Chicken Ovalbumin Upstream Promoter Transcription Factor Binds to a Negative Regulatory Region in the Human Immunodeficiency Virus Type 1 Long Terminal Repeat

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The human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) contains a negative regulatory element (NRE) which downregulates the rate of LTR-directed transcription and HIV-1 replication. Within the NRE is a GGTC A palindrome, which binds a possible member of the steroid/thyroid hormone receptor superfamily. Mutation of this site leads to an increase in LTR-directed transcriptional activity compared with the wild type, consistent with this element's being a functional part of the NRE. The palindrome contains significant identity to the chicken ovalbumin upstream promoter (COUP) element to which COUP transcription factors (COUP-TFs), members of the steroid/thyroid hormone receptor superfamily, bind. We demonstrate here that human COUP-TFs can bind specifically to this HIV-1 COUP-like element in a manner identical to binding to ovalbumin COUP. We show that the predominant COUP-TF family member synthesized in T cells is the 68-kDa form, which is likely to be responsible for any *in vivo* function of the HIV-1 COUP-like element in these cells. Finally, we have identified three HIV-1 variant strains that contain mutations in the HIV-1 COUP-like element which affect the binding affinity of COUP-TF for these variant COUP elements.

The rate of replication of the human immunodeficiency virus type 1 (HIV-1) is governed in large part by the level of expression from the HIV-1 5' long terminal repeat (LTR). The rate of transcription from this promoter is dependent upon a number of cellular and viral parameters mediated by factors encoded by the virus and the host cell (3). These factors include Tat (2, 38), Rev (6, 37), and Nef (21, 41) encoded by HIV-1 and TFIID (8), Spl (15), and NF- κ B (24) present in uninfected cells, in addition to other less well characterized factors. These host transcription factors interact with a number of discrete response elements within the HIV-1 LTR promoter to increase the level of transcription in response to a number of cellular signals induced by antigens, lymphokines, and mitogens, such as phorbol esters (1). In addition to these positive response elements, the sequence between -420 and -154 of the HIV-1 LTR has been shown to contain a functional negative response element (NRE) (28, 35). Deletion of this sequence leads to a threefold increase in HIV-1 LTR-driven chloramphenicol acetyltransferase (CAT) activity in transient transfections and a corresponding increase in HIV-1 replication (18). The precise role of the NRE in HIV-1 replication has not been clearly defined. A number of factors, SP-50 (36), USF (9), NFAT-1 (34), and AP-1 (7), have been shown to bind to sequences within this general region (Fig. 1); the function of these interactions remains unclear. Recent evidence has shown that an additional factor binds within an AP-1 site between -356 and -323 (25). This factor was identified as a potential member of the steroid/thyroid hormone receptor superfamily on the basis that the protein-DNA complex formed in a gel mobility shift assay could be inhibited weakly with a large molar excess of oligonucleotides containing estrogen response elements (EREs) or thyroid response elements (TREs) (25). Mutation of sequences important to the binding of this factor led to an increase in LTR-directed CAT activity relative to the wild-

type LTR (25). Thus, this DNA region is functionally important and contributes to the action of the HIV-1 NRE.

Inspection of this element revealed a palindrome containing a chicken ovalbumin upstream promoter (COUP)-like sequence (Fig. 1). COUP transcription factors (COUP-TFs) bind to the COUP element within the ovalbumin promoter and stimulate the initiation of transcription *in vitro* in conjunction with a second protein (S300-II) (29, 42, 42a, 44). COUP-TF exists in at least three M_r forms, 46,000, 47,000, and 68,000. Low- M_r COUP-TFs (46,000 and 47,000) have been cloned and shown to be members of the steroid/thyroid hormone receptor superfamily (16, 27, 43). The GGTC A half-site has been proposed as the COUP-TF recognition sequence, based on the comparison of a number of binding sites within several promoters (unpublished data). COUP-TF binds to an imperfect direct repeat of this sequence in the ovalbumin promoter (Fig. 1). The HIV-1 LTR COUP-like element is an imperfect palindrome that has nine nucleotides in common with the ovalbumin COUP element. With the knowledge that many steroid receptors have both positive and negative regulatory functions (5, 10, 30), we set out to determine whether COUP-TF binds to this functional sequence within the HIV-1 NRE.

MATERIALS AND METHODS

DNA probes. A *Sall*-*AvaI* restriction fragment (-453 to -153 HIV-1 LTR plus 20 bp of vector), spanning the entire NRE, was used for gel mobility shift assays, DNase I footprinting, and methylation interference. It was end labeled on the bottom strand at the *Sall* site with [α - 32 P]dTTP and on the top strand at the *AvaI* site with [α - 32 P]dCTP by using Klenow enzyme (Boehringer Mannheim Biochemicals) (31). The following specific double-stranded oligonucleotides were formed by annealing the appropriate chemically synthesized oligonucleotides: COUP, oligonucleotides spanning -92 to -70 of the ovalbumin promoter (44), 5'-TTCTAT GGTGTCAAAGGTCAAAC and 5'-AGAAGTTTGACCTT

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FIG. 1. Map of the HIV-1 LTR NRE, identifying the positions of the binding sites for AP-1 (7), NFAT-1 (34), USF (9), SP-50 (36), and COUP-TF and comparing the chicken ovalbumin COUP element and the HIV-1 LTR NRE COUP-like element. The solid bars indicate a region of 9 bp of identity between the ovalbumin COUP and the HIV-1 LTR NRE COUP-like sequences. The arrows indicate the positions and directions of perfect and imperfect GGTC half-sites.

TGACACCAT; mutated COUP (mCOUP), oligonucleotide containing a G to C change (13), 5'-TATGGTGTCAAAGC TCAAATTCT and 5'-AGAAGTTTGAGCTTTGACACCA; and AP-1 oligonucleotide, based on the collagenase promoter (17), 5'-GATCTAAGCATGAGTCAGACAG and 5'-GATCCTCTGTCTGACTCATGCTTA. An oligonucleotide based on the progesterone receptor element (PRE) of the tyrosine aminotransferase gene (40), 5'-GATCCTGTACAG GATGTTCTAGCTACG and 5'-GATCCGTAGCTAGAATCCTGTACA; HIV-1 variant A COUP element oligonucleotides 5'-GATCCCAGGGGTCAGATATCCACTGACCTTTGG and 5'-GATCCCAAAGGTCAGTGGATATCTGACCCCTGG; HIV-1 variant B COUP element oligonucleotides 5'-GATCCCAGGAGTCAGATATCCACTGACCTTTGG and 5'-GATCCCAAAGGTCAGTGGATATCTGACTCTGG; and HIV-1 variant C COUP element oligonucleotides 5'-GATCCCAGGGATCAGATATCCACTGACCTTTGG and 5'-GATCCCAAAGGTCAGTGGATATCTGATCCCTGG. Oligonucleotides used for probes in gel mobility shift assays were end labeled and blunt ended with Klenow enzyme and the appropriate radionucleotide.

Gel mobility shift assays. The gel mobility shift assays were performed as described previously (29) except poly(dI-dC) · poly(dI-dC) (Pharmacia) was used as a nonspecific competitor instead of pBR322/HinfI. HeLa and Jurkat nuclear extracts and partially purified COUP-TF were prepared as described previously (29).

DNase I footprint analysis. The *SalI-AvaI* HIV-1 NRE fragment was used for the DNase I footprint analysis. Bovine serum albumin (BSA) or partially purified COUP-TF was incubated with the labeled DNA under the conditions employed for the gel mobility shift assays in the presence of 50 ng of poly(dI-dC) · poly(dI-dC) nonspecific competitor. Reaction mixes to which competitor oligonucleotides were added were incubated at room temperature for 5 min prior to the addition of probe. The reactions were treated with 0.1 μ g of DNase I for 1 min at room temperature. The reaction mixes were analyzed on a 7% polyacrylamide sequencing gel.

Methylation interference analysis. The *SalI-AvaI* NRE restriction fragment was partially methylated as described previously (29) with dimethyl sulfate for 6 min at room temperature. A preparative gel mobility shift assay, employing conditions similar to those outlined above but scaled up fivefold, was performed to isolate the C_1 and C_2 complexes. The DNA was recovered, purified, cleaved under alkaline conditions, and analyzed on a 7% polyacrylamide sequencing gel.

FIG. 2. Gel mobility shift analysis of the *SalI-AvaI* NRE restriction fragment with partially purified COUP-TF, prepared as described previously (29). Lane 1, No protein; lanes 2 through 8, 0.4 μ g of partially purified COUP-TF. The competitor oligonucleotides at a 10- or 50-fold molar excess were incubated with protein for 5 min at room temperature prior to the addition of probe as indicated. Each reaction mix contained 2 μ g of nonspecific competitor. F, Free probe.

Western immunoblot analysis. Jurkat and HeLa nuclear extracts (150 μ g) were separated by sodium dodecyl sulfate-10% (wt/vol) polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to an Immobilon polyvinylidene difluoride membrane (Millipore Corp., Bedford, Mass.). After blocking with 3% nonfat milk-50 mM Tris-HCl [pH 7.5]-150 mM NaCl, the membrane was incubated with a 500-fold dilution of COUP-TF antiserum in the blocking solution (43). 125 I-labeled protein A (ICN, Irvine, Calif.) (1.25 μ Ci) was used to visualize the signal. The membrane was washed in 50 mM Tris-HCl (pH 7.5)-150 mM NaCl-0.05% Tween 20 for 60 min with five changes of buffer.

RESULTS

COUP-TF binds to the NRE region of the HIV-1 LTR. To investigate the possible interaction of authentic COUP-TF with the HIV-1 LTR NRE, we employed gel mobility shift assays, DNase I protection, and methylation interference studies. The probe used for this purpose was the *SalI-AvaI* restriction fragment spanning the entire HIV-1 NRE. Our first step was to determine if indeed COUP-TF had the ability to bind to the HIV-1 NRE. It is evident from the gel mobility shift assay (Fig. 2) that upon addition of partially purified COUP-TF (lane 2), characteristic retarded C_1 and C_2 complexes (43) were formed on the HIV-1 NRE probe. The C_1 complexes were formed by the binding of low- M_r COUP-TFs of 46,000 and 47,000, and the C_2 complex was formed by the binding of a high- M_r COUP-TF of 68,000 (43). Low- M_r COUP-TFs were the predominant form in this preparation. Both complexes were eliminated upon the addition of 10- and 50-fold molar excesses of unlabeled ovalbumin COUP oligonucleotide competitor (lanes 3 and 4), but not with a mutated COUP oligonucleotide (mCOUP) having a point mutation that destroys COUP-TF binding (lanes 5 and 6). In addition, AP-1 oligonucleotides also did not compete (lanes 7 and 8). Furthermore, addition of COUP-TF-specific antiserum to the gel mobility shift assay further decreased the mobility of the C_1 and C_2 complexes (see Fig. 4B). Thus, the protein-DNA complexes C_1 and C_2 were specific for and dependent upon a COUP element within the HIV-1 NRE. Lack of competition by the AP-1 oligonucleotide indicated that these complexes were not due to contaminating AP-1 in

the protein preparations, since Franza et al. (7) have shown an interaction between AP-1 and this region.

DNase I footprinting and methylation interference analyses were used to locate the site of interaction of COUP-TF within the NRE. With partially purified COUP-TF in DNase I protection studies on the top strand of the NRE, a number of footprints of various strengths were identified (Fig. 3A, lanes 2 and 3); all footprints were eliminated upon preincubation with excess unlabeled COUP oligonucleotide competitor (lanes 4 and 5) but not with the mCOUP oligonucleotide (lanes 6 and 7). The strongest footprint (FP1) spanned the 3' GGTCA half-site of the imperfect palindrome and extended weakly over the 5' AGTCA half-site. FP2 and FP3 may be the result of weak binding of COUP-TF to sequences which have slight homology to COUP sequences.

Methylation interference of COUP-TF binding to the HIV-1 NRE (Fig. 3B) was only observed in the region encompassed by the DNase I footprint FP1. There was strong methylation interference (compare lanes 2 and 3 with lanes 4 and 5) on the G residues at positions -329 and -330 of the 3' GGTCA for both the C₁ and C₂ complexes on the bottom strand. There was also interference of binding at the G residue at position -344 of the 5' AGTCA motif, though it was somewhat weaker. Weak methylation interference was evident at the G residue at position -332 on the top strand, and there was no interference by methylation of the AG dinucleotide of the upstream AGTCA sequence (data not shown). This methylation interference pattern, summarized in Fig. 3C, is characteristic of an interaction of COUP-TF with a GGTCA motif (44). It would appear that COUP-TF binds with higher affinity to the 3' GGTCA half-site of the palindrome than the 5' AGTCA half-site (Fig. 3C). It has been demonstrated previously (13) that the GG dinucleotide of the GGTCA half-site is absolutely required for the binding of COUP-TF, as witnessed by the inability of mCOUP oligonucleotide to eliminate the formation of C₁ or C₂ in gel mobility shift assays (Fig. 2) or in DNase I footprints (Fig. 3A). Thus, transition of the G to an A in the upstream half-site of the palindrome would in theory and did in practice reduce the binding of COUP-TF at this site. These experiments show that COUP-TF binds to the functionally important palindrome within the NRE defined by Orchard et al. (25).

Sequence polymorphisms of HIV-1 COUP-like elements and binding to COUP-TFs. Comparison of our DNase I footprint and methylation interference data (variant B) with those obtained by Orchard et al. (25) (variant A) revealed some interesting differences (Fig. 3C). They used an LTR derived from the HIV-1 strain HXB2 (39). They detected stronger binding of the factor with both halves of the palindrome, and this was confirmed by methylation interference at both GGTCA half-sites on both strands and by DNase I footprinting (Fig. 3C). This pattern is, however, consistent with a COUP-TF-COUP element interaction at this site. Comparison of the sequences of both probes in this region revealed a polymorphism in the upstream portion of the palindrome, an A to G transition at position -347 (Fig. 3C). This polymorphism yielded a perfect GGTCA palindrome in their sequence. The base transition, occurring in the GG dinucleotide of the GGTCA half-site, which has been shown to be essential for binding of COUP-TF, could account for the differences in the observations of the two groups.

This observation raised some intriguing questions and possibilities. A search of the GenBank data base revealed a number of HIV-1 strains (BH10 [26], MNCG [12], NL43 [1], PV22 [23], and SF2CG [32]) with an additional mutation in

FIG. 3. Binding of partially purified COUP-TF to the HIV-1 NRE. (A) DNase I footprinting of the *SalI*-*AvaI* restriction fragment of the HIV-1 LTR NRE with COUP-TF. Lane 1, BSA control; lane 2, 1 μ g of partially purified COUP-TF; lanes 3 through 7, 2 μ g of partially purified COUP-TF; lanes 4 and 5, COUP oligonucleotide competitor; lanes 6 and 7, mCOUP oligonucleotide. Competitors were incubated for 5 min at room temperature in a 10- and 50-fold molar excess, as indicated. (B) Methylation interference analysis of COUP-TF binding to the *SalI*-*AvaI* HIV-1 LTR restriction fragment. Lane 1, G and A Maxam and Gilbert sequencing ladder; lane 2, G Maxam and Gilbert sequencing ladder; lane 3, no protein; lane 4, C₁ complex (low-M_r COUP-TF); lane 5, C₂ complex (high-M_r COUP-TF). (C) Diagrammatic summary of the DNase I footprinting and methylation interference data from our experiments with variant B and those of Orchard et al. with variant A (25) and the sequence polymorphisms in the region of the COUP element within the NRE of three variant HIV-1 strains. The brackets indicate regions of strong DNase I protection; dashed brackets indicate regions of weak protection. Asterisks represent points of strong methylation interference, and plus signs represent points of weak methylation interference.

FIG. 4. (A) Comparison of COUP-TF binding in HeLa nuclear extracts to COUP and HIV-1 variant COUP oligonucleotide probes by gel mobility shift analysis. HeLa nuclear extracts (N.E.) (2 μ g) were incubated with a 50-fold molar excess of either the COUP or PRE oligonucleotide as indicated before addition of the appropriate probes in the presence of 4 μ g of nonspecific competitor. F, Free probe. (B) Analysis of binding to COUP and HIV-1 oligonucleotide probes with HeLa nuclear extracts with COUP-TF antiserum (Ab.) in a gel mobility shift assay. COUP-TF antiserum (0.2 μ l) (43) was incubated with 2 μ g of HeLa nuclear extracts for 15 min on ice prior to the addition of the indicated probes. Unmarked arrow indicates the position of the antibody-COUP-TF-probe complex.

the upstream GGTCA half-site, another G to A transition at G -346 (Fig. 3C). Thus, to date three different HIV-1 variants with respect to the COUP element have been identified, which we refer to as variants A to C (Fig. 3C). The base variations are clustered in the GG dinucleotide of the upstream COUP GGTCA half-site. We predict that COUP would bind with lower affinity to the GATCA variant (variant C) than the GGTCA variant (variant A) (25), similar to what was seen for the AGTCA variant (variant B) which we have used.

To determine the differences in binding of COUP-TFs for each of the HIV-1 variant NRE COUP elements, we synthesized oligonucleotides corresponding to each sequence for analysis in gel mobility shift assays. These assays were initially performed with HeLa nuclear extract, a known source of both low- and high- M_r COUP-TFs. Elimination of the mobility shift complexes (Fig. 4A, lanes 2 and 6) with

unlabeled COUP oligonucleotide competitor (lanes 3 and 7) identified COUP-specific complexes formed with both the ovalbumin COUP and HIV-1 variant A probes. C_1 and C_2 were the major retarded complexes, with a minor intermediate complex detectable. The difference in migration of the retarded complexes formed with the two probes was due to differences in the sizes of the probes. The complexes formed with the variants B and C (lanes 10 and 14) probes were somewhat different. There was decreased formation of the C_1 and C_2 complexes, and a portion of the " C_2 " complex on these probes was considered nonspecific because it was not eliminated with excess COUP oligonucleotide competitor (lanes 11 and 15).

COUP-TF antibody analysis of the complexes formed with these probes further highlighted their differences. COUP-TF antibodies decreased the mobility of all three complexes on the COUP and variant A probes (Fig. 4B, lanes 3 and 6), but only decreased the mobility of the C_1 and the weak intermediate complexes on the variant B and C probes (lanes 9 and 12). The mobility of only a small portion of the upper band was decreased; this indicated that although this protein-DNA complex migrated with similar mobility to the C_2 complex, the majority of the complex was not high- M_r COUP-TF. Thus, in HeLa nuclear extracts, the binding of both low- and high- M_r COUP-TFs to the COUP and variant A probes was strong and of identical specificity. However, low- M_r COUP-TF (i.e., C_1) bound weakly, by comparison, to the variant B and C probes, while high- M_r COUP-TF (C_2) bound even more weakly under those conditions.

Comparison of COUP-TF binding in HeLa and Jurkat cells.

A direct comparison of the binding of COUP-TF species in HeLa and human T cells (Jurkat cell line) to the COUP and variant A probes in gel mobility shift assays (Fig. 5A) was performed to determine whether these interactions between COUP-TF species and the HIV-1 NRE could be physiologically significant in T cells. Figures 4A and 5A show the formation of both C_1 and C_2 COUP-specific complexes with both probes with HeLa nuclear extracts (Fig. 5A, lanes 2 to 4 and 9 to 11). In contrast to complex formation in HeLa nuclear extracts, the major COUP-specific complex formed on both probes in Jurkat nuclear extracts was C_2 (compare lanes 5 and 6 with lanes 12 and 13). To confirm that high- M_r COUP-TF was the major species which bound to the variant A COUP element was analyzed with COUP-TF antibody (Fig. 5B). The mobility of the C_2 complex formed with HeLa and Jurkat nuclear extracts (lanes 2 and 4) was decreased to the same extent in both cases after incubation with COUP-TF antibody (lanes 3 and 5). Thus, high- M_r COUP-TF was the major species which bound to the variant A COUP element in T cells. These results agreed with the gel mobility shift data of Orchard et al. (25), who also found only a single major complex formed on a similar probe with Jurkat nuclear extract.

The presence of very low amounts of C_1 suggested that low- M_r COUP-TF was expressed at very low levels in T cells. To confirm this, we subjected HeLa and Jurkat nuclear extracts to Western analysis with specific COUP-TF antiserum (Fig. 5C). Both low- M_r (46,000 and 47,000) and high- M_r (68,000) COUP-TF species were detected in HeLa cells (lane 1). In Jurkat cells, the major COUP-TF present was the large species, while only extremely low levels of the low- M_r forms were detected (lane 2). High- M_r COUP-TF was present at a level similar to that in HeLa cells. It was evident from these experiments that high- M_r COUP-TF was the predominant form in T cells.

FIG. 5. (A) Gel mobility shift analysis of HeLa and Jurkat cell COUP-TF binding to COUP and HIV-1 variant A oligonucleotide probes. HeLa or Jurkat nuclear extracts (N.E.) (2 μ g) were incubated as described in the legend to Fig. 4A prior to the addition of the indicated probes with competitor oligonucleotides. F, Free probe. (B) Analysis of binding of variant A oligonucleotide probe with HeLa and Jurkat nuclear extracts with COUP-TF antiserum (Ab) in a gel mobility shift assay. COUP-TF antiserum was incubated for 15 min at room temperature with the nuclear extracts prior to addition of the probe. (C) Western analysis of HeLa and Jurkat nuclear extracts with COUP-TF antiserum. Lane 1, HeLa; lane 2, Jurkat. Positions of high-M_r and low-M_r species are indicated. Unmarked arrows indicate the positions of the antibody-COUP-TF-probe complexes.

COUP-TF binds with different affinities to the variant HIV-1 COUP elements. To examine the binding of high-M_r COUP-TF to the different HIV-1 NRE COUP variants in more detail, we used the variant COUP probes A to C in a gel mobility shift assay with Jurkat nuclear extract (Fig. 6A). High-M_r COUP-TF formed a strong COUP-specific C₂ complex with the variant A probe (lanes 6 and 7), which was comparable to that observed with the COUP probe (lanes 2 and 3). In contrast, there was very little mobility shift of the variant B and C probes (lanes 10 and 14). The small amount of complex formed which migrated at the C₂ position was composed of an unknown and nonspecific protein-DNA complex, as was observed in the COUP-TF antibody analysis with HeLa cell extracts (Fig. 4B, lanes 9 and 12). Thus, it would appear that high-M_r COUP-TF also had a higher affinity for the variant A sequence than for the B and C sequence variants.

To further investigate the relative affinity of high-M_r COUP-TF for each of the variant sequences, we used increasing amounts of unlabeled oligonucleotides containing the three different sequences as competitors in a gel mobility shift assay, with variant A as the probe (Fig. 6B). COUP-TF clearly had a greater affinity for the variant A oligonucleotide (lanes 3 to 6) than either of the other variants. Competition with variant A eliminated the C₂ complex at a fivefold molar excess, while the amounts of the variant B (lanes 7 to 10) and variant C (lanes 11 to 14) oligonucleotides had to be increased to at least a 100-fold molar excess to achieve a similar level of competition. Thus, there is at least a 20-fold difference in the binding affinity of high-M_r COUP-TF for the variant A COUP element than for the other variants.

DISCUSSION

Orchard et al. have shown a strong correlation between the binding of a potential member of the steroid receptor superfamily to the ERE/TRE-like element in the HIV-1 LTR and the functional activity of the HIV-1 NRE (25). Linker

mutation of this element led to an increase in LTR-directed CAT activity compared with the wild-type LTR, showing that it is a functional part of the NRE. We have shown, by COUP oligonucleotide competition in gel mobility shift assays, that this ERE/TRE-like element is in fact a COUP element. Analysis of these retarded complexes with antiserum specific to COUP-TF, estrogen receptor (ER) and thyroid receptor (TR) (ER and TR data not shown) showed that the factor binding to this element is COUP-TF, a member of the steroid/thyroid hormone receptor superfamily and not either ER or TR. COUP-TFs bind in an identical fashion to the variant A HIV-1 COUP element and to the ovalbumin COUP element. However, in T cells the low-M_r forms of COUP-TF are present in very low amounts, and thus the high-M_r COUP-TF is likely to be the physiologically important COUP-TF species. The binding of high-M_r COUP-TF to the HIV-1 NRE COUP element in Jurkat cells is likely to be an important step in the negative functional activity of this element. The vanishingly small amounts of low-M_r COUP-TF in T cells is interesting in that this factor has been detected in all tissues examined to date (22). Alternatively, one might raise a question as to the biologic relevance of the vanishingly low concentration of low-M_r COUP-TF in T cells.

These results also raise the question of how a transcription factor such as COUP-TF binds to an element in a promoter in one cell type and behaves as a positive regulator, while binding of the same factor to another promoter in a different cell type results in negative behavior. Notably, this phenomenon has been observed with many members of the steroid/thyroid hormone receptor superfamily (5, 10; reference 30 and references therein). There may be a number of possible mechanisms leading to the negative effect of COUP-TF on the HIV-1 LTR. The NRE COUP element itself may determine the negative effect. The opposite orientation and increased spacing of the GGTC motifs compared with the ovalbumin COUP element (Fig. 1) may alter the conformation of COUP-TF when it binds, masking an activation

FIG. 6. (A) Comparison of COUP-TF binding to COUP and HIV-1 variant oligonucleotide probes in Jurkat cells. Reaction mixes to which a 50-fold molar excess of either COUP or PRE competitor oligonucleotide was added were incubated with the Jurkat nuclear extract (N.E.) (2 μ g) for 5 min at room temperature prior to addition of probes. F, Free probe. (B) Comparison of the relative binding affinity of COUP-TF for the three HIV-1 COUP variant oligonucleotide probes A to C by gel mobility shift assay. Increasing molar excesses of unlabeled variant oligonucleotide competitors were incubated as described for Jurkat nuclear extract (2 μ g) prior to the addition of the variant A probe.

domain in the polypeptide. Alternatively, COUP-TF may interact with additional factors that bind to the LTR to achieve this negative effect. Such interactions could interfere with the interaction of COUP-TF with S300-II, thereby preventing its transformation to a positive regulator of transcription.

Recent publications have delineated the interaction of the AP-1 transcription factor and its components c-Jun and c-Fos with the glucocorticoid receptor. There is a mutual repression of transcriptional induction based on protein-protein interactions between these factors. The glucocorticoid receptor represses the AP-1 activation of certain target genes (e.g., collagenase gene) and vice versa (4, 14, 20, 33, 45). A similar interaction between COUP-TF and AP-1 could occur in T cells. This may account for the intriguing data (7) showing that AP-1 binds to the same HIV-1 NRE element as high- M_r COUP-TF, even though Orchard et al. (25) have found no evidence for a direct interaction between AP-1 and this element. However, the AP-1 purified by using the HIV-1

NRE COUP element and microscale DNA affinity precipitation may have been isolated on the basis of protein-protein interactions between AP-1 and COUP-TF bound to this element. Such interactions will be the subject of future investigation.

The identification of HIV-1 variants with sequence polymorphisms in the NRE COUP element has implications not only for the promoter. This region of the HIV-1 LTR has a dual function, as a promoter and part of the coding sequence for Nef (21, 41). However, these polymorphisms produce conservative amino acid changes in Nef. The 5' GGGTCA half-site of variant A and that of variant B encodes Gly and Val, whereas the variant C half-site encodes Gly and Ile. Thus, these nucleotide polymorphisms are probably more important to the HIV-1 LTR in its promoter function than in coding for Nef.

The results showing that COUP-TF binds with different affinities to the variant COUP sequences in different HIV-1 strains raises important possibilities. Differences in the affinity of COUP-TF for these sequences may lead to different levels of activity for these elements, with a consequent effect on the rate of transcription. It would be predicted that the HIV-1 COUP sequence to which COUP-TF binds with high affinity (variant A) would display a strong negative effect on the rate of transcription, while the sequences (variants B and C) to which COUP-TF binds with lower affinity would have a weak negative effect on transcription. These latter elements may in fact be nonfunctional, since the binding affinity of COUP-TF for them is 20-fold lower than for variant A. This may explain the recent data from Lu et al. (19), which conflicts with that of Orchard et al. (25) on the functional significance of this element. The LTR used by Lu et al. may contain a nonfunctional COUP element with the sequence of variant B or C, whereas the LTR used by Orchard et al. contains the variant A sequence.

Lu et al. have shown that increases in transcriptional rate due to deletion of the NRE are reflected in increases in replication rates of the whole virus (18). Thus, differences in binding affinity of COUP-TF to the variant NRE sequences may in turn lead to different rates of viral replication. These mutations in the HIV-1 COUP element may represent an evolutionary step whereby the HIV-1 variants B and C may have escaped some or all of the negative effect of the NRE, allowing them to replicate faster. Alternatively, variant A could have acquired a positive mutation which increases the activity of the NRE, making replication slower and the virus less lethal. However, this is a simplification of the potential effect of COUP-TF binding on viral replication, the rate of which is dependent on many host and virus parameters. It is generally considered that HXB2 is one of the faster-replicating HIV-1 strains. Thus, in addition to the potential negative effect of COUP-TF, there may be additional mutations which are dominant and increase the rate of replication. Golub et al. have shown that of two HIV-1 variants isolated from one patient, the one that exhibits the highest level of LTR-directed transcriptional activity had the slower rate of infection and replication kinetics due to a defective *vif* gene (11). In this highly polymorphic retrovirus, the rate of replication will depend on the cumulative effect of mutations in several elements and genes which contribute to the overall rate. Thus, to understand and predict the rate of replication of different virus variants, we need to understand the interplay of all the host and viral factors involved and the effect of mutations during the viral life cycle.

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REFERENCES

- Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin. 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and non-human cells transfected with an infectious molecular clone. *J. Virol.* 59:284-291.
- Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal. 1985. *trans*-activator gene of human T-lymphocyte virus type III (HTLV-III). *Science* 229:69-73.
- Cullen, B. E., and W. C. Greene. 1989. Regulatory pathways governing HIV-1 replication. *Cell* 58:423-426.
- Diamond, M. I., J. N. Miner, S. K. Yoshinaga, and K. R. Yamamoto. 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science* 249:1266-1272.
- Drouin, J., M. A. Trifiro, R. K. Plante, M. Nemer, P. Ericksson, and O. Wrangé. 1989. Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of proopiomelanocortin gene transcription. *Mol. Cell. Biol.* 9:5305-5314.
- Feinberg, M. B., R. F. Jarrett, A. Aldovini, R. C. Gallo, and F. Wong-Staal. 1986. HTLV-III expression and production involve complex regulation at the level of splicing and translation of viral RNA. *Cell* 46:807-817.
- Franza, B. R., Jr., F. J. Rauscher III, S. F. Josephs, and T. Curran. 1988. The Fos complex and Fos-related antigens recognize sequence elements that contain AP-1 binding sites. *Science* 239:1150-1153.
- Garcia, J. A., D. Harrich, E. Doultsanakis, F. Wu, R. Mitsuyasu, and R. B. Gaynor. 1989. Human immunodeficiency virus type 1 LTR TATA and TAR region sequences required for transcriptional regulation. *EMBO J.* 8:765-778.
- Garcia, J. A., F. K. Wu, R. Mitsuyasu, and R. B. Gaynor. 1987. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. *EMBO J.* 6:3761-3770.
- Glass, C. K., J. M. Holloway, O. V. Devay, and M. G. Rosenfeld. 1988. The thyroid hormone receptor binds with opposite transcription effects to a common sequence motif in thyroid hormone and estrogen response elements. *Cell* 54:313-323.
- Golub, E. I., G. Li, and D. J. Volsky. 1990. Differences in the basal activity of the long terminal repeat determine different replicative capacities of two closely related human immunodeficiency virus type 1 isolates. *J. Virol.* 64:3654-3660.
- Gurgo, C., H.-G. Guo, G. Franchini, A. Aldovini, E. Collalti, K. Farrell, F. Wong-Staal, R. C. Gallo, and M. S. Reitz, Jr. 1988. Envelope sequences of two new United States HIV-1 isolates. *Virology* 164:531-536.
- Hwung, Y.-P., D. T. Crowe, L.-H. Wang, S. Y. Tsai, and M.-J. Tsai. 1988. The COUP transcription factor binds to an upstream promoter element of the rat insulin II gene. *Mol. Cell. Biol.* 8:2070-2077.
- Jonat, C., H. J. Rahmsdorf, K.-K. Park, A. C. B. Cato, S. Gebel, H. Ponta, and P. Herrlich. 1990. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 62:1189-1204.
- Jones, K. A., J. T. Kodonaga, P. A. Luciw, and R. Tjian. 1986. Activation of AIDS retrovirus promoter by the cellular transcription factor Sp1. *Science* 232:755-759.
- Ladiaz, J. A. A., and S. K. Karathanasis. 1991. Regulation of the apolipoprotein AI gene by ARP-1, a novel member of the steroid receptor superfamily. *Science* 251:561-565.
- Lee, W., P. Mitchell, and R. Tjian. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell* 49:741-752.
- Lu, Y., M. Stenzel, J. G. Sodroski, and W. A. Haseltine. 1989. Effects of long terminal repeat mutations on human immunodeficiency virus type 1 replication. *J. Virol.* 63:4115-4119.
- Lu, Y., N. Touzjian, M. Stenzel, T. Dorfman, J. G. Sodroski, and W. A. Haseltine. 1990. Identification of *cis*-acting repressive sequences within the negative regulatory element of human immunodeficiency virus type 1. *J. Virol.* 64:5226-5229.
- Lucibello, F. C., E. P. Slater, K. U. Jooss, M. Beato, and R. Muller. 1990. Mutual transrepression of Fos and the glucocorticoid receptor: involvement of a functional domain in Fos which is absent in Fos B. *EMBO J.* 9:2827-2834.
- Luciw, P. A., C. Chang-Mayer, and J. A. Levy. 1987. Mutational analysis of the human immunodeficiency virus: the *orf*-B region down-regulates virus replication. *Proc. Natl. Acad. Sci. USA* 84:1434-1438.
- Miyajima, N., Y. Kadowaki, S.-I. Fukushige, S. I. Shimizu, K. Semba, Y. Yamanashi, K.-I. Matsubara, K. Toyoshima, and T. Yamamoto. 1988. Identification of two novel members of *erbA* superfamily by molecular cloning: the gene products of the two are highly related to each other. *Nucleic Acids Res.* 16:11057-11074.
- Muesing, M. A., D. H. Smith, C. D. Cabradilla, C. V. Benton, L. A. Lasky, and D. J. Capon. 1985. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. *Nature (London)* 313:450-458.
- Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* 326:711-713.
- Orchard, K., N. Perkins, C. Chapman, J. Harris, V. Emery, G. Goodwin, D. Latchman, and M. Collins. 1990. A novel T-cell protein which recognizes a palindromic sequence in the negative regulatory element of the human immunodeficiency virus long terminal repeat. *J. Virol.* 64:3234-3289.
- Ratner, L., A. Fisher, L. L. Jagodzinski, H. Mitsuya, R.-S. Liou, R. C. Gallo, and F. Wong-Staal. 1987. Complete nucleotide sequences of functional clones of the AIDS virus. *AIDS Res. Hum. Retroviruses* 3:57-69.
- Ritchie, H. H., L.-H. Wang, S. Y. Tsai, B. W. O'Malley, and M.-J. Tsai. 1990. COUP-TF gene: a structure unique for the steroid/thyroid receptor superfamily. *Nucleic Acids Res.* 18:6857-6862.
- Rosen, C. A., J. G. Sodroski, and W. A. Haseltine. 1985. The location of *cis*-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. *Cell* 41:813-823.
- Sagami, I., S. Y. Tsai, H. Wang, M.-J. Tsai, and B. W. O'Malley. 1986. Identification of two factors required for transcription of the ovalbumin gene. *Mol. Cell. Biol.* 6:4259-4267.
- Sakai, D. D., S. Helms, J. Carlstedt-Duke, J.-A. Gustafsson, F. M. Rottman, and K. R. Yamamoto. 1988. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. *Genes Dev.* 2:1144-1154.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw. 1985. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). *Science* 227:484-492.
- Schule, R., P. Rangaran, S. Kliever, L. J. Ransone, J. Bolado, N. Yang, I. Verma, and R. M. Evans. 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 62:1217-1226.
- Shaw, J.-P., P. J. Utz, D. B. Durand, J. J. Toole, E. A. Emmel, and G. R. Crabtree. 1988. Identification of a putative regulator of early T cell activation genes. *Science* 241:202-205.
- Siekevitz, M., S. F. Josephs, M. Dukovich, N. Peffer, F. Wong-Staal, and W. C. Greene. 1987. Activation of the HIV-1 LTR by T cell mitogens and the *trans*-activator protein of HTLV-1. *Science* 238:1575-1578.

36. Smith, M. R., and W. C. Greene. 1989. The same 50-kDa cellular protein binds to the negative regulatory elements of the interleukin 2 receptor alpha-chain gene and the human immunodeficiency virus type 1 long terminal repeat. *Proc. Natl. Acad. Sci. USA* **86**:8526–8530.
37. Sodroski, J., W. C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. A. Haseltine. 1986. A second post-translational *trans*-activator gene required for HTLV-III replication. *Nature (London)* **321**:412–417.
38. Sodroski, J., R. Patarca, C. Rosen, F. Wong-Staal, and W. Haseltine. 1985. Location of the *trans*-activating region on the genome of human T-cell lymphotropic virus type III. *Science* **229**:74–77.
39. Starcich, B., L. Ratner, S. F. Josephs, T. Okamoto, R. C. Gallo, and F. Wong-Staal. 1985. Characterization of long terminal repeat sequences of HTLV-III. *Science* **227**:538–540.
40. Strahle, U., G. Klock, and G. Schutz. 1987. A DNA sequence of 15 base pairs is sufficient to mediate both glucocorticoid and progesterone induction of gene expression. *Proc. Natl. Acad. Sci. USA* **84**:7871–7875.
41. Terwilliger, E., J. G. Sodroski, C. A. Rosen, and W. A. Haseltine. 1986. Effects of mutations within the 3' *orf* open reading frame region of human T-cell lymphotropic virus type III (HTLV-III/LAV) on replication and cytopathogenicity. *J. Virol.* **60**:754–760.
42. Tsai, S. Y., I. Sagami, H. Wang, M.-J. Tsai, and B. W. O'Malley. 1987. Interactions between a DNA-binding transcription factor (COUP) and a non-DNA binding factor (S300-II). *Cell* **50**:701–709.
- 42a. Wang, L.-H., and N. Ing. Personal communication.
43. Wang, L.-H., S. Y. Tsai, R. G. Cook, W. G. Beattie, M.-J. Tsai, and B. W. O'Malley. 1989. COUP transcription factor is a member of the steroid receptor superfamily. *Nature (London)* **340**:163–166.
44. Wang, L.-H., S. Y. Tsai, I. Sagami, M.-J. Tsai, and B. W. O'Malley. 1987. Purification and characterization of chicken ovalbumin upstream promoter transcription factor from HeLa cells. *J. Biol. Chem.* **262**:16080–16086.
45. Yang-Yen, H.-S., J.-C. Chambard, Y.-L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin. 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* **62**:1205–1215.