The high-affinity Sp1 binding site in the HTLV-1 promoter contributes to Tax-independent basal expression

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ABSTRACT

Transcriptional activation of human T-cell leukemia virus type 1 (HTLV-1) requires many cellular proteins and the virally encoded transcription factor Tax. Tax binds the three viral cAMP-response elements (CREs) with ATF/CREB (activating transcription factor/cAMP-response element-binding protein) and recruits the cellular coactivators CBP/p300. HTLV-1 also utilizes other cellular transcription factors that bind to the promoter to regulate transcription. One of these factors, Sp1, has been shown to bind to the viral promoter at two elements; one located within the third viral CRE, and the second located between the second and third viral CREs. The functional significance of Sp1 binding at each of these regions of the viral promoter is not completely understood. We set out to characterize Sp1 binding and to evaluate the functional significance of Sp1, both in the absence and presence of Tax. We found that Sp1 binds preferentially to the element located between the second and third viral CREs, and modestly activates transcription in vitro and in vivo. Sp1 was detected at the integrated HTLV-1 promoter in vivo. Surprisingly, point mutagenesis of the strong Sp1 binding site rendered the HTLV-1 reporter plasmid insensitive to Sp1 activation, and dramatically reduced basal transcription in vivo. These data indicate a role for Sp1 in basal level transcription of HTLV-1.

INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of an aggressive form of cancer called adult T-cell leukemia/lymphoma (1,2). The virus is also the causative agent of other diseases, including tropical spastic paraparesis (TSP/HAM), a neurodegenerative disorder similar to multiple sclerosis (reviewed in 3±5). After infection, HTLV-1 integrates randomly into the host cell genome, and is generally expressed at very low levels. High-level expression of the virus requires strong transcriptional activation mediated by the virally encoded transactivator Tax. Many cellular proteins have also been implicated in transcriptional regulation of the virus. The best characterized promoter elements of the virus are the three imperfectly conserved 21-bp repeats, called viral cAMP-response elements (CREs). These elements carry an octanucleotide CRE core immediately flanked upstream and downstream by GC-rich sequences. The viral cAMP-responsive elements (CREs) serve as binding sites for Tax in complex with the cellular transcription factor CREB (cAMP-responsive element-binding protein) [or other members of the activating transcription factor (ATF)/CREB family of transcription factors] (6±9). Tax binds to the viral CREs through protein±DNA interactions with the GC-rich sequences (10±13) and protein±protein interactions with CREB (6,8). The formation of this promoter-bound Tax±CREB complex is critical for the recruitment of the cellular coactivators CBP and p300 (6,7,9,14±19).

It has also been reported that the cellular transcription factor Sp1 binds to the promoter proximal viral CRE, and competes with CREB for binding to this sequence (20,21). In addition, the region between the second and third viral CRE (−117 to −163) binds several cellular proteins, including Sp1 and Ets (22±27). Specifically, Sp1 has been shown to bind a GC-rich sequence within this region (22,24). Studies have also suggested that this region may be responsive to Tax (26,28). Although Sp1 has been shown to bind these two distinct regions within the HTLV-1 transcriptional control region, the significance of each site remains to be fully elucidated.

Sp1 is a cellular transcription factor involved in a wide variety of processes, and has been shown to bind to >1000 different promoters to regulate transcription (29,30). It was originally identified as the protein that binds GC elements in the SV40 promoter (31). Sp1 is expressed in all tissue types examined (32). It is an essential protein, as homozygous knockouts of Sp1 in mice result in embryonic lethality (33). The C-terminal DNA binding domain of Sp1 contains three zinc fingers that bind with high affinity to sequences with high GC content, called GC boxes (34). Sp1 has been shown to play a role in transcriptional activation, repression and maintenance of basal transcription of both cellular and viral genes (35). Sp1 has also been shown to be important for the transcription of genes with promoters that do not contain TATA boxes (36±39).

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In this study, we set out to characterize the role of Sp1 in HTLV-1 transcription further. We were first interested in establishing the recognition elements on the HTLV-1 promoter responsible for high-affinity Sp1 binding. Quantitative DNA binding assays demonstrated that Sp1 had the highest affinity for the region between the second and third viral CREs. Chromatin immunoprecipitation assays revealed that Sp1 was present at the chromosomally integrated HTLV-1 promoter. However, Sp1 only modestly activated HTLV-1 transcription when examined both in vivo and in vitro. Finally, a double point mutation of the site responsible for high affinity Sp1 binding dramatically reduced basal level expression of the virus in vivo, while having no effect on Tax transactivation. These results define a role for Sp1 in Tax-independent, constitutive expression of HTLV-1.

MATERIALS AND METHODS

Electrophoretic mobility shift assay

End-labeled, double-stranded (ds) oligonucleotide probes (0.15 mM) were incubated with increasing amounts of purified Sp1 (Promega) for 30 min on ice as described previously (15). Protein–DNA complexes were resolved by electrophoresis on 5% non-denaturing polyacrylamide gels. The top strand sequences of the oligonucleotide probes used in the electrophoretic mobility shift assays (EMAS) are as follows: consensus Sp1, 5'-ATTTCGATCGGCGGGAGGACC-3'; vSp1, 5'-GATGTCACCCAGACACGACCCATTTCCGTA-3' (--149 to --127) (the Sp1 binding GC-box is underlined); vCRE-1 (--251 to --231), 5'-GATCCTCAAGGCTCTGACACTCCCGGA-3'; vCRE-2 (--203 to --183), 5'-GATCTGCTAGCCCTGTGGCTCCCCCTGA-3'; and vCRE-3 (--103 to --83), 5'-GATCCTCAAGGCTCTGACACTCCCGGA-3'. The octanucleotide CRE sequence is underlined in each CRE. For the determination of relative binding affinities, we ensured that the concentration of free Sp1 approximated total concentration of Sp1 by keeping the amount of labeled DNA probe constant and at a low level. Both bound and free probe were quantitated to determine the percent of DNA in complex with Sp1. Analysis of the data was performed with ImageQuant software and Kaleidagraph.

In vitro transcription templates

All DNA templates have been described previously (40). The pHTLV-1/G-less cassette carries the full promoter (upstream to --306), driving expression of a 380-bp G-less cassette. The p4TxRE/G-less cassette carries four reiterated copies of the third viral CRE cloned immediately upstream of the HTLV-1 core promoter (--52), driving expression of a 380-bp G-less cassette.

Chromatin assembly

Nucleosomes were assembled on DNA templates as described previously (41). Following the addition of the DNA, ATP (3 mM), creatine phosphokinase (1 µg/ml) and phosphocreatine (30 mM) were added in a 70-µl reaction containing 10 mM HEPES (K+)(pH 7.6), 50 mM KCl, 5 mM MgCl2 and 5% (v/v) glycerol. Briefly, histone octamers were preassembled with Drosophila NAP-1 (8:1 dNAP-1/core histones) on ice for 30 min. The supercoiled plasmids were assembled into chromatin using dAcf1 and Xenopus histones, at a 0.6:1.0 histone to DNA ratio, overnight at 27°C (42).

In vitro transcription assays

Following chromatin assembly, preinitiation complexes were formed on 150 ng of the plasmid DNA, as described previously (42). All reactions contained 100 µM acetyl CoA (United States Biochemical). CEM (an HTLV-1-negative T-cell line) cell nuclear extract (70 µg) was added immediately following the addition of the activators and/or coactivator. Optimal amounts of Sp1 were empirically determined by titration over a wide concentration range of purified protein. Transcription from the pHTLV-1/G-less chromatin template was analyzed in the presence of Sp1 (16 nM), p300 (20 nM) and Tax (280 nM), as indicated. Following a 60-min preincubation reaction at 30°C, RNA synthesis was initiated by the addition of 250 µM ATP, GTP, CTP and 12 µM UTP plus 0.8 µM [32P-³]UTP (3000 Ci/mmol; New England Nuclear). Transcription reactions were processed and analyzed as described previously (12). Molecular weight markers (radiolabeled HpaII-digested pBR322) were used to estimate the size of the RNA products.

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) assays were performed as described previously (43). Formaldehyde cross-linked chromatin from 10⁶ (SLB-1) or 10⁷ (CHOK1-Luc) cells/antibody was used for immunoprecipitation. Cross-linking reactions were quenched with 125 mM glycine, cells were lysed, and chromatin was sonicated to obtain an average DNA length of 500 bp. Following centrifugation, the recovered DNA was used for PCR amplification (27–30 cycles). Primer-protein complexes were washed and eluted twice with 200 µl of elution buffer. The protein–DNA cross-links were reversed by heating at 65°C overnight, and 10% of the recovered DNA was used for PCR amplification (27–30 cycles).

Antibodies

For the ChIP assays, antibodies against Sp1 and CBP were purchased from Santa Cruz Biotechnology. Tax monoclonal antibody (Hybridoma 168B17-46-92) was obtained from the National Institutes of Health (NIH) AIDS Research and Reagent Program.

ChIP primers

The HTLV-1 promoter primer set for PCR amplification of chromatin from SLB-1 cells was as follows: --290, 5'-TTTCGAGAACAGAGCTCGTAC-3'; --31, 5'-CTCTGACTAGTTATTGGAC-3'. The HTLV-1 promoter primer set for PCR amplification of chromatin from CHOK1-Luc cells was as follows: --349, 5'-GTGAGGGTTGTCGTCA-3'; --81, 5'-AATGACCATGACCCCCA-3'.
Cell culture
CEM cells, Jurkat T-cells and HTLV-1-transformed SLB-1 cells were cultured in Iscove’s modified Dulbecco’s medium supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine and penicillin–streptomycin. Hamster CHOK1-Luc cells (44) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS, 2 mM l-glutamine, penicillin–streptomycin and 500 μg/ml of G418 (Geneticin; Invitrogen).

Mammalian expression plasmids and transient transfection assays
Sp1 was expressed from the pCMV-Sp1 plasmid (courtesy of Robert Tjian). The HTLV-1 Tax expression plasmid has been described previously (45). The reporter plasmid, pHTLV-1/Luc, carries the HTLV-1 promoter driving the luciferase gene. For transient cotransfection assays, cells were grown to a density of 10⁶ cells/ml and transfected with Lipofectamine (Life Technologies, Inc.) and a constant amount of DNA for 5 h. The cells were incubated for 19 h before harvest. Cells were lysed and luciferase activity was measured using the Dual-Luciferase reporter Assay System with a Turner Designs model TD 20-e luminometer. Luciferase activity was normalized to pRL-TK vector (Promega), which encodes the Renilla luciferase from HSV-TK promoter, as an internal control.

Site-directed mutagenesis
The pHTLV/ΔvSp1-Luc reporter plasmid was prepared by PCR-based site-directed mutagenesis. The top strand of the primer set was as follows: 5′-GGGAAGCCACCAA-GAACCAACATTTCTCTCCC-3′. The two underlined nucleotides in the GC box were changed from C (wild type) to A. The mutation was verified by DNA sequence analysis of the full promoter and the 5′ end of the luciferase gene.

RESULTS
Quantitative analysis of Sp1 binding to HTLV-1 promoter elements
Previous studies have shown that Sp1 binds to two distinct sites in the HTLV-1 promoter, one located within a short GC-rich region between the second and third viral CRE (22,24), and the second located within the third (promoter proximal) viral CRE (20,21). We were interested in determining the relative binding affinities of Sp1 for each of these sites. For these studies, we used EMSA to characterize Sp1 binding. Although Sp1 binding was previously shown to occur at the third viral CRE, we tested all three viral CREs (vCRE-1, -2 and -3), since they each contain highly conserved GC-rich sequences. We also tested Sp1 binding to the GC-rich region located between the second and third viral CRE (vSp1; -149 to -127) (see Fig. 1A). As a control we tested Sp1 binding to the consensus site. In these experiments, the amount of radioactively labeled DNA was kept constant, and purified recombinant Sp1 was titrated over a wide concentration range. The binding reactions were analyzed on non-denaturing polyacrylamide gels (Fig. 1B). To determine the apparent binding affinities, the fraction of probe bound versus the total Sp1 concentration was presented graphically (Fig. 1C). The concentration of protein required for 50% binding was used to determine the apparent Kₐ of Sp1 for the indicated DNA sequence (Table 1).

EMSA of Sp1 binding at each of these sequences is shown in Figure 1B. As expected, Sp1 bound the consensus site with the highest apparent affinity (10 nM), with Sp1 binding to the vSp1 site only ~2-fold less than that observed for the consensus site (23 nM) (Fig. 1B, lanes 1–14, and C; Table 1). Surprisingly, Sp1 bound the vSp1 site with 3-fold higher affinity than that observed for the vCRE-3 site (75 nM), suggesting that the vSp1 site may represent the most physiologically relevant Sp1 binding site on the HTLV-1 promoter. Sp1 binding to vCRE-3 was lower than expected, and is much lower than the reported 5 nM binding affinity of CREB for this same element (46).

Sp1 binds to the HTLV-1 promoter in vivo
Although it has been clearly established that Sp1 binds to the HTLV-1 promoter in vitro, we were interested in investigating whether Sp1 binds to the proviral promoter under physiological conditions in the HTLV-1 productively infected human T-cell line SLB-1. ChIP was used to investigate the binding of Sp1 in these cells. Cross-linked SLB-1 chromatin was immunoprecipitated with an antibody to Sp1 and the purified genomic DNA was amplified with primers specific to the HTLV-1 promoter (~31/–290). As shown in Figure 2A, Sp1 is detected at the HTLV-1 promoter in SLB-1 cells (lane 4). As controls, Tax and the coactivator CBP were also immunoprecipitated, and it was found that both proteins were present on the HTLV-1 promoter as described previously (Fig. 2A, lanes 5 and 6) (43). These data indicate that Sp1 binds to the integrated provirus in vivo.

Since SLB-1 cells express large amounts of Tax protein, the previous experiment revealed Sp1 binding to the HTLV-1 promoter in the presence of Tax. However, since Sp1 has been shown to be involved in basal transcription, we were interested in comparing Sp1 binding in the absence and presence of Tax. For these experiments, we used a cell line that contained the HTLV-1 5′ promoter, driving luciferase expression, stably integrated in the genome of the cell line CHOK1 (44). In the absence of Tax, the HTLV-1 promoter in this cell line is transcriptionally repressed, exhibiting low levels of luciferase activity [data not shown and (44)]. Upon transfection of a plasmid that expresses Tax, strong activation of the HTLV-1 promoter was observed (44). Figure 2B revealed the binding of Sp1 to the inactive HTLV-1 promoter (lane 3). Upon transfection of a Tax expression plasmid, the relative amount of Sp1 found at the HTLV-1 promoter remained relatively unchanged. Since the transfection efficiency of these cells was ~50%, we would expect that substantial changes in Sp1 binding would be detected in this assay. Inspection of the data shown in Figure 2B suggests that transfection of Tax actually increased Sp1 binding to the integrated HTLV-1 promoter (Fig. 2B, compare lanes 1 and 3 with 4 and 6). However, quantitative real-time PCR analysis revealed a slight decrease (0.5-fold) in Sp1 binding to the HTLV-1 promoter in the presence of Tax. These data suggest that Sp1 is persistently bound at the HTLV-1 transcriptional control region, and its binding is unaffected by Tax transactivation.
To determine the functional role of Sp1 in living cells, we examined the transcriptional effects of Sp1 on the HTLV-1 promoter in transient transfection assays. In the ChIP assays shown in Figure 2, we detected Sp1 binding at the chromosomally integrated HTLV-1 promoter in CHOK1-Luc cells, indicating that as expected these cells contain endogenous Sp1. We were interested in determining whether enforced overexpression of Sp1 would activate the viral promoter in these cells. Figure 3A shows that the highest amount of an Sp1 expression plasmid increased HTLV-1 transcription ~5-fold. We also tested Sp1 activation of HTLV-1 in the human T-cell line Jurkat. For these experiments, we cotransfected the HTLV-1 luciferase reporter plasmid together with the Sp1 expression plasmid. Comparison of Figure 3A and B reveals that the modest effect of Sp1 on HTLV-1 transcription is similar in both cell types tested. We were also
interested in testing whether Sp1 might cooperate with Tax in transcriptional activation from the HTLV-1 promoter. Figure 3C shows that cotransfection of expression plasmids for Sp1 had essentially no effect on the extent of Tax transactivation.

To examine further the role of Sp1 in HTLV-1 transcription, the function of purified, recombinant Sp1 in an in vitro transcription assay was measured. A DNA template carrying the natural HTLV-1 promoter driving synthesis of a 380-nucleotide guanine-less transcript was used (see Fig. 4A). We chose to analyze the activity of Sp1 in a chromatin context, as we found Sp1 had no activity on non-nucleosomal DNA (data not shown). Chromatin assembly of the HTLV-1 G-less template was performed using the recombinant Drosophila assembly proteins Acf1/ISWI, GST-yNAP-1 and purified Xenopus core histones, as described previously (41,42,47). These assembly proteins are sufficient for the ATP-dependent formation of evenly spaced nucleosomal arrays. Topological assays were performed to determine the optimal ratio of core histones to DNA (data not shown). We performed in vitro transcription assays on the chromatin templates using a nuclear extract from CEM cells (a human T-lymphocyte cell line) as a source of RNA polymerase and general transcription factors. Figure 4B shows that the addition of Sp1 modestly activated (2-fold) HTLV-1 transcription, relative to transcription observed in the absence of activators (compare lanes 1 and 2). The addition of the cellular coactivator p300 only slightly enhanced the activation observed in the presence of Sp1, suggesting that p300 and Sp1 do no cooperate in transcriptional activation (Fig. 4B, lane 4). Strong transcription activation was observed in the presence of purified Tax (Fig. 4B, lane 6); the addition of Sp1 did not significantly enhance Tax transactivation (lane 5). These data suggest that the transcriptional activation seen in the presence of Tax and Sp1 is not synergistic.

Since the vSp1 site has been identified as the high-affinity binding site for Sp1 on the HTLV-1 promoter, we were interested in testing whether this site contributes to the 2-fold Sp1 activation observed in the in vitro transcription assay.

### Table 1. Apparent dissociation constants of various recognition elements

<table>
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<th>Recognition element</th>
<th>Apparent $K_d$</th>
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<tr>
<td>Consensus Sp1 site</td>
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</tr>
<tr>
<td>vSp1 site</td>
<td>23 nM</td>
</tr>
<tr>
<td>vCRE-1</td>
<td>NA</td>
</tr>
<tr>
<td>vCRE-2</td>
<td>NA</td>
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<tr>
<td>vCRE-3</td>
<td>75 nM</td>
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*Apparent $K_d$ is defined as the concentration of Sp1 at the midpoint of binding.

NA, not available.

Figure 2. Sp1 binds the chromosomally integrated HTLV-1 promoter. (A) ChIP analysis showing Sp1 binding to the HTLV-1 promoter in infected SLB-1 cells. PCR results from coimmunoprecipitation reactions using no antibody, preimmune rabbit serum (IgG), and antibodies against Sp1, CBP and Tax are shown. Each panel shows amplification of 0.4% of the total input chromatin (input). Purified DNA was analyzed by PCR using primer sets that amplify the viral promoter (–31 to –290). DNA size standards are indicated. (B) ChIP analysis showing Sp1 proteins bound to the integrated HTLV-1 promoter in CHOK1-Luc cells (44) in the absence and presence of transfected Tax. PCR results from input, preimmune serum (IgG), and antibody against Sp1 are shown. The cells were either transfected with pUC (as a control) or the HTLV-1 Tax expression plasmid, as indicated. Transfection efficiencies of ~50% were obtained with these cells. Purified DNA was analyzed by PCR using primer sets that amplified the viral promoter (–349–81).

Figure 3. Sp1 modestly activates HTLV-1 in transient transfection assays (A). An expression plasmid for Sp1 (pCMV-Sp1; 400 ng and 800 ng) was cotransfected into CHOK1-Luc cells. Luciferase expression was driven by the integrated HTLV-1 5′ promoter (44). The values shown are luciferase units (in duplicate) ± standard deviation. (B) The pHTLV-1/Luc reporter plasmid (200 ng) was cotransfected into Jurkat cells with increasing amounts of the expression plasmid for Sp1 (pCMV-Sp1; 400 ng and 800 ng). (C) The pHTLV-1/Luc reporter plasmid (200 ng) was cotransfected into Jurkat cells with the expression plasmid for Tax (pHTLV/Tax; 200 ng) and/or the expression plasmid for Sp1 (pCMV-Sp1; 300 ng), as indicated.
described above. To perform this experiment, we used a DNA template that carries four reiterated copies of the third viral CRE, cloned upstream of the first 52 bp of the core HTLV-1 promoter (see Fig. 4C). This transcription template carries the four lower affinity Sp1 binding sites (vCRE-3) previously identified (20,21), but does not contain the vSp1 site. This construct is highly responsive to Tax/CREB activation (19,42). The 4TxRE/G-less plasmid was assembled into chromatin, and transcription was assayed in the presence of purified Sp1. Figure 4D shows that Sp1 has no effect on this template (compare lanes 1 and 2). As expected, p4TxRE/G-less was strongly activated by Tax (Fig. 4D, lane 5). These results suggest that the vSp1 site is the major cis-acting element for Sp1 function on the HTLV-1 promoter.

**Sp1 plays a role in constitutive expression of HTLV-1**

As shown above, functional assays with Sp1 revealed only modest activation of HTLV-1 transcription both in vitro and in vivo. We reasoned that this modest activation may be due
to near saturating levels of endogenous Sp1 present in the Jurkat cells and the CEM nuclear extracts. This endogenous Sp1 may have been sufficient for HTLV-1 transcription, thereby masking the effects of exogenous Sp1. To evaluate endogenous Sp1 levels, western blot analysis was performed. Figure 5A shows that both Jurkat and CEM whole-cell extracts contain comparable levels of Sp1. This endogenous Sp1 may therefore play a significant role in activating HTLV-1 transcription in each of the functional assays performed above, rendering the HTLV-1 promoter essentially insensitive to exogenous Sp1.

To circumvent this problem, and to test directly the role of Sp1 in HTLV-1 transcription, site-directed mutagenesis was used to create a double point mutation in the high-affinity vSp1 site. This site was selected as Sp1 binds to this region with a 3-fold higher affinity than vCRE-3, as shown above. This mutation left the remainder of the HTLV-1 reporter plasmid fully intact. We then compared this construct, named pHTLV/ΔvSp1-Luc, with the wild-type reporter plasmid in transient transfection assays. Surprisingly, the basal level of transcription from pHTLV/ΔvSp1-Luc was significantly reduced (15-fold) compared with the wild-type HTLV-1 reporter plasmid (Fig. 5B). Furthermore, pHTLV/ΔvSp1-Luc was unresponsive to increasing amounts of the Sp1 expression plasmid (Fig. 5B, lanes 5–8). To ascertain whether this mutation in vSp1 influenced Tax transcription function, we transfected an expression plasmid for Tax. Figure 5C shows that both the wild-type and pHTLV/ΔvSp1-Luc reporter constructs are similarly responsive to Tax. These data suggest that Sp1, through specific binding to the vSp1 site, plays a prominent role in basal level expression of HTLV-1. Furthermore, the data also indicate that the vSp1 does not participate in Tax transactivation.

DISCUSSION

Previous studies have shown that Sp1 binds to two distinct promoter elements in the HTLV-1 promoter. The first is a GC box located between the second and third viral CRE (22,24). This site was originally identified by DNase I footprinting, and purified Sp1 was shown to bind between nucleotides −149 and −127 of the HTLV-1 promoter (vSp1) (22). This sequence is functionally responsive to a transfected Sp1 expression vector in Sp1-deficient Schneider cells, and cooperates with the cellular transcription factor Ets in HTLV-1 transcriptional activation (24). The second Sp1 binding site is located within the upstream GC-rich sequence of the third viral CRE (vCRE-3) (20,21). This sequence has also been shown to bind purified Sp1, and is functionally responsive to Sp1. Unfortunately, the respective contribution of these two sites to HTLV-1 transcription by Sp1 has not been addressed.

In this study, we set out to compare the relative binding affinities of Sp1 for these two regions. Quantitative EMSAs revealed that Sp1 binds with 3-fold higher affinity to the vSp1 site relative to the vCRE-3 site, a result corroborated by oligonucleotide competition assays. The affinity of Sp1 for the vSp1 site was within 2-fold of that observed for a consensus Sp1 site, indicating relatively high affinity binding. To establish the physiological relevance of Sp1 binding, we also tested whether Sp1 bound to the chromosomally integrated HTLV-1 promoter in vivo. Using ChIP, Sp1 binding on the integrated HTLV-1 promoter was detected in two cell lines. Sp1 binding was comparable in the absence and presence of Tax, suggesting that under conditions of strong HTLV-1 transcriptional activation, Sp1 binding remains unchanged. Together, these binding data suggest that Sp1 plays a physiological role in the regulation of basal HTLV-1 transcription, and that the effects of Sp1 are likely mediated primarily through the vSp1 site.

The two previous studies that defined the promoter proximal viral CRE as the major Sp1 binding site did not simultaneously examine Sp1 binding at vSp1 (20,21), and therefore may have been unaware of the significance of this element. Although the affinity of Sp1 for vCRE-3 is 75 nM (compared with 23 nM for vSp1) under conditions of high Sp1 protein concentrations, it is probable that Sp1 also binds to the third viral CRE. However, since CREB displaces Sp1 from this sequence (21), and since the binding affinity of CREB for
vCRE-3 is only 5 nM, it is unlikely that Sp1 occupies this site under most conditions in vivo.

We also examined the transcriptional effects of Sp1 both in transient transfection assays and in vitro transcription assays using chromatin-assembled DNA templates. We found that in all cases, Sp1 only modestly activated HTLV-1 transcription. However, we found that endogenous Sp1 was present in the cell lines and extracts used in the assays, and reasoned that this may account for the low-level activation observed upon Sp1 addition. To address this issue, we prepared a double point mutation in the GC box at the vSp1 binding site (CC → 135→136 → AA) and found that basal transcription levels in the transient transfection assay were dramatically reduced. Interestingly, this mutation had no effect on Tax transactivation.

These data support a prominent role for Sp1 in basal HTLV-1 expression and raise the question of whether Sp1 participates in activated viral transcription. We have not observed a synergistic effect of Sp1 on Tax transactivation in vivo or in vitro (data not shown), however it is has been shown that Sp1 cooperates with the cellular transcription factors Ets and p53 in Tax-independent activated transcription (24,48). Perhaps the significantly higher levels (15-fold) of basal transcription observed with the wild-type HTLV-1 promoter, relative to the vSp1 mutant HTLV-1 promoter, reflect a synergy between Sp1 and other cellular factors binding within this region. Together, the data indicate that Sp1, specifically via the vSp1 site, is required for supporting physiological expression of the HTLV-1 genome in the absence of Tax.

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