JOURNAL OF VIROLOGY, June 1989, p. 2585–2591
0022-538X/89/062585-07$02.00/0
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Vol. 63, No. 6

Role of SP1-Binding Domains in In Vivo Transcriptional Regulation of the Human Immunodeficiency Virus Type 1 Long Terminal Repeat

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Received 13 December 1988/Accepted 1 March 1989

Five regions of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) have been shown to be important in the transcriptional regulation of HIV in HeLa cells. These include the negative regulatory, enhancer, SP1, TATA, and TAR regions. Previous studies in which purified SP1 was used showed that the three SP1-binding sites in the HIV LTR were important in the in vitro transcription of this promoter. However, no studies to ascertain the role of each of these SP1-binding sites in basal and *tat*-induced transcriptional activation in vivo have been reported. To determine the role of SP1 sites in transcriptional regulation of the HIV LTR in vivo, these sites were subjected to oligonucleotide mutagenesis both individually and in groups. The constructs were tested by DNase I footprinting with both oligonucleotide affinity column-purified SP1 and partially purified HeLa extract and by chloramphenicol acetyltransferase assays in both the presence and absence of the *tat* gene. Mutagenesis of each SP1-binding site resulted in minimal changes in basal and *tat*-induced transcriptional activation. Mutations involving alterations of SP1 sites I and II, I and III, or II and III also resulted in minimal decreases in basal and *tat*-induced transcriptional activation. However, mutagenesis of all three SP1-binding sites resulted in a marked decrease in *tat* induction. The latter mutation also greatly decreased DNase I protection over the enhancer, TATA, and TAR regions when partially purified HeLa nuclear extract was used. Mutagenesis of the HIV LTR SP1 sites which converted them to consensus high-affinity SP1-binding sites with the sequence GGGGCGGGGC resulted in increased *tat*-induced gene expression compared with the wild-type HIV LTR template. These results suggest that SP1, through its interaction with other DNA-binding proteins, is critical for in vivo transcriptional regulation of HIV.

Human immunodeficiency virus type 1 (HIV-1) is an etiologic agent of acquired immunodeficiency syndrome (3, 16, 36). Important regulatory regions which serve as the binding sites for cellular proteins (9, 17, 27, 28, 33, 34, 38, 42, 48, 50, 51) and viral proteins such as those encoded by *tat* (2, 6, 7, 12, 14, 18, 19, 24, 32, 40, 42, 49), *rev* (12, 45) and *nef* (1) are important in the gene expression of this virus. At least five portions of the viral long terminal repeat (LTR), including the negative regulatory, enhancer, SP1, TATA, and TAR regions, serve as binding sites for cellular proteins in studies using HeLa cell nuclear extracts (17). Mutagenesis of the LTR has revealed that each of these regions is important for transcriptional regulation of the LTR (13, 17, 23, 26–28, 33, 34, 38, 42, 48, 49). Six DNA-binding proteins, including EBP-1 and NF-KB, which bind to the enhancer region (34, 51); UBP-1, LBP, and CTF, which bind to the TAR region (28, 51); and SP1 (27), have been purified and shown to interact with important HIV regulatory domains.

The HIV LTR is activated at the transcriptional level by the 86-amino-acid nuclear protein encoded by *tat* (2, 6, 12, 14, 24, 32, 40, 41, 51). *tat* has been reported to increase HIV gene expression by increasing steady-state RNA levels (6, 17, 19, 37, 40). In vitro nuclear runoff experiments have shown that *tat* increases HIV nuclear mRNA levels, further supporting a role for *tat* in transcriptional activation of the HIV LTR (24, 41). However, *tat* has also been reported to increase the translation of HIV mRNA (6, 12, 37, 42). Thus, *tat* may have dual mechanisms of action (6). Complete activation of the HIV LTR by the *tat* protein requires both upstream regulatory regions, such as the enhancer, SP1, and TATA elements, and the downstream TAR region (13, 17, 23, 26–28, 37, 38, 40, 43). In the TAR region, a stem-loop structure has been described which appears to be important for HIV gene expression (13, 26, 37, 39). Studies have defined a region between +19 and +44 in the TAR domain required for activation by the *tat* protein (13, 23, 26). In addition, TAR DNA-binding proteins appear to be important in *tat* induction (17, 28). These results suggest that multiple HIV LTR regulatory elements play a role in HIV gene expression.

Prior data have shown that the binding sites for the cellular transcription factor SP1 are important for both in vivo and in vitro function of the HIV LTR (17, 27). The HIV LTR was shown to bind highly purified preparations of SP1 between −43 and −83 in the viral LTR (17, 27). Examination of the sequences in this region indicated the presence of three potential SP1-binding sites. The third SP1-binding sequence has the highest affinity for purified SP1 (27). Oligonucleotide mutagenesis of the third SP1-binding site did not affect in vitro transcriptional activation of the HIV LTR, but mutagenesis of all three SP1-binding sites resulted in a 10-fold decrease in transcription of the HIV LTR in vitro with uninfected HeLa cell extracts (27). Another study showed that deletion of SP1-binding sites resulted in a decrease in in vivo basal and *tat*-induced transcriptional activation and altered protection over the TATA region (17). In contrast to results with highly purified SP1, partially purified HeLa cell nuclear extracts showed protection over only the third

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SP1-binding site (17). Thus, both in vitro studies and in vivo deletion analysis of the HIV LTR have revealed that the SP1 sites appear to have a role in HIV transcriptional regulation and may interact with other HIV LTR DNA-binding proteins.

To determine the role of SP1 sites in transcriptional regulation of the HIV LTR in vivo, oligonucleotide-directed mutagenesis of these sites either individually or in pairs was performed. DNase I footprinting with partially purified SP1 indicated that each of these mutations altered the binding of SP1. The individual mutations were also tested for effects on HIV LTR gene expression both in the presence and in the absence of the *tat* gene. Mutagenesis of each of the three SP1-binding sites resulted in minimal changes in *tat*-induced activation, as did mutations of sites I and II, I and III, or II and III. However, mutation of all three SP1-binding sites resulted in a marked decrease in *tat*-induced activation. Mutations of all three SP1-binding sites also decreased DNase I protection patterns with partially purified HeLa extract over the enhancer, TATA, TAR, and negative regulatory regions. When the SP1-binding sites were converted to high-affinity consensus SP1-binding sites with the sequence GGGGCGGGGC, *tat*-induced gene expression increased two- to threefold over that for the wild-type HIV LTR template. These results suggest an important role for SP1-binding sites in factor binding and HIV transcriptional regulation.

MATERIALS AND METHODS

Mutagenesis. A *Mae*I (−177)-*Hin*dIII (+83) fragment from the HIV LTR of ARV2 was cloned into *Hin*cII-*Hin*dIII mp18, and single-stranded DNA template was prepared as described elsewhere (51). Oligonucleotides (19- to 45-mer) were synthesized on an Applied Biosystems DNA synthesis machine (Molecular Biology Institute and UCLA Jonsson Comprehensive Cancer Center Fermenter/Preparation Core Facility, courtesy of Thomas Sutherland). The oligomers were gel purified, quantitated, and kinased with ATP. The kinased oligomers were used in conjunction with a commercial site-directed mutagenesis kit (Amersham Corp.) according to conditions described by the manufacturer. Positive clones were identified by screening and confirmed by sequencing, and *Bam*HI-*Hin*dIII fragments were then isolated for ligation into pJGFCAT18.

Plasmid constructions. The *Eco*RI-*Hin*dIII fragments from each of the single SP1 mutations, the double mutation NSP23, and the high-affinity SP1 mutations were gel isolated and ligated into pUC19. The multiple mutations NSP12, NSP13, and NSPALL were joined at convenient restriction sites and confirmed by sequencing. All mutations were then ligated into the *Bam*HI-*Hin*dIII sites of pJGFCAT18, which is a Rous sarcoma virus (RSV) chloramphenicol acetyltransferase (CAT) derivative (51).

Construction of the expression plasmid has been described elsewhere (17). RSV *tat* contains the first 72 amino acids of the ARV2 *tat* protein (contained within the second exon of the *tat* message) in place of the beta-globin sequence of the control expression plasmid RSV beta-globin.

Preparation of HeLa and SP1 extracts. Nuclear extract was prepared from 20 ml of packed HeLa cells as described elsewhere (8) and applied to a heparin-agarose column. The column was washed with 0.1 M KCl and eluted with 0.5 M KCl, and these fractions were used in DNase I footprinting. For partial purification of the SP1 protein, 10 mg of HeLa cell extract from the 0.5 M KCl eluate of a heparin-agarose column was dialyzed and then applied to a 1-ml column of Sepharose CL-2B coupled with double-stranded oligonucleotides containing a high-affinity binding site of SP1 (31). The column was washed extensively with buffer containing 0.1 M KCl, and the SP1 was eluted in buffer containing 1.0 M KCl. This material was dialyzed and reapplied to the column, and the material from the second elution of the oligonucleotide affinity column was used in DNase I footprinting.

DNase I footprinting. To footprint the HIV LTR on the coding strand, the pUC19 constructs were digested with *Eco*RI, treated with alkaline phosphatase, and end labeled with γ-^{32}P. The *Eco*RI-*Hae*II fragments were gel isolated, electroeluted, and used for the DNase I footprinting assay (15). To footprint the noncoding strand, the pUC19 constructs were cut with *Hin*dIII, treated with phosphatase, and end labeled with γ-^{32}P. The *Hin*dIII-*Bgl*I fragments were gel isolated, electroeluted, and used for the DNase I footprinting. One to five nanograms of end-labeled probe was added to each 50-μl reaction mixture along with partially purified HeLa extract or partially purified SP1 extract (31), poly(dI-dC) (3 μg), and final concentrations of 10 mM Tris (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol. The DNA and extract were allowed to bind for 20 min at room temperature, and then the reaction mixture volume was increased to 100 μl and final concentrations of DNase I (0.4 to 2.0 μg/ml), 5 mM $MgCl_2$, and 2.5 mM $CaCl_2$ were added. The reaction was stopped after 30 s with phenol-chloroform, the mixture was precipitated with ethanol, and the precipitant was loaded onto a 10% polyacrylamide–8 M urea sequencing gel. G+A and C+T Maxam-Gilbert sequencing reactions were performed for each probe. All gels were then subjected to autoradiography.

Transfections and CAT assays. HeLa cells were maintained on complete Iscoves medium with 5% newborn calf serum containing penicillin and streptomycin. Plates were split on the day prior to transfection so that each 100-mm culture was 50 to 70% confluent at the time of transfection. The calcium phosphate precipitation technique was used to cotransfect 5 μg of each HIV LTR CAT construct and 5 μg of the RSV *tat* or RSV beta-globin expression plasmid onto identically prepared plates (17). Four hours after transfection the cells were glycerol shocked, and 48 h after transfection the cells were harvested and extracts were prepared and used in CAT assays as described previously (22).

RESULTS

Oligonucleotide-directed mutagenesis of the HIV LTR SP1-binding sites. To determine the role of the SP1 sites in the transcriptional regulation of HIV, a series of oligonucleotide-directed mutations were made in each of the three SP1-binding sites in the HIV LTR. These mutations were made in nucleotides previously reported to be critical in SP1 binding to the HIV LTR (27). Mutations that interrupted each of the three SP1-binding sites were constructed, as were mutations of sites I and II, II and III, and I and III. In addition, a mutation of sites I, II, and III was constructed by coupling plasmids containing each of the individual mutations. Since the SP1-binding sites in HIV differ from the high-affinity consensus SP1-binding sites found in simian virus 40 (SV40) (20, 21), mutations were also made that converted the SP1 sites to the high-affinity SP1 consensus sequence GGGGCGGGGC. The SP1-binding region and the mutations introduced are illustrated in Fig. 1.

SP1 mutants did not bind partially purified SP1. The oligonucleotide-directed mutations in the HIV LTR SP1-

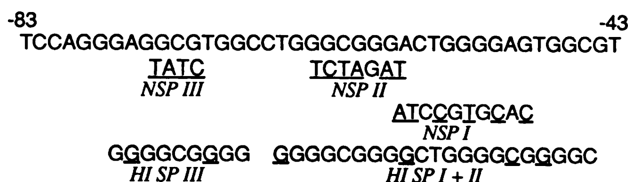


FIG. 1. HIV LTR SP1-binding sequences and oligonucleotide-directed mutations. The sequences in the SP1-binding region extending from −83 to −43 are shown, and the base substitutions introduced by oligonucleotide-directed mutagenesis are indicated. To generate double and triple SP1 mutations, the individual mutants were joined via convenient restriction sites. The mutations indicated as NSP III, NSP II, and NSP I interrupt each of the three SP1-binding sites in the HIV LTR, while the mutations indicated as HISP III and HISP I+II convert the SP1-binding sites to the high-affinity consensus SP1-binding site GGGGCGGGGC. The underlined sequences indicate those nucleotides changed in the mutagenesis.

binding domains were tested by DNase I footprinting with partially purified SP1 eluted from an SP1 sequence-specific oligonucleotide affinity column as previously described for the purification of SP1 (31). Mutations of each of the three SP1-binding sites altered the binding of partially purified SP1 on both the coding (Fig. 2A) and noncoding (Fig. 2B) strands. Mutagenesis of site III (ΔSP1-III) decreased protection between −82 and −70, mutagenesis of site II (ΔSP1-II) decreased protection between −69 and −57, and mutagenesis of site I (ΔSP1-I) decreased protection between −56 and −44 (Fig. 2A and B). The effect of the site II mutation was more clearly seen with longer exposures of this gel (data not shown). Mutagenesis of both sites I and II (ΔSP1-I+II) decreased protection between −82 and −57, while mutagenesis of all three SP1-binding sites (ΔSP1-I+II+III) eliminated protection between −82 and −44 (Fig. 2A and B). Thus, as in a previous study, mutations of each SP1-binding domain specifically interrupted SP1 binding (27).

Creation of high-affinity SP1-binding sites increased binding of SP1. The SP1-binding sites in the HIV LTR differ in sequence from those sites in the SV40 promoter (20, 21, 27). In SV40, sequences are similar to the SP1 high-affinity consensus sequence GGGGCGGGGC (20, 21). To determine if converting the SP1 sequences in the HIV LTR to this high-affinity consensus SP1 sequence would alter their binding, a series of mutants were constructed that contained this sequence. These mutants changed either site III or both sites I and II to this high-affinity consensus SP1-binding sequence.

DNase I footprinting of the HIV LTR-coding strand with partially purified SP1 for the high-affinity consensus SP1 sites I and II construct resulted in protection extending from −82 to −36 (Fig. 3, lanes 5 to 8). The high-affinity consensus SP1 site III construct did not alter the region of protection compared with the wild-type construct (Fig. 3, lanes 9 to 12). Thus, these mutations do not change the amount of partially purified SP1 protein at which complete clearing of the HIV SP1 region occurs, but the high-affinity consensus SP1 sites I and II construct increases the extent of protection.

SP1-binding sites altered binding to TAR, TATA, and enhancer regions. Previously, we showed that DNase I footprinting of the HIV LTR-coding strand with partially purified HeLa extracts resulted in protection over the negative regulatory element (−173 to −159), the enhancer and a portion of the third SP1-binding site (−97 to −78), and the TATA-TAR region (−42 to +28). Clearing over the first two SP1-binding sites was not detected by using partially purified HeLa extracts, though these extracts resulted in protection of five of six SP1 sites in the SV40 promoter (17). However, DNase I protection of all three SP1-binding sites was detected by using partially purified SP1 (17, 27) (Fig. 2A and B). In addition to SP1, a cellular protein purified from HeLa cells, known as EBP-1, results in protection between −97 and −78. Another protein, known as UBP-1, in conjunction with a TATA-binding factor and a recently described factor known as UBP-2 (J. A. Garcia et al., unpublished observations), is responsible for protection between −42 and +28 (51). DNase I protection with this partially purified HeLa extract revealed that mutations of both SP1-binding sites I and II resulted in slightly decreased protection over the enhancer, TATA, and TAR regions compared with the wild-type HIV LTR template (Fig. 4). Mutations of sites I, II, and III resulted in even further decreases in protection over the enhancer, TATA, and TAR regions (Fig. 4). The difference in binding was not due to differences in the specific activity of the probes used, nor could complete clearing of the enhancer and TATA-TAR regions be detected with protein concentrations as high as 200 μg per reaction mixture (data not shown). The conversion of site III or sites I and II to high-affinity consensus SP1-binding sequences did not significantly alter the binding of the enhancer, TATA, or TAR regions compared with the wild-type HIV LTR construct (Fig. 5). These results suggest that the SP1-binding sites play a role in regulating the binding of proteins to other HIV regulatory regions.

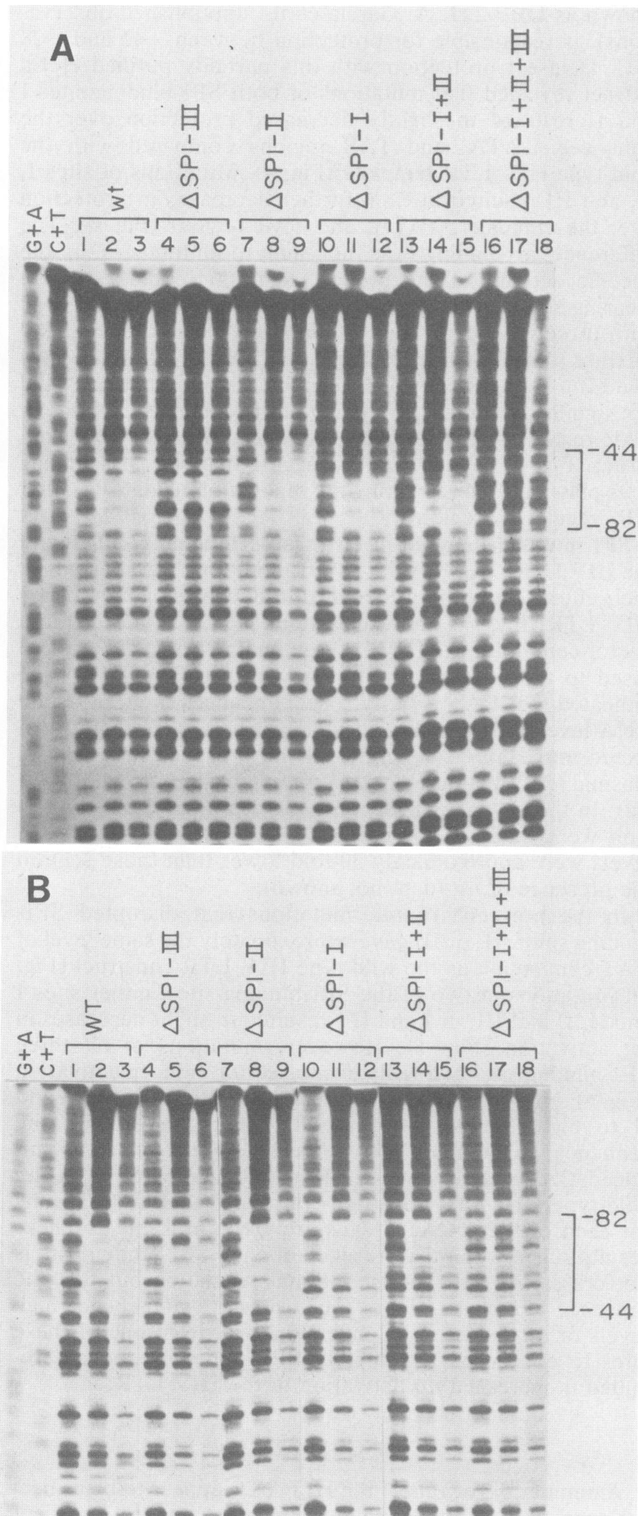
SP1 mutations altered in vivo transcriptional regulation of the HIV LTR. The HIV LTR SP1 mutations were tested for their effects on both basal and *tat*-induced regulation of the HIV LTR. Each of these mutations was introduced into a vector containing the HIV LTR extending from −177 to +83 fused to the CAT gene. Studies with the HIV LTR have indicated that CAT activity reflects changes in steady-state RNA levels (17, 19, 40). These constructs were then transfected into HeLa cells either in the presence of a control plasmid (RSV beta-globin) or a *tat* expression plasmid (RSV *tat*). In the absence of *tat*, only low levels of CAT conversion were detected for each of the constructs, and these levels were approximately 30-fold lower than those seen in the presence of *tat* (data not shown).

In the presence of *tat*, mutations that disrupted SP1-binding site I, II, or III gave approximately the same level of CAT conversion as the wild-type HIV LTR construct (Fig. 6). Mutations of two of the SP1-binding sites, either sites I and II, II and III, or I and III, resulted in slight decreases in *tat* activation (Fig. 6). However, mutations of all three SP1-binding sites markedly decreased *tat*-induced activation (Fig. 6). Mutations that converted either site III or sites I and II to high-affinity consensus SP1-binding sites with the sequence GGGGCGGGGC increased the level of *tat*-induced CAT activity two- to threefold compared with the wild-type HIV LTR construct (Fig. 6). Quantitative results for each of these CAT assays are given in Table 1. These results suggest that the presence of each SP1-binding site is not critical for HIV transcriptional regulation but that the presence of at least one of these sites is sufficient for high levels of *tat* induction. Conversion of at least one of these sites to consensus high-affinity SP1-binding sequences resulted in increased *tat* activation of the HIV LTR.

DISCUSSION

A number of regions of the HIV LTR appear to be critical for regulation of this promoter when assayed by transient

expression assays in HeLa cells (9, 17, 23, 26–28, 33, 34, 38, 43, 48, 50). Previous mutagenesis experiments have indicated a critical role for the enhancer, TATA, and TAR regions for basal and *tat*-induced induction. The results presented here support those of previous in vitro transcription assays showing that SP1-binding sites also play a role in the transcriptional regulation of the HIV LTR (28).



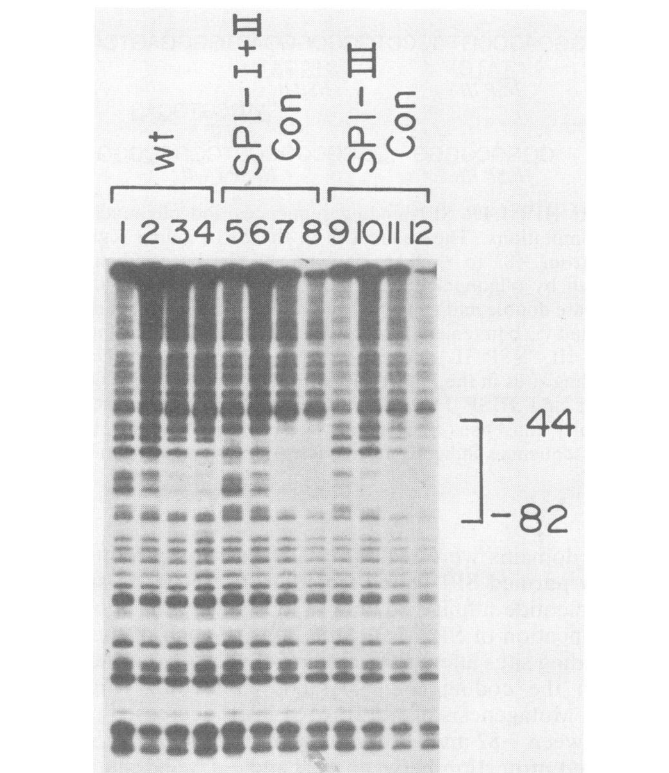


FIG. 3. DNase I footprinting of the coding strand of the HIV LTR from −177 to +83 with the wild type (lanes 1 to 4), ΔSP1-I+II Con (lanes 5 to 8), and ΔSP1-III Con (lanes 9 to 12) with partially purified SP1. Lanes 1, 5, and 9 contain no added extract; lanes 2, 6, and 10 contain 1 μl of extract; lanes 3, 7, and 11 contain 5 μl of extract; and lanes 4, 8, and 12 contain 10 μl of extract. G+A and C+T indicate Maxam-Gilbert sequencing lanes. The region of protection for the wild-type construct extending from −44 to −82 in the HIV LTR is indicated.

Transcription factor SP1 is a protein that binds DNA and activates transcription from a number of different cellular and viral promoters (10, 11, 20, 21, 27, 29, 35). SP1 was originally identified as a protein present in HeLa cell extracts that binds to multiple GGGCGG sequences (GC boxes) in the 21-base-pair repeat elements of SV40 and that activates in vitro transcription from the SV40 early and late promoter (10, 11, 15, 21). SP1-responsive promoters usually contain multiple GC box recognition sites of variable binding affinity that are usually located 40 to 70 base pairs upstream of the mRNA start site. However, a single SP1 site may be sufficient for transcriptional stimulation by SP1 (10, 11, 20, 21, 28, 29, 35). SP1-binding sites are frequently found near binding sites of other transcription factors, such as CTF-

FIG. 2. DNase I footprinting of ΔSP1 mutant constructs. DNase I footprinting of both the coding strand (A) and the noncoding strand (B) of the HIV LTR extending from −177 to +83 with partially purified SP1 is shown for the wild type (lanes 1 to 3), ΔSP1-III (lanes 4 to 6), ΔSP1-II (lanes 7 to 9), ΔSP1-I (lanes 10 to 12), ΔSP1-I+II (lanes 13 to 15), and ΔSP1-I+II+III (lanes 16 to 18). Lanes 1, 4, 7, 10, 13, and 16 contain no added extract; lanes 2, 5, 8, 11, 14, and 17 contain 5 μl of extract; and lanes 3, 6, 9, 12, 15, and 18 contain 10 μl of extract. G+A and C+T indicate Maxam-Gilbert sequencing lanes. The region of protection extending from −44 to −82 in the HIV LTR is indicated.

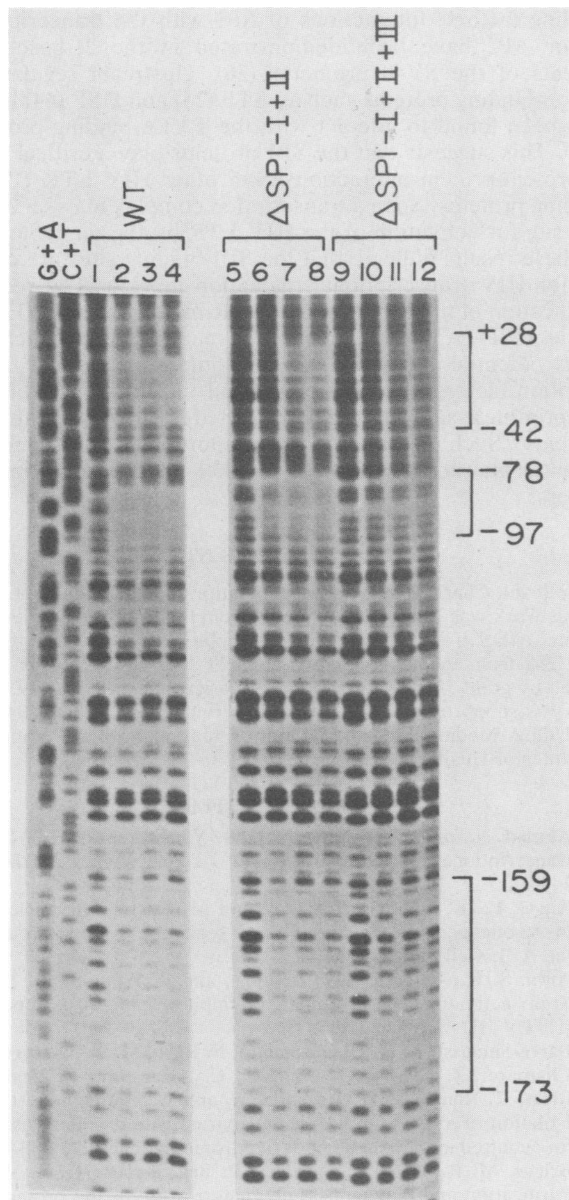


FIG. 4. DNase I footprinting of the HIV LTR-coding strand with partially purified HeLa extract for the wild type (lanes 1 to 4), ΔSP1-I+II (lanes 5 to 8), and ΔSP1-I+II+III (lanes 9 to 12). Either no extract was added (lanes 1, 5, and 9) or 25 μg (lanes 2, 6, and 10), 50 μg (lanes 3, 7, and 11), or 100 μg (lanes 4, 8, and 12) of extract was added. The regions of protection for the TATA-TAR regions (+28 to −42), the enhancer and third SP1-binding site (−78 to −97), and the negative regulatory element (−159 to −173) are indicated. G+A and C+T indicate Maxam-Gilbert sequencing lanes.

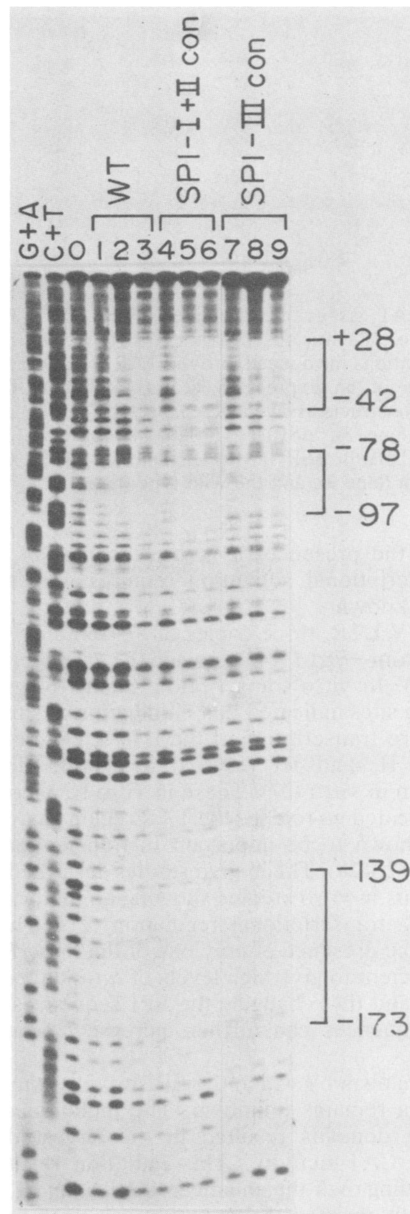


FIG. 5. DNase I footprinting of the HIV LTR-coding strand with partially purified HeLa extract for the wild type (lanes 1 to 3), SP1-I+II Con (lanes 4 to 6), and SP1-III Con (lanes 7 to 9). Either no extract was added (lane 0) or 25 μg (lanes 1, 4, and 7), 50 μg (lanes 2, 5, and 8), or 100 μg (lanes 3, 6, and 9) of extract was added. G+A and C+T indicate Maxam-Gilbert sequencing lanes. The regions of protection for the TATA-TAR regions (+28 to −42), the enhancer and third SP1-binding site (−78 to −97), and the negative regulatory element (−159 to −173) are indicated.

NF1 and AP1 (27, 35). This suggests that SP1 may act in conjunction with other transcription factors to activate transcription.

A partial cDNA clone encoding the SP1-binding protein has been identified, and the binding properties of this protein have been studied in vitro (30). These studies have shown that SP1 contains three zinc fingers that are critical for its DNA-binding activity. This motif has been shown to be involved in the DNA binding of other cellular transcription factors (4). Elimination of zinc results in the failure of this protein to bind DNA and stimulate in vitro transcription (30). The mechanism by which this protein stimulates transcription and its potential interactions with other transcription factors are not known. However, its proximity to other cellular transcription factor-binding sites such as NF1 (26) and AP1 (33) suggests that SP1 may interact with neighboring transcription factors. The presence of multiple copies of SP1 sites has been demonstrated upstream of a number of promoters (20, 21, 29, 35). During SV40 infection variable occupancy of these sites occurs (20, 21). However, the

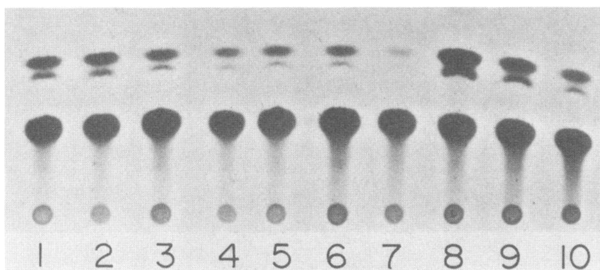


FIG. 6. CAT assays of SP1 mutants. The HIV LTR extending from −177 to +83 was fused to the CAT gene. Oligonucleotide-directed mutations introduced into the TAR region were assayed in the presence of an expression vector containing the *tat* gene (RSV-*tat*). Constructs assayed were ΔSP1-III (lane 1), ΔSP1-II (lane 2), ΔSP1-I (lane 3), ΔSP1-I+II (lane 4), ΔSP1-II+III (lane 5), ΔSP1-I+III (lane 6), ΔSP1-I+II+III (lane 7), SP1-III Con (lane 8), SP1-I+II Con (lane 9), and the wild type (lane 10).

reason for the presence of multiple copies of these sites when transcriptional activation requires only a few such sites is not known.

In the HIV LTR, three copies of SP1-binding sites occur, extending from −78 to −43 between the enhancer and TATA regions (27). In vitro transcription studies of mutations in SP1-binding sites indicated that elimination of site III did not affect in vitro transcription of the HIV LTR, but mutations of sites I, II, and III resulted in 10-fold decreases in transcription in vitro (27). These in vitro transcription studies also indicated no role for the TAR region past +21, which has been shown to be important in transfection assays in vivo (13, 23, 26–28). The in vivo studies described here agree with previous in vitro studies showing an important role for SP1 in HIV transcriptional regulation (27). They demonstrate that the presence of only one of the three SP1-binding sites is sufficient to give high levels of *tat*-induced HIV gene expression and that changing the SP1 sequences to consensus SP1 sequences can further increase *tat*-induced gene expression.

The mechanism by which the SP1 protein stimulates HIV transcription remains unknown. The mutation of all three SP1-binding domains resulted in the largest decrease in *tat*-induced CAT activity. This mutation resulted in decreased binding over the enhancer, TATA, and TAR regions when partially purified HeLa extract was used, suggesting a potential role for SP1 in interactions with the other DNA-binding factors. Interactions of SP1 with the transcription factor AP2 have been demonstrated in the 21-base-pair repeats of the SV40 promoter (36). Upstream regulatory region-binding proteins such as ATF (25) and USF (44) have also been found to interact with the TATA-binding protein II-D. This suggests that the SP1 proteins play a critical role in protein-protein interactions with other HIV LTR DNA-binding proteins. Such a transcription complex may serve as one site for activation of the HIV LTR by the *tat* protein.

These results indicate that the SP1-binding sites are critical for HIV transcriptional regulation in vivo. The recent purification of the enhancer and TAR-binding proteins EBP-1 and UBP-1 (51) should provide reagents with which to study potential interactions of these important cellular transcription factors with SP1 by careful titrations in DNase I footprinting assays and in reconstituted in vitro transcription systems. Such studies will be important in determining potential interactions of SP1 with other cellular transcription factors.

TABLE 1. *tat*-induced CAT activity of SP1 mutants

Construct	CAT[a]	
	% Conversion	Relative activity
ΔSP1-III	4.65	1.02
ΔSP1-II	4.38	0.96
ΔSP1-I	4.08	0.90
ΔSP1-I+II	1.88	0.41
SP1-II+III	1.74	0.38
SP1-I+III	1.80	0.40
ΔSP1-I+II+III	0.30	0.07
SP1-III Con	14.35	3.15
SP1-I+II Con	9.68	2.13
Wild type	4.55	1.00

[a] Both unacetylated and acetylated chloramphenicol (^{14}C) were determined by scintillation counting of CAT assays in the presence of the *tat* gene product to determine percent CAT conversion. A 4.55% conversion for the wild-type construct was assigned a CAT activity of 1.00.

ACKNOWLEDGMENTS

We thank Charles Leavitt for preparation of the manuscript.

This work was supported by grants from the California Universitywide AIDS Task Force and by Public Health Service grant AI-25288 from the National Institutes of Health. R.G. was supported by grant JFRA-146 from the American Cancer Society, and J.G. was supported by Public Health Service grant GMO-80942 to the UCLA Medical Scientist Training Program from the National Institutes of Health.

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