstream Sp1 element(s) exist. The basal activity of the HIV-2 LTR is 5-to 10-fold higher than that of the HIV-1 LTR (see legend of fig. 2). While removal of upstream negative response elements (NRE) in the HIV-1 LTR resulted in an increase in activity in Jurkat T-cell lines [16], removal of sequences 5′ to nucleotide −112 from the HIV-2 LTR did not alter basal activity in SL2 cells, suggesting that SL2 cells may be deficient in certain inhibitory factors that bind to the NRE, or the functions of upstream *cis*-acting elements differ in the LTRs of HIV-1 and HIV-2.

Figure 2B shows that T_3R activated both types of LTRs ~5-fold, and RXR activated ~10-fold in response to their cognate ligands. With both ligands or with T_3 alone, T_3R and RXR together activated LTR activities

Fig. 2. Stimulation of HIV LTRs by Sp1 and by ligand-activated T_3R and RXR. SL2 cells were transfected in 12-well dishes with the indicated luciferase reporter plasmids and expression plasmids which utilize the actin 5C promoter. pPacβgal was used as an internal control and each data point represents triplicate wells ± standard error. **A** Effect of increasing amounts of pPacSp1 expression plasmid on HIV-1 and HIV-2 LTR activities. HIV-2·LTRΔBP-LUC was prepared by digestion with *Bgl*II and *Pst*I to remove sequences upstream of nucleotide −112 of the HIV-2 LTR. For HIV-1 LTR-LUC 1 = 1.8 × 10^5 and for HIV-2 LTR-LUC 1 = 9.0 × 10^5 light units of luciferase activity/OD_{414} unit of β-galactosidase activity·min^{-1}, respectively. **B, C** Effect of ligand-activated T_3R and RXR on HIV LTRs. 50 ng of each expression plasmid was used and where indicated transfected cells were treated with 100 n*M* L-T_3 and/or 100 n*M* 9-*cis*-RA. ΔDBD = T_3RΔ aa 100–171, defective in DNA binding; S = T_3RS with deletion of Thr^{332}, defective in ligand binding; ΔC = T_3R truncated at residue 332 in the C terminus, defective in ligand binding and dimerization. For HIV-1 LTR-LUC (**B**), 1 = 2.1 × 10^5 and for HIV-2 LTR-LUC (**C**), 1 = 2 × 10^6 light units of luciferase activity/OD_{414} unit of β-galactosidase·min^{-1}, respectively.

20- to 30-fold, which is in agreement with reports that T_3R and RXR form heterodimers which are functionally more active than T_3R monomers or homodimers on thyroid hormone response elements [13, 15, 36, 37]. However, 9-*cis*-RA treatment stimulated LTR activities to a lower extent in the presence of both receptors than with RXR alone. This implies that in the absence of T_3, T_3R formed inactive heterodimers with RXR and prevented RXR from functioning through its endogenous partner or by itself. T_3R with deletion of DNA binding or COOH-terminal domains ($T_3R\Delta DBD$ and $T_3R\Delta C$) and T_3R defective in ligand binding (T_3RS) did not increase HIV LTR activities, indicating that the activation is specific for ligand-activated wild-type T_3R. These results suggest that T_3R and RXR activate HIV LTRs by direct binding to *cis*-acting elements in the LTRs. Figure 3 shows that HIV-2 LTRΔBP retained the same pattern of T_3R and RXR regulation, suggesting that critical elements that interact with T_3R and RXR are contained in the proximal portion of the HIV-2 LTR.

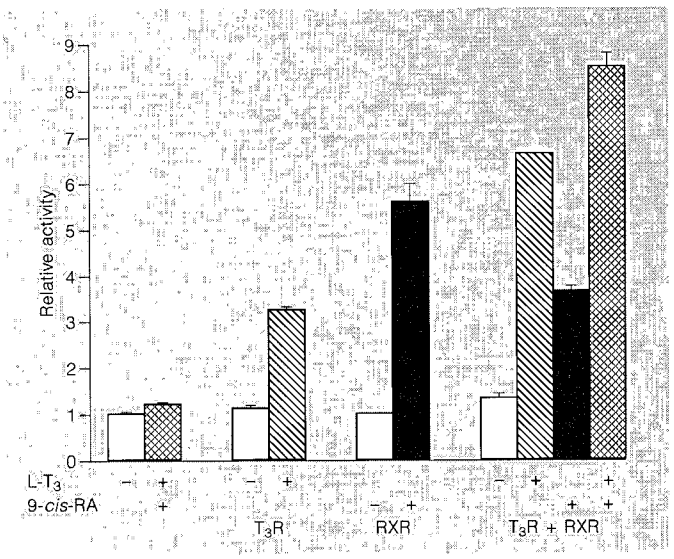


Fig. 3. Activation of HIV-2 LTRΔBP by ligand-activated T_3R and RXR. SL2 cells were transfected in 12-well dishes wit 0.5 μg of the reporter plasmid HIV-2 LTRΔBP-LUC, 50 ng of pPacβgal, 1.5 μg of pUC 18, without or with 50 ng of pPacT_3R, and without or with 50 ng of pPacRXR in each well. Where indicated, transfected cells were treated with 100 n*M* L-T_3 and/or 100 n*M* 9-*cis* RA. Activity of the reporter plasmid alone was set to 1, which equaled 2.1×10^6 light units of luciferase activity/OD_{414} unit of β-galactosidase activity·min^{-1}. Each data point represents triplicate wells ± SE.

Binding of T_3R Homodimers and T_3R·RXR Heterodimers to the NF-κB and Sp1 Sites in the HIV-1 LTR

Because HIV-2 LTRΔBP contains a single NF-κB site and a related K site followed by three Sp1 sites 5′ to the TATA box [22], the NF-κB and Sp1 motifs were examined as binding sites for T_3R and RXR and compared to the NF-κB site of HIV-1 [5]. Figure 4A shows that T_3R bound to NF-$κB_I$, NF-$κB_{II}$ and $Sp1_{II}$ and formed one specific complex (designated as A). RXR greatly enhanced the binding of T_3R on these elements (only one third the amount of T_3R was used for binding reactions in the presence of RXR) and formed a complex which migrated more slowly. Both complexes were competed by the same unlabeled probes, but not by oligonucleotides corresponding to the proximal promoter of *erb* B-2 (see fig. 4C). No specific DNA-protein complex was detected with RXR alone, although this purified RXR is able to bind to an RXR specific element from the retinol-binding protein type II promoter. Adding the ligand, 9-*cis*-RA [9] did not promote any specific complex formation (data not shown). To identify the complexes, antisera against T_3R·RXR were included in the binding reactions. As shown in figure 4B, formation of complex A was blocked by T_3R antiserum, while formation of complex B was blocked by both T_3R and RXR antisera. The preimmune serum for RXR did not affect complex B formation. Complex A thus represents T_3R homodimers and complex B represents T_3R·RXR heterodimers.

Specificity was further confirmed by comparing nonspecific and mutant oligonucleotides as competitors. As shown in figure 4C, left panel, unlabeled oligonucleotides corresponding to the NF-$κB_{II}$ and $Sp1_{II}$ sites effectively competed T_3R·RXR heterodimer binding to the corresponding ^{32}P-labeled oligonucleotides whereas oligonucleotides corresponding to the *erb* B-2 promoter showed no displacement. As shown in figure 4C, right panel, equimolar concentrations of mutant NF-$κB_{II}$ and $Sp1_{II}$ oligonucleotides showed only minimal competition compared to wild-type oligonucleotides.

Although all three elements bound T_3R homodimers and T_3R·RXR heterodimers, their affinities varied. In order to measure their relative affinities, T_3R·RXR binding to NF-$κB_{II}$ was competed by increasing concentrations of unlabeled probes of itself, $Sp1_{II}$ and NF-$κB_I$. Figure 4D shows that among the three probes, NF-$κB_I$ has the highest affinity for T_3R·RXR heterodimers, being at least 4-fold higher than the affinity of NF-$κB_{II}$. The affinities of NF-$κB_I$ and $Sp1_{II}$ are comparable, differing by less than 2-fold.

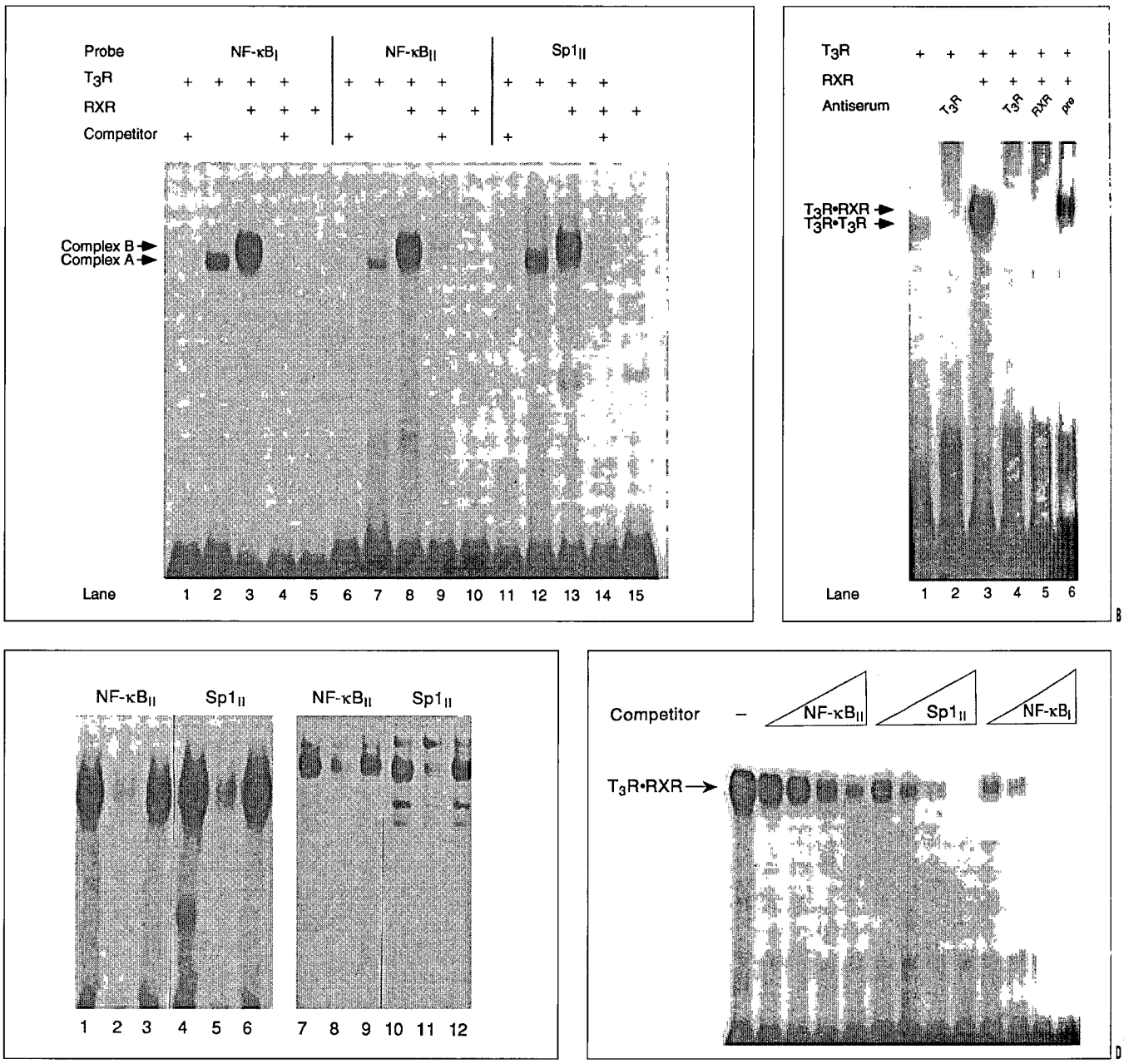


Fig. 4. Binding of T_3R and RXR to the NF-κB and Sp1 sites on HIV LTRs. Electrophoretic mobility shift assays were used to study the binding of purified T_3R and RXR to NF-κB$_I$, NF-κB$_{II}$ and Sp1$_{II}$. **A** Each reaction contained 3×10^4 cpm of probe. When only T_3R was included, 21, 192, and 64 ng were used for NF-κB$_I$, NF-κB$_{II}$, and Sp1$_{II}$, respectively; when only RXR was included, 48 ng was used. When both T_3R and RXR were added, one third the above amounts of T_3R was used together with 24 ng of RXR. 100-fold excess of unlabeled oligonucleotide was used as competitor where indicated. Shifted complexes, designated as A and B, are indicated by arrows. **B** T_3R (64 ng) alone or with 24 ng of RXR were used for binding to 3×10^4 cpm of NF-κB$_{II}$. Where indicated, a 1:20 dilution of antisera raised against either purified T_3R or RXR were included along with 0.5 μg of poly(dI-dC). T_3R homodimer and $T_3R \cdot$RXR heterodimer are indicated by arrows. pre = Preimmune. **C** T_3R (64 ng) plus RXR (48 ng) were incubated with ^{32}P-labeled NF-κB$_{II}$ and Sp1$_{II}$ oligonucleotides without (lanes 1, 4, 7 and 10) or with a 40-fold excess of unlabeled NF-κB$_{II}$ (lanes 2 and 8), mutant NF-κB$_{II}$ (lane 9), Sp1$_{II}$ (lanes 5 and 11), mutant Sp1$_{II}$ (lane 12) or an oligonucleotide corresponding to the erb B2 promoter (lanes 3 and 6). Complexes were resolved by polyacrylamide gel electrophoresis and autoradiographed. **D** To study the relative affinities of NF-κB$_I$, NF-κB$_{II}$, and Sp1$_{II}$ for $T_3R \cdot$RXR heterodimers, 3×10^4 cpm of NF-κB$_{II}$ was incubated with 64 ng of purified T_3R and 48 ng of purified RXR. Increasing concentrations (12, 24, 48 and 96 ng) of the indicated oligonucleotide competitor were added and incubated to equilibrium. $T_3R \cdot$RXR heterodimer is indicated by the arrow.

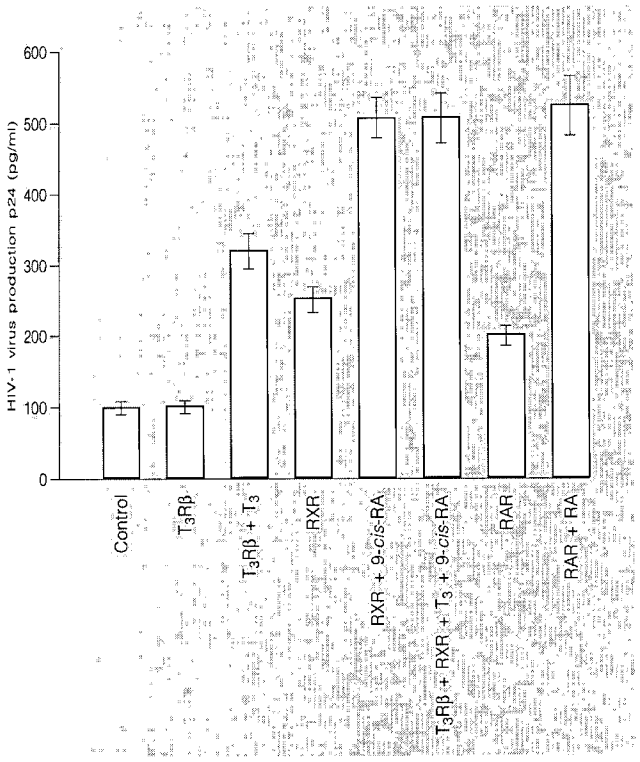


Fig. 5. Activation of HIV-1 HXB-2 with ligand-activated T_3R and RXR. U937 cells were transfected with the HIV-1 HXB-2 clone without or with the expression plasmids pRSVT_3R, pRSVRAR or pRSVRXR. The total amount of DNA in each transfection was equalized with pUC-19 DNA. Where indicated, transfected cells were treated with 150 n*M* L-T_3 and/or 150 n*M* 9-*cis*-RA or all-trans-RA. Values presented represent triplicates ± SE. The experiment was repeated three times with values that varied less than 10%.

Ligand-Activated T_3R and RXR Stimulation of HIV-1 Production

To determine whether T_3R and RXR activated the HIV LTR in the context of virus expression, U937 cells were cotransfected with HIV-1 HXB-2 DNA and the receptor expression plasmids pRSVT_3R, pRSVRAR, pRSVRXR or pRSVT_3R + pRSVRXR. The cells were maintained in the presence or absence of cognate ligands for 72 h before measuring virus in the supernatant. Cells transfected with T_3R alone did not show increased virus production compared to untreated cells, but addition of ligand T_3 increased viral production 3-fold. Transfection with RAR or RXR resulted in a 3-fold increase in viral production, suggesting the presence of endogenous ligands for these receptors despite the use of stripped serum. Stimulation with the corresponding ligands further enhanced viral production up to 5-fold compared to control cells. Stimulation with ligands in the absence of receptors showed no increase in viral production in transient assay compared to control cells (data not shown). Cells cotransfected with RXR and T_3R and treated with both T_3 and 9-*cis*-RA did not show any further increase in viral production compared to that seen with 9-*cis*-RA-activated RXR alone. This likely represents the maximal transcriptional activation effect obtained in short-term assays with T_3R and RXR in the presence of the potent viral transactivator, *tat*, in the context of expression of the complete viral genome. It was not feasible to measure HIV-2 production using similar transient cotransfections because active virus is not encoded in a single plasmid.

Discussion

The present studies confirm reported effects of Sp1 on HIV LTR transcription in vitro [11] and those shown in mammalian cells using chimeric Sp1 constructions [12] by demonstrating strong effects of exogenous Sp1 on HIV LTR activity in SL2 cells with HIV-2 > HIV-1. We confirm the report of Desai-Yajnik and Samuels [5] that the NF-κB and Sp1 wites in HIV-1 LTR function as TREs and indicate that comparable sites in the HIV-2 LTR also function as TREs. Moreover, hormone receptor effects on reporter gene constructions are paralleled by increased viral production in U937 cells.

The T_3R·RXR heterodimer has a higher affinity for the NF-κB_I site that contains two functional NF-κB elements compared to NF-κB_{II} that contains one functional and one nonfunctional NF-κB site. NF-κB sites of both HIV-1 and HIV-2 LTRs contain a 6-bp direct repeat TRE with an 8-bp spacing. There is no homology to a consensus PuGGTCA TRE site within the Sp1 sites, but the ability of this fragment to bind T_3R and T_3R·RXR indicates that T_3R has the potential to recognize a wide variety of DNA sequences [14, 34]. The paradox that RXR activated LTR activity but the purified protein failed to bind to the NF-κB and Sp1 motifs in EMSA suggests that RXR functioned through heterodimerization with other cellular transcription factors such as the endogenous *Drosophila* steroid/thyroid hormone-receptor superfamily members, e.g. *Ultraspiracle* or the ecdysone receptor in SL2 cells [35] or with RAR or PPAR in U937 cells. Alternatively, it is possible that RXR bound to an RXR response element in the LTRs outside of the NF-κB or SP1 elements.

Our results with the retinoids are in agreement with a previous study which showed that retinoids increased HIV replication in monocytes as measured by an in-

creased amount of HIV mRNA and virus production [30]. Studies in human teratocarcinoma and myeloid cell lines treated with retinoids have shown similar results in transient assays [19]. Other studies have reported that retinoic acid exerts effects on HIV expression in monocytic cells similar to the endogenous cytokine TGF-β [28]. The present studies provide evidence that T_3R and RXR bind Sp1 and NF-κB sites of the HIV-1 and HIV-2 through the formation of heterodimers and that introduction of the hormone receptors into U937 cells conferred responsiveness to ligand stimulation of virus production. Maciaszek et al. [18] found synergistic activation of the LTRs of SIV_{mac} and HIV-1 by RA and phorbol esters. Synergism was independent of a putative RA response element located 5′ to the NF-κB and Sp1 sites in the SIV LTR. Activation was markedly decreased but not abolished by deletion of the NF-κB sites, suggesting that indirect effects of RA may also occur. It is difficult to assess the importance of hormone stimulation on HIV replication and pathogenesis in vivo as this will depend on the relative concentrations of receptors and ligands within different cells. However, these results indicate that in model systems the T_3R and RXR response elements in both HIV-1 and HIV-2 have the capacity to influence transcription and viral replication.

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