

Transcription of the human immunodeficiency virus type 1 (HIV-1) promoter in central nervous system cells: effect of YB-1 on expression of the HIV-1 long terminal repeat

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Transcription of human immunodeficiency virus type 1 (HIV-1) is regulated by a series of host DNA-binding proteins which interact with the upstream viral promoter sequences, and the viral regulatory protein Tat which associates with TAR RNA sequences located in the leader of the viral transcript. Here we have examined the ability of the cellular protein YB-1 to modulate transcription of the HIV-1 promoter in a human astrocytic cell line (U-87MG), a neuronal cell line (SK-N-MC) and lymphoid cells (Jurkat) by transfection assay. Ectopic expression of YB-1 in U-87MG and SK-N-MC augments basal transcriptional activity of the viral sequence located between 80 and 43, which encompasses the GC-rich motif. In accord with the previous report, in Jurkat cells YB-1-mediated activation of the HIV-1 promoter required sequences which are located further upstream from the GC-rich motif. Combined overexpression of YB-1 and the GC-rich binding protein, SP1, in the transfected cells decreased the

level of activation of the viral promoter, suggesting that YB-1 and SP1 may exert negative effects on each other's function. Results from band shift assay with purified YB-1 and SP1 indicated that SP1 and YB-1 bind to the GC-rich DNA sequence in the double-stranded and single-stranded configurations, respectively. However, efficient binding of SP1 to the double-stranded GC-rich motif corresponding to the HIV-1 long terminal repeat (LTR) is diminished in the presence of YB-1. Similarly, in the presence of SP1, YB-1 loses its ability to become associated with its target single-stranded DNA probe. No evidence for direct association of YB-1 and SP1 either in the presence or in the absence of DNA was observed. These data suggest that while YB-1 stimulates expression of the LTR in central nervous system cells, the level of activity of other cellular proteins, such as SP1, may dictate binding of YB-1 to its target sequence, and therefore affect the regulatory function of this protein.

Introduction

Regulation of human immunodeficiency virus type 1 (HIV-1) gene transcription during the viral lytic cycle is mediated by multiple regulatory modules positioned at upstream and downstream regions with respect to the transcription start site within the long terminal repeat (LTR) (reviewed in Kingsman & Kingsman, 1996; Garcia & Gaynor, 1994). The upstream regulatory DNA modules exert their function by binding to the cellular DNA-binding proteins, facilitating interaction of the bound proteins with each other (Garcia *et al.*, 1987; Harrich *et al.*, 1989). Results from mutagenesis studies suggested that the two upstream regulatory elements, TATA box (nucleotides -28 to -24) and the GC-rich motif (nucleotides -77 to

-46), and the downstream responsive region TAR (nucleotides +3 to +59) are critical for activation of viral gene expression by Tat (Feng & Holland, 1988; Harrich *et al.*, 1996; Kingsman & Kingsman, 1996). Tat is a potent transcription transactivator which upon interaction with TAR and/or with transcription factors which are engaged with the LTR sequences stimulates transcription of the HIV-1 genome (reviewed in Gaynor, 1995).

Located immediately upstream of the GC-rich motif is the HIV-1 enhancer region which contains two binding sites for the transcription factor nuclear factor kappa B (NF- κ B) (Nabel & Baltimore, 1987; Roulson *et al.*, 1995). This enhancer functions as the final common target for a wide variety of stimuli including cytokines such as tumour necrosis factor- α (TNF- α) and mitogens which simultaneously activate T lymphocytes and up-regulate HIV-1 transcription (Poli & Fauci, 1992). The HIV-1 LTR also contains binding sites for

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numerous other cellular factors including YY1, TCF-1, HIP, LBP, HIV-1 TF1, USF, NFAT, c-Myb, AP-1 and COUP-TF (Gaynor, 1992; Liu & Latchman, 1997). In general, the role of these transcription factors in HIV-1 gene expression during lytic infection is not well established.

During the past 10 years our laboratory has been focused on understanding the regulatory mechanism which mediates regulation of HIV-1 gene transcription in the central nervous system (CNS). In one series of studies we demonstrated that a novel regulatory pathway operative in human glial cells facilitates transcriptional activation of the HIV-1 LTR by Tat in the absence of the TAR sequence (Taylor & Khalili, 1994; Taylor *et al.*, 1992a, 1993, 1994). Evidently, TAR-independent transcriptional activation of the LTR in glial cells is mediated through NF- κ B, as mutations within the κ B motif of Δ TAR LTR abrogated the ability of Tat to exert its activity.

The complexity of the viral regulatory region has led us to believe that in addition to the known regulatory proteins which recognize the viral DNA sequence, other cellular and viral proteins which may share a common target or interact with known regulatory proteins may be involved in determining the activity of HIV-1 LTR (Sawaya *et al.*, 1996; Wang *et al.*, 1995). Recently, we and others identified and molecularly cloned the gene for a DNA-binding protein which belongs to the YB-1 family (Didier *et al.*, 1988; Kerr & Khalili, 1991). YB-1 is a gene family initially isolated from a human B-cell expression library based on its ability to interact with a GT-rich sequence within the Y box regulatory motif of the class II major histocompatibility gene (Barberis *et al.*, 1987; Wolffe, 1994). The presence of a CCAAT motif within the Y box of the class II major histocompatibility complex gene and many other YB-1-responsive genes (Benoist & Mathis, 1990; Dorn *et al.*, 1987; Kashanchi *et al.*, 1994) led to speculation that the YB-1 family, like C/EBP and CTF/NF-1, belongs to a class of CCAAT box binding proteins. However, several later studies have reported virtually no association of C/EBP with the YB-1 protein (Cohen & Reynolds, 1991; Hasegawa *et al.*, 1991; Kolluri *et al.*, 1992; Yan & Tamm, 1991). The binding of a recombinant YB-1 protein to a CT-rich DNA sequence that can adopt an intramolecular triplex, single-stranded structure suggests that the topological configuration of the DNA molecule may play an important role in determining YB-1 binding to DNA (Kolluri *et al.*, 1992). The presence of multiple DNA elements resembling the YB-1 binding sequence within the HIV-1 LTR prompted us to determine transcriptional activity of the viral promoter in the presence of YB-1 protein.

Methods

Plasmids. The various promoter deletion reporter constructs were made using specific restriction enzymes, as described previously (Taylor *et al.*, 1992a; Sawaya *et al.*, 1996). Plasmid $-458/+80(\Delta-75/-40)$, kindly provided by S. Zeichner (NCI/NIH, Bethesda, Md., USA), was generated using LTR deletion constructs, $-75/-58$ and $-57/-40$ (Zeichner *et al.*, 1991). To generate the $-117/+80(\Delta$ GC-

rich region) LTR-chloramphenicol acetyltransferase (CAT) and the $-117/+3(\Delta$ GC-rich region) LTR-CAT, the plasmid $-458/+80(\Delta-75/-40)$ LTR-CAT was digested with *Bgl*II/*Taq*I, *Bgl*II/*Taq*I and *Mae*III/*Hind*III, respectively. The YB-1 expression plasmid driven by a cytomegalovirus (CMV) promoter has been described previously (Chen *et al.*, 1995). The SP1 expression vector driven by a CMV promoter was kindly provided by Robert J. Tjian (Howard Hughes Medical Institute, Univ. California, Berkeley, Calif., USA). The plasmid, pAct-Tat (Taylor *et al.*, 1992b) was used as a Tat expression vector.

Cells, tissue culture, transfection and CAT assay. Human astrocytic cells (U-87MG) and neuroblastoma-derived cells (SK-N-MC) were maintained in Dulbecco's minimal essential medium supplemented with 10% foetal calf serum (FCS, Gibco). The medium of the neuroblastoma SK-N-MC cells was supplemented with non-essential amino acids. Jurkat T cells were grown in RPMI 1640 with 10% FCS. U-87MG and SK-N-MC cells (5×10^5) were seeded on 60 mm plates and grown overnight prior to transfection by the calcium phosphate precipitation method (Graham & van der Eb, 1973). Jurkat cells were grown in Ti35 flasks and transfected by electroporation. Cells were transfected with 5 μ g of the reporter plasmid in the absence or presence of 5 μ g of the CMV expression plasmid (pCMV-YB-1, pCMV-SP1) or the empty CMV construct. Each transfection was performed in duplicate and repeated several times with at least two different plasmid preparations. Cell extracts were prepared 48 h after transfection, and CAT assays were performed essentially as described previously (Gorman *et al.*, 1982).

Preparation of bacterially expressed YB-1. Plasmid pGEX2T-YB-1 was kindly provided by G. MacDonald (MacDonald *et al.*, 1995). The procedure for preparing GST-fusion proteins was previously described (MacDonald *et al.*, 1995). Briefly, bacteria containing the expression plasmids were grown to an OD₆₀₀ of 0.6 and induced with 300 μ l IPTG (1 M) for 90 min at 37 °C. Protein extract was prepared and the fusion protein was purified with glutathione agarose beads and eluted with freshly made 50 mM Tris (pH 7.4) containing 15 mM glutathione. For long storage at -70 °C, glycerol was added to a 10% final concentration.

Electrophoretic mobility shift assays. Protein-DNA binding reactions were performed with 10 ng of SP1 protein (purchased from Santa Cruz Biotechnology) and 50 ng of GST or GST-YB-1 fusion proteins. The binding reaction contained 1 ng of ³²P-5'-end labelled oligonucleotide, 0.25 mg/ml poly(dI-dC), 50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 2.5 mM dithiothreitol, 2.5 mM EDTA, 5 mM MgCl₂, 20% glycerol. Reaction mixtures were incubated for 15 min at 4 °C and protein-DNA complexes were analysed by electrophoresis on a 6% polyacrylamide gel in 0.25 \times Tris-buffered EDTA.

Results and Discussion

Identification of YB-1 responsive element within the HIV-1 LTR

We examined the effect of YB-1 on transcription of the HIV-1 LTR promoter using a reporter plasmid expressing CAT under the direction of the full-length HIV-1 LTR promoter (LTR-CAT) ($-458/+80$), by co-transfection assay. In this study, the human astrocytic cell line (U-87MG), the human neuronal cell line (SK-N-MC) and the human T-cell line (Jurkat) were transfected with the reporter LTR-CAT alone or with a plasmid expressing YB-1 under the cytomegalovirus promoter

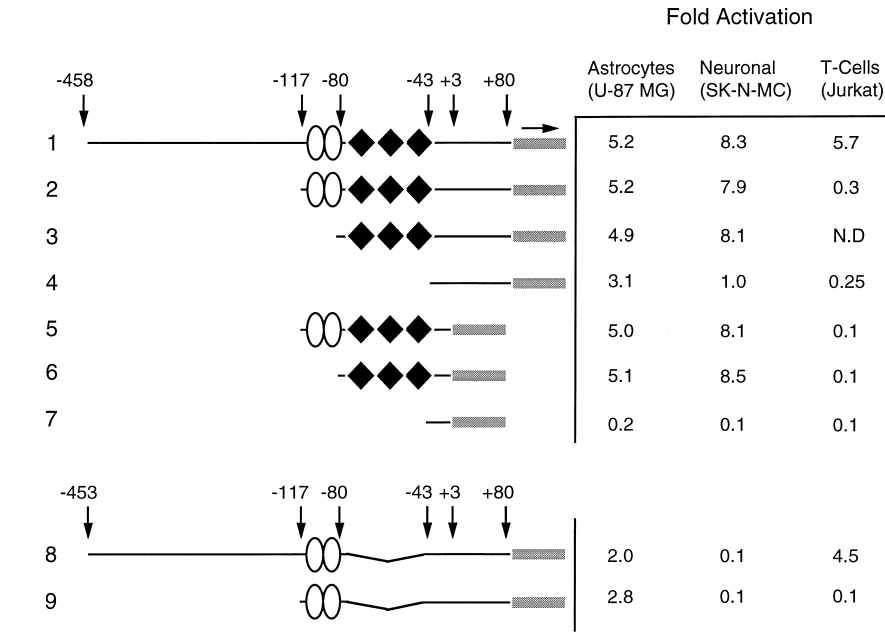


Fig. 1. YB-1 induces HIV-1 LTR transcriptional activity through GC-rich sequences. LTR CAT full-length promoter construct containing two NF- κ B binding sites between -117 and -80 and three GC-rich sequences between -80 and -43 are depicted (not shown to scale) encompassing sequences from -458 to $+80$ and the various mutant variants. Approximately $5 \mu\text{g}$ of the reporter CAT constructs alone or together with $5 \mu\text{g}$ of pCMV-YB-1 expression plasmid were introduced into astrocytic cells (U-87MG), neuronal cells (SK-N-MC) and T cells (Jurkat). The total amount of DNA present in each transfection was normalized using an empty pCMV plasmid. Each transfection was repeated at least three times and the values represent the average fold activation of LTR by YB-1 as determined by CAT assay (Gorman *et al.*, 1982). Average standard deviations for U-87MG, SK-N-MC and Jurkat cells were $\pm 15\%$, $\pm 20\%$ and $\pm 19\%$, respectively.

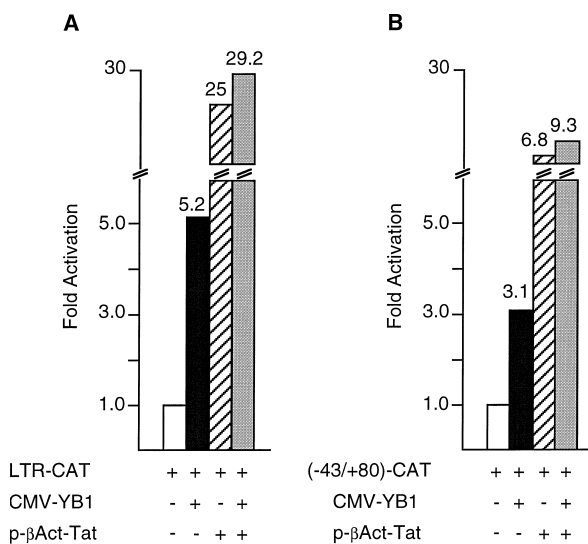


Fig. 2. Effect of YB-1 and Tat on transcriptional activity of HIV-1 LTR. Approximately $5 \mu\text{g}$ of the reporter construct containing the full-length LTR (LTR-CAT) or a region of the LTR encompassing sequences between -43 and $+80$ ($-43/+80$ -CAT) fused to the CAT gene were introduced into U-87MG cells alone or together with $5 \mu\text{g}$ of CMV-YB-1 and $5 \mu\text{g}$ of pAct-Tat. The final amount of DNA in each transfection mixture was kept constant by the addition of pCMV vector plasmid. Protein extracts were prepared from transfected cells at 48 h post-transfection and the levels of CAT activity were measured. Bar graphs represent average fold activation of five independent experiments for each transfection with a standard deviation of $\pm 15\%$.

(CMV-YB-1). We observed that ectopic expression of YB-1 enhances transcription from the LTR promoter in all three cell lines (Fig. 1). In previous studies it was shown that the nucleotide sequences 370 bases upstream of the transcription start site of the HIV-1 LTR are responsive to YB-1 in transiently transfected Jurkat cells (Kashanchi *et al.*, 1994). Therefore, in the next series of studies we utilized a series of 5' deletion

promoter constructs that remove sequences between -458 and -117 (pCD23; Taylor *et al.*, 1992a), -458 and -80 , and -458 and -43 to determine the ability of YB-1 in activating promoter mutant constructs in U-87MG, SK-N-MC and Jurkat cells. As shown in Fig. 1, removal of the sequences between -458 and -80 had no significant effect on the level of HIV-1 LTR activation by YB-1 in the two CNS-derived cell lines, i.e. U-87MG and SK-N-MC. It should be noted that the removal of κ B had no drastic effect on the absolute transcription of the LTR promoter construct (data not shown). This is consistent with recent reports indicating that under certain conditions κ B sequences may be dispensable for viral transcription/replication (Laughlin *et al.*, 1995; Valerie *et al.*, 1995; Zhang *et al.*, 1997). In contrast to these observations, the level of transactivation by YB-1 was drastically decreased in the transfected Jurkat cells upon removal of the sequences between -458 and -117 . This result is consistent with the previous report suggesting that the distal regulatory sequences are important to confer YB-1 responsiveness of the HIV-1-LTR promoter in Jurkat cells (Kashanchi *et al.*, 1994). Deletion of the nucleotide sequence between -80 and -43 , which spans the GC-rich region, decreased the level of activation of the LTR in U-87MG and completely abrogated YB-1-induced activation of the LTR in SK-N-MC cells. It is evident that in U-87MG cells, deletion of GC sequences can be partially compensated by retention of the κ B sites (Fig. 1, compare constructs 2 and 9). To address this issue more specifically experiments are in progress to test the effect of point mutations within GC and κ B sequences on YB-1-induced viral transactivation.

To examine the importance of the sequences spanning the TAR region, we utilized a 3' deletion construct that removes sequences between $+3$ and $+80$, but retains sequences between -117 and $+3$, -80 and $+3$, and -43 and $+3$.

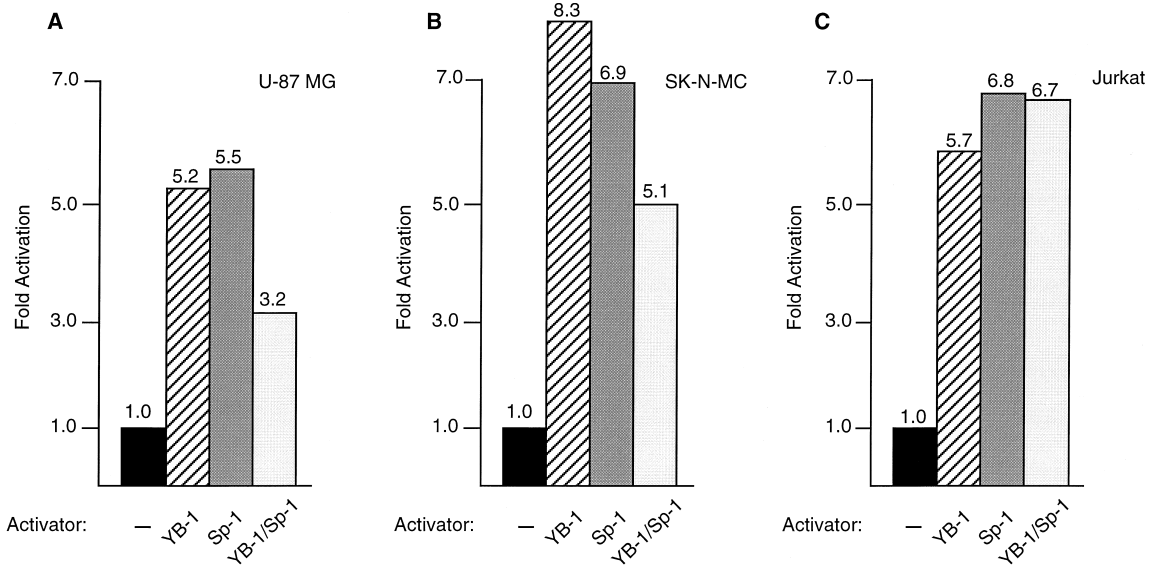


Fig. 3. Effect of YB-1 and SP1 on transcriptional activity of HIV-1 LTR. The LTR-CAT construct (5 µg) was introduced into U-87MG (A), SK-N-MC (B) and Jurkat (C) alone or with expression plasmids pCMV-YB-1 and pCMV-SP1 (5 µg each). The final concentration of DNA was kept constant by the addition of pCMV-DNA. Extracts were prepared 48 h after transfection and CAT enzymatic activity was determined (Gorman *et al.*, 1982). Fold activation of the LTR by YB-1 and SP1 is shown by bar graphs and the values represent an average of five experiments with an average standard deviation of ± 15%.

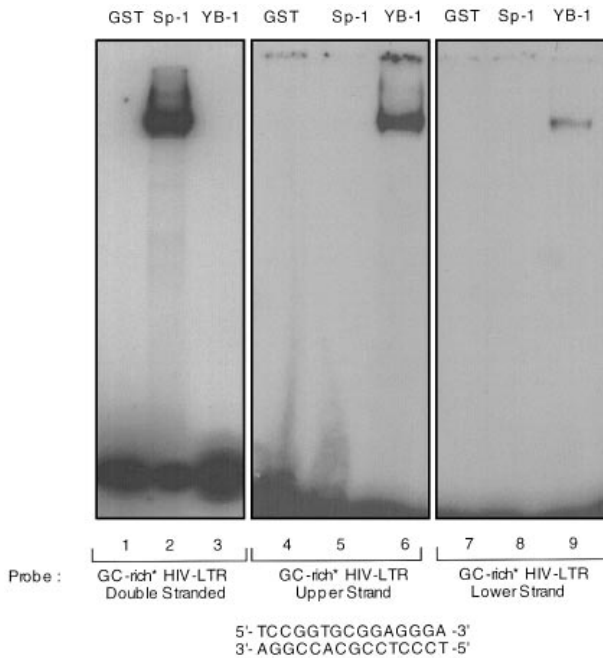


Fig. 4. Binding of YB-1 and SP1 to the GC-rich motif of the LTR. DNA-protein binding reactions were performed with 10 ng of SP1 protein (Santa Cruz Biotechnology) and 50 ng of GST-YB-1 fusion protein or 50 ng of GST as described previously (Chen *et al.*, 1995). The probe in lanes 1–3 was double-stranded DNA representing the GC-rich motif of the LTR whereas the probes in lanes 4–6 and lanes 7–8 were derived from the coding and non-coding single-stranded DNAs of the GC-rich motif. The primary structure of the DNA probe is shown below.

Results from co-transfection studies indicated that in the absence of the nucleotide sequences between +3 and +80, the upstream LTR sequence spanning the GC-rich domain is sufficient to respond to YB-1 induction in both U-87MG and SK-N-MC cells.

In the next series of studies we utilized internal deletion mutants that remove the GC-rich sequences between –80 and –43 of the LTR. Results from transfection studies indicated that internal removal of the GC-rich sequence (Δ GC-LTR) from the full-length (–453/+80) and the truncated (–117/+80) promoter decreased the level of activation by YB-1. Low but detectable levels of activation of the Δ GC-LTR constructs by YB-1 in U-87MG astrocytic cells suggest that in addition to the GC-rich region, nucleotides within the TAR region may be responsive to YB-1 in astrocytic but not in neuronal or T cells. This observation was supported by the results obtained using the 5' deletion mutant construct, where removal of the GC-rich sequences decreased the level of YB-1 activation in glial cells and eliminated LTR activation by YB-1 in the neuronal and T cells.

Stimulation of the HIV-1 LTR by YB-1 and Tat

The ability of YB-1 to enhance, although modestly, transcription of the HIV-1 LTR through the TAR sequence prompted us to investigate the combined effect of YB-1 and Tat proteins on viral gene transcription. In this respect, U-87MG cells were transfected with the reporter construct containing the full-length viral promoter (LTR-CAT), or a truncated promoter construct encompassing nucleotides –43 to +80 (–43/+80-CAT), alone or together with expression plasmids for YB-1 and Tat. As shown in Fig. 2, the level of

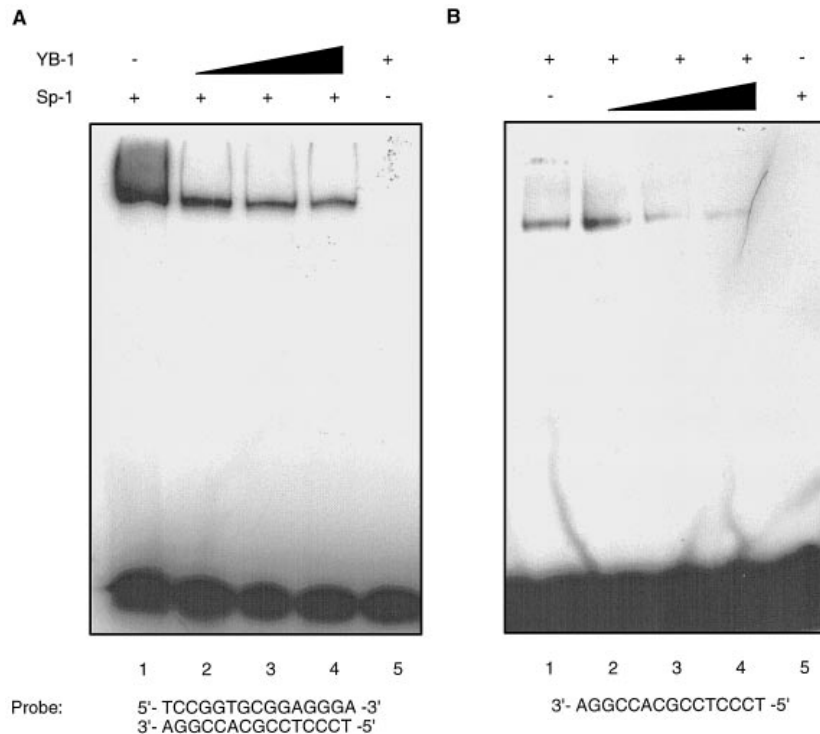


Fig. 5. Effect of YB-1 and SP1 on each other's DNA binding activity. (A) Double-stranded GC-rich probe was incubated with 10 ng of SP1 alone (lane 1) or with 25, 50, or 100 ng of GST-YB-1 (lanes 2-4, respectively). Lane 5 represents binding of GST-YB-1 (100 ng) to the double-stranded DNA. Protein concentration in the binding reaction was kept constant by the addition of GST protein. (B) Interaction of 50 ng of GST-YB-1 with single-stranded GC-rich sequence (non-coding strand) in the absence (lane 1) or presence of 25, 50, or 100 ng of SP1 (lanes 2-4). In lane 5, the binding reaction contained 50 ng of SP1 and the single-stranded DNA probe.

transcription from the full-length and truncated LTR promoters was increased in cells expressing YB-1 and Tat. It was evident that co-expression of Tat and YB-1 in the transfected cells increased the LTR activity and that the level of activation was equal to the sum of enhancements seen by each protein alone. These observations suggest that YB-1 and Tat may not function synergistically, and exert no influence, positively or negatively, on each other's function. Perhaps it should be mentioned that our preliminary results from *in vitro* protein-binding studies showed that bacterially produced YB-1 has the ability to form a complex with the TAR RNA sequence. The importance of YB-1/TAR interaction in relation to the ability of YB-1 to enhance LTR through TAR sequences is currently under investigation.

Regulation of the HIV-1 LTR by YB-1 and SP1

In the next series of studies we focused our attention on the upstream target for YB-1 that spans the GC-rich motif. As previous studies pointed to the functional association of the ubiquitous transcription factor, SP1, with the GC-rich sequence of LTR (Jones *et al.*, 1986; Majello *et al.*, 1994), we chose to investigate the functional interaction of SP1 and YB-1 in U-87MG and SK-N-MC cells. As illustrated in Fig. 3, ectopic expression of YB-1 or SP1 in transfected U-87MG (Fig. 3A), SK-N-MC (Fig. 3B) and Jurkat (Fig. 3C) cells results in enhancement of LTR transcription. Of interest, U-87MG and SK-N-MC cells co-transfected with both YB-1 and SP1 exhibited reduced CAT activity, suggesting that YB-1 and SP1

may modulate each other's ability to activate transcription of the HIV-1 LTR promoter. This effect may not be attributed to the effect of one activator on expression of the other as both SP1 and YB-1 are expressed by the CMV promoter. In Jurkat cells, combined overproduction of SP1 and YB-1 showed no drastic inhibitory effect in the level of LTR transcription in comparison to the level seen by each activator alone (Fig. 3C). This is an interesting observation in the light of data shown above suggesting that whereas YB-1 utilizes the GC-rich motif, the target for SP1 action in CNS cells, an alternative sequence located further upstream from the GC-rich region, is important for YB-1-mediated activation of the LTR in Jurkat cells.

Association of YB-1 and SP1 with the upstream GC-rich motif of the LTR

In order to gain some insight into the mechanism by which YB-1 and SP1 may influence each other's activity, we examined the ability of YB-1 and SP1 to form complexes with their target DNA sequences within the GC motif. A double-stranded oligonucleotide from the GC-rich motif of the LTR was ³²P-labelled and mixed with bacterially produced SP1 and YB-1. As shown in Fig. 4, whereas SP1 (lane 2) exhibited strong binding affinity to the duplex DNA probe, YB-1 was unable to form a complex with the double-stranded DNA probe (lane 3). The control bacterially produced GST showed no binding activity to the probe (Fig. 4, lane 1). Of note, we have previously reported on the ability of YB-1 to bind double-stranded

sequences, although to a much lesser degree than single-stranded DNA, corresponding to the JCV late promoter (Chen *et al.*, 1995). These data provide experimental support that the lack of binding of YB-1 to double-stranded GC elements of the HIV-1 LTR appears to be promoter-dependent, and may not be due to the absence of an adaptor protein. Next, the ability of YB-1 and SP1 to associate with the single-stranded DNA probes from the GC-rich motif was examined. As anticipated, SP1 showed virtually no binding affinity to either the upper or lower strand probes, whereas YB-1 formed a complex with both strands (Fig. 4, lanes 4–9).

To examine the possible effect of YB-1 on binding of SP1 to the double-stranded GC-rich motif, increasing amounts of GST–YB-1 were included in the SP1-binding mixture. As shown in Fig. 5(A), addition of YB-1 to the binding reaction decreased the intensity of the band corresponding to the SP1 complex and no apparent new complex was obtained. In the reciprocal experiment, the effect of SP1 on binding of YB-1 to its single-stranded DNA probe was evaluated. As shown in Fig. 5(B), inclusion of SP1 in the YB-1 binding reaction abrogated association of YB-1 with the single-stranded probe. Again, no new complex implying association of SP1 with single-stranded DNA probes or formation of SP1–YB-1 DNA complex was detected. Thus far, we have been unable to detect direct interaction of YB-1 and SP1 on their target DNA suggesting that either these two proteins do not co-associate with the GC-rich sequence, or such a complex may not remain intact during gel analysis. As such, one may speculate that YB-1 by associating with SP1 forms a heterodimer complex with a reduced binding activity to their target GC-rich sequence. Studies are in progress to investigate the functional interaction of YB-1 and SP1 on the HIV-1 promoter and to determine regions within YB-1 and SP1 which play important roles in the observed event.

From mutational analysis of LTR reporter constructs, promoter elements spanning GC-rich motifs were identified as the targets for YB-1-induced activation. Because this activation function depends on SP1-binding sites, YB-1 may function in concert with transcription factor SP1. At the present time it is unclear whether YB-1 interacts with SP1 directly or indirectly. It is known that the GC-rich region within the HIV-1 LTR contains in addition to the three putative SP1 binding sites, an overlapping p53 binding site (Gualberto & Baldwin, 1995) and a T3R binding motif (Rahman *et al.*, 1995). It is conceivable that YB-1 can exert its positive regulatory effect through interaction with other regulatory proteins present at this region. In agreement with this notion, our preliminary results indicate an interaction between p53 and YB-1 (G. L. Gallia & B. E. Sawaya, unpublished observations).

Another peculiar finding that emerged from our studies was the observation that YB-1 exhibited higher affinity for binding G/A-rich DNA within the HIV-1 regulatory domain. This is dissimilar to C/T-rich sequences that were traditionally considered target sequences for this protein. Incidentally, this

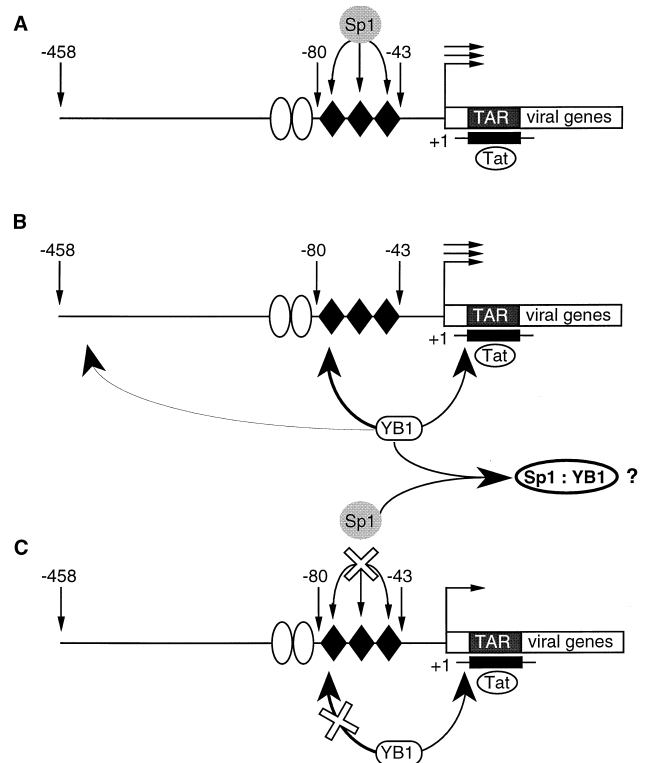


Fig. 6. Model for regulation of the HIV-1 LTR by SP1 and YB-1. (A) SP1 binding to the GC-rich sequence positioned between -80 and -43 stimulates transcription of the HIV-1 LTR. (B) YB-1 has the ability to stimulate transcription of the HIV-1 LTR via its association with the single-stranded GC-rich sequence (nucleotides -80 to -43) and to a lesser extent via the TAR region in CNS-derived cells. In Jurkat T cells, YB-1 exerts a positive effect on transcription of the HIV-1 LTR through a region located further upstream from the GC-rich domain. (C) Co-overexpression of YB-1 and SP1 in CNS cells results in a decrease in their ability to stimulate HIV-1 LTR transcription and their capacity to bind to the target DNA sequence is greatly diminished. Whether or not YB-1 and SP1 form a complex remains to be determined.

observation was reported by MacDonald *et al.* (1995), who demonstrated preferential binding of YB-1 to the GA-rich sequences within the DRA promoter.

The data presented here demonstrate that two cellular transcriptional activators with a common binding site may modulate each other's activity on the responsive promoters. As illustrated in Fig. 6, association of SP1 with the double-stranded GC-rich motif of the HIV-1 LTR positioned between nucleotides -43 and -80 (Fig. 6A) or interaction of YB-1 with the single-stranded DNA sequence encompassing the same region (Fig. 6B) augment HIV-1 LTR activity in CNS cells. Once these two proteins are co-overexpressed, the binding affinity of these proteins to their target sequences and their transcriptional activities are drastically decreased. Whether or not these two proteins interact with each other remains to be determined. Fig. 6(B) also depicts the ability of YB-1 to stimulate the HIV-1 LTR via an upstream element in T cells and TAR regulatory element. Furthermore, cooperativity of YB-1 with Tat protein in enhancing viral

transcription, points to its possible influence through Tat-responsive elements within the viral LTR.

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