Transcriptional activity of the human immunodeficiency virus-1 LTR promoter in fission yeast

Schizosaccharomycodes pombe

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ABSTRACT
We have analyzed the transcriptional activity of the human immunodeficiency virus type I (HIV-1) LTR promoter in the fission yeast Schizosaccharomycodes pombe (S.pombe). The ability of a series of 5'-deleted forms of the HIV-1 LTR promoter to direct transcription of the chloramphenicol acetyltransferase reporter gene was studied. We found that the HIV-1 promoter is functional in S.pombe and that deletion of sequences upstream of the NF-kB binding site previously identified to contain the negative regulatory element (NRE) in mammalian cells, resulted in an about thirty-fold increase in transcriptional activity. Sequences in the HIV-1 promoter that bind NF-kB were found to be essential for transcriptional activation in S.pombe. In mammalian cells, transactivation of the HIV-1 LTR requires TAR sequences and the viral Tat protein. In fission yeast, Tat failed to transactivate the HIV-1 LTR, suggesting that S.pombe may lack a cellular factor(s) required for the Tat transactivation process.

INTRODUCTION
The human immunodeficiency virus type 1 (HIV-1) encodes several regulatory genes, which are essential for virus production (1-4). The HIV-1 Tat protein is essential for transactivation of the HIV-1 LTR promoter (2, 5-8). The Tat protein requires a cis-acting RNA sequence called TAR which is located downstream of the transcription start site (+19 to +42) (9-11). The TAR region is capable of forming a stable RNA stem-loop secondary structure (6). In vitro, the Tat protein binds to the bulge region of the TAR RNA, and mutations within this region abolish transactivation (12, 13). Mutations within the loop region abolish Tat transactivation even though the Tat protein is still capable of binding to the TAR RNA (12). Since a 68 kD cellular protein has been shown to bind to the loop region of TAR RNA (14), the data would imply that both Tat and a cellular factor(s) are required for transactivation. Regulation of the HIV-1 promoter activity by Tat has been proposed to occur by several mechanisms. Tat has been found to increase the rate of initiation of HIV-1 transcription and also to stimulate elongation of short transcripts within the HIV-1 LTR leader sequences, possibly by antitermination mechanisms (7, 8, 10, 11, 14-16). Binding sites for the transcriptional factor Sp1 have been shown to be required for Tat-dependent transactivation (17) leading to the suggestion that the interaction of Tat with Sp1 may be one mechanism responsible for the increased rate of transcription initiation.

Studies of HIV-1 transcription in mammalian cells have identified a number of cis-acting regulatory elements such as TATA box and NF-kB and Sp1 binding sites. The HIV-1 LTR contains a negative regulatory element (NRE) upstream of the NF-kB binding site (position -420 to -157 as defined in ref. 18; Figure 1), deletion of which results in an increased rate of Tat-dependent transcription (18). Specific sequences within the NRE which bind the transcription factors NFAT-1 and USF have been shown to contribute to the inhibitory effect of NRE on HIV-1 promoter activity (18).

Recently, the transcriptional machinery of yeast and mammalian cells has been shown to be highly conserved (19, 20). For example, the TATA box binding factor TF-IID of yeast will complement for the mammalian TF-IID factor in in vitro transcription assays (21). Also, mammalian enhancer sequences and cellular transcription factors will activate transcription in yeast (19). A number of mammalian promoters, including the SV40 early, human cytomegalovirus (CMV), human chorionic gonadotropin α-subunit (CGα) and Adenovirus region 3 promoters, have been shown to be functionally active in the fission yeast Schizosaccharomycodes pombe (S.pombe) (20). The transcription start sites of these promoters in S.pombe correspond precisely to the authentic 5' RNA ends observed in mammalian cells (20).

In this study, we have characterized the transcriptional activity of the HIV-1 LTR promoter in S.pombe. We found that the HIV-1 LTR promoter has a weak activity in this system. Deletion of NRE sequences within the HIV-1 LTR resulted in a
considerable increase in promoter activity which was dependent on the presence of the NF-kB binding sites located downstream of the NRE. Unlike in mammalian cells, expression of the Tat protein in S. pombe did not lead to an increase in HIV-1 LTR promoter, activity.

METHODS

Strains and media

The S. pombe genotypes ura4-294 h- (ATC38436) and ura4-294 leu1-32 h- (gift from K. Okazaki) were used as hosts for transformation. A modified minimal medium (22) was used for growing S. pombe.

Plasmid construction

JB12 (kindly provided by Julie Brown), a modified yeast replicating vector, was derived from S. pombe replicating vector pIRT1 containing the URA3 gene of Saccharomyces cerevisiae (S. cerevisiae) as a selectable marker for transformation (kindly provided by A. Klar). A 146 bp DNA fragment containing a SacI restriction site at its 5' end followed by two poly A signals in opposite orientation (underlined), restriction sites for Ball, BglIII, XhoI, KpnI, EcoNI, NruI, NotI, and EagI, a second stretch of two poly A signals in opposite orientation (underlined), and a BamHI site at its 3' end (5'-AGCTCGCGC AATAAA AGATA-TTTATTTCATAGATATGTGGTGTTGTTTGTGGTGCGCAAGATCTTCTCAGGTAAGCGCCCGGC-AAAAATAAAATCAATGGCAATATGGTTGTTA ACTTG-TTATTGTGACGGATC-3') was inserted at the SacI and BamHI sites of the pIRT1 multiple cloning site to create JB12. A series of HIV-1 LTR promoter constructs, containing different 5' promoter deletions, the CAT reporter gene, and SV40 early splice and poly A signals (23) were subcloned into plasmid pBR322 using Xbal (5' end) and BamHI (3' end) linkers (kindly provided by J. Rappaport). The Xbal-BamHI fragments were cut out from the different HIV-1 LTR promoter deletion constructs, blunt-ended, and cloned into the Ball site present in the polylinker region of JB12. The clones were designated pCHIV452 through pCHIV65, the numbers corresponding to the number of nucleotides upstream of the RNA start site (+1) present in these constructs (Figure 1). Clone pCHIV80 was generated by PCR using clone pCHIV452 as a template. The upstream primer contained a Stul restriction site at the 5' end followed by a sequence corresponding to nucleotides -80 to -66 of the HIV-1 LTR. The downstream primer was 5'-GCTTCCTGG GCATGGACTGAGAAGCGCCCGGC-AAAATAAAATCAATGGCAATATGGTTGTTA ACTTG-TTATTGTGACGGATC-3'. The resulting 325 bp PCR product was inserted into an active BamHI site at the 5' end, downstream (5'-TGGCCATGGCCTCAGGT-CACAACGCTAGGTAATCTGGAACATCGTGATGGTA-TTCTTCCGGGTCTGC-3'), HA sequence is underlined, XhoI site at the 5' end). The resulting 325 bp PCR product was cloned into pCMVL at the HindIII (5') and XhoI (3') sites of the CMV promoter.

Transformation

A high efficiency transformation protocol was used as previously described (22). HIV-1 LTR promoter deletion constructs were transformed into S. pombe ura4-294 h- (Figures 2 and 5) or ura4-294 leu1-32 h- (Figure 3) either alone or together with pCMVL-tatHA or pCMVL. S. pombe transformants containing pCMVL-tatHA showed no detectable changes in growth rate.

Extract preparation and CAT assay

Transformants (approximately 1.5×10^6 cells) were plated on minimal medium plates and cultured for 5 days at 30°C. Transformants were harvested, washed twice with water, and suspended in 1 ml of a solution containing 1 M sorbitol, 0.1 M EDTA, and 14 mM 2-mercaptoethanol. The cells were then treated with lyticase (Sigma, 0.1 units per μl) at 30°C for 1 hr.

![Diagrammatic representation of the HIV-1 LTR promoter showing upstream promoter deletion mutants. The positions of the regulatory domains including the negative regulatory element (NRE), NF-kB, Sp1, TATA and TAR sequences are indicated. The 5'-end of the RNA corresponds to position +1. The numbering of the pCHIV deletion constructs refers to the 5'-end points of the LTR sequences. pCHIV452 contains the entire HIV-1 LTR, with 452 nucleotides upstream of the 5' end of the RNA. All constructs were cloned into the Ball restriction site within the polylinker region of JB12, an pIRT1 modified yeast replicating vector containing the URA3 gene of Saccharomyces cerevisiae as a selectable marker. The polylinker is flanked by mammalian poly A sites at both ends and in both orientations.](image)
The spheroplasts were harvested by centrifugation at 3000 rpm (Beckman table top centrifuge) for 10 min at 4°C, and resuspended in 1.2 ml of 92 mM Tris–HCl (pH 7.4) containing 42 mM EDTA. The cells were sonicated on ice by a Branson sonifier 450 (output control 2.5) for 1 min and cell debris was removed by centrifugation. Protein concentrations of the extracts were measured according to Bradford (26) using the BioRad protein assay kit. CAT assays were performed according to published protocols using 1 μg of protein extract (20).

35S labelling of proteins and immunoprecipitation

Exponentially growing cultures (100 ml in minimal medium) of S. pombe transformed with pCMVL or pCMVL-tatHA were harvested by centrifugation at 3000 rpm (Beckman table top centrifuge) for 20 min at 4°C. The cells were resuspended in 10 ml of minimal medium and labelled with 35S-Cysteine (100 μCi/ml) for 1 hr. Cells were collected by centrifugation then washed 2 times with 1 ml of the cell breakage buffer (0.1 M Tris–HCl [pH 7.5], 1 mM DTT, 20% glycerol and a mixture of the protease inhibitors (100 μg/ml each) N-Tosyl-L-lysine chloromethyl ketone (TLCK), N-Tosyl-L-phenylalanine chloromethyl ketone (TPCK) and Phenylmethanesulfonyl fluoride (PMSF)). Cells were resuspended in 0.3 ml of cell breakage buffer, and acid washed glass beads (Sigma, 425—600 μm) were added to just below the meniscus. Cells were vortexed vigorously in 1.5 ml microfuge tubes 6 times for 1 min, keeping the cells on ice during the intervals. Cell extracts were separated from glass beads, and about 0.3 ml of extract was obtained. The extract was diluted with modified RIPA buffer (20 mM MOPS, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% deoxycholate, 0.1% SDS; pH 7.0) containing 1% apoprotein. Cell debris was removed by centrifugation. 1 ml of extract was incubated with 2 μl of monoclonal antibody 12CA5 (obtained from Berkeley Antibody Inc.) directed against the 11 amino acid HA epitope (see above) for 4 hr at 4°C. 50 μl of protein A Sepharose CL-4B beads (Pharmacia) were added and extracts were allowed to incubate for an additional 90 min. Following five times washes in modified RIPA buffer, Sepharose beads were resuspended in 30 μl of sample buffer for electrophoresis, heated at 100°C for 5 min, and separated on 1.5% SDS–polyacrylamide gels. Gels were then fixed with glacial acetic acid/methanol, treated with Enlightening (NEN), dried and autoradiographed.

Northern blot analysis and primer extension

Total RNA was extracted from S. pombe transformants as previously described (20). 10 μg of RNA was denatured and fractionated on 1% agarose gels containing 6.7% formaldehyde. RNA was transferred to nitrocellulose filters and hybridized to a probe (32P-labelled by nick translation) containing either the whole CAT coding sequence, the entire tatHA coding sequence or a fragment of the S. pombe actin gene. The DNA fragments were cut out from the blot.

Figure 2. Transcriptional activity of HIV-1 LTR promoter deletion mutants. S. pombe cells were transformed with the different HIV-1 LTR promoter deletion constructs shown in Figure 1. The results of CAT assays (A) and Northern blot analysis (B) are shown. Protein (A) or total RNA (B) was extracted from transformants carrying the following plasmids: pCHIV452 (lane 1, full length LTR); pCHIV278 (lane 2); pCHIV176 (lane 3); pCHIV117 (lane 4); pCHIV80 (lane 5); pCHIV65 (lane 6). 1 μg of protein extract was used for the CAT assays. Conversion rates of 14C chloramphenicol product (given as % acetylation/30 min; mean values ± S.E.M.; n = 3) were the following: pCHIV452 (0.78 ± 0.03), pCHIV278 (1.3 ± 0.2), pCHIV176 (1.2 ± 0.1), pCHIV117 (29 ± 7), pCHIV80 (1.4 ± 0.1), pCHIV65 (0.12 ± 0.02). In panel B (upper section), a 0.9 kb BamHI restriction fragment containing the entire CAT coding sequence (cut out from pDCAT; H.Okayama, unpublished data) was used as a probe. The lower section of panel B shows the same blot reprobed with a fragment of the S. pombe actin gene (see Methods) as a control. The molecular size markers (kb) are shown on the left of the blot.

Figure 3. Transcriptional activity of the HIV-1 LTR promoter in the presence and absence of Tat. S. pombe cells were transformed with different HIV-1 LTR promoter deletion constructs together with pCMVL-tatHA or PCMVL (control). S. pombe extracts were assayed for CAT activity (A) and the presence of CAT mRNA (B). pCHIV452 (lane 1), pCHIV117 (lane 2), or pCHIV65 (lane 3) were cotransformed with pCMVL-tatHA. In parallel, pCHIV452 (lane 4), pCHIV117 (lane 5), or pCHIV65 (lane 6) were cotransformed with PCMVL which does not contain the tat gene. 1 μg of protein extract was used for the CAT assays (A). Conversion rates of 14C chloramphenicol product (given as % acetylation/30 min; mean values ± S.E.M.; n = 3) were: pCHIV452 + pCMVL-tatHA (0.59 ± 0.2), pCHIV117 + pCMVL-tatHA (10.2 ± 1.2), pCHIV65 + pCMVL-tatHA (0.15 ± 0.03), pCHIV452 + PCMVL (0.36 ± 0.10), pCHIV117 + PCMVL (17.4 ± 3.5), pCHIV65 + PCMVL (0.21 ± 0.01). (B) The probes used for the Northern analysis are described under Methods. A fragment of the S. pombe actin gene was used as a control probe (panel B, lower section). The molecular size markers (kb) are shown on the left of the blot. (C) Immunoprecipitation of TatHA fusion protein. S. pombe cells transformed with pCMVL-tatHA (lane 1) or PCMVL (lane 2, control) were labelled with 35S-cysteine as described under Methods. TatHA fusion protein was immunoprecipitated with monoclonal antibody 12CA5 directed against the HA epitope (see Methods). The molecular size markers (kD) are shown on the left of the panel.
used as probes were prepared as follows: The entire CAT coding region (0.9 kb) was excised from pDCAT (gift from H.Okayama) with BamHI. For the tat probe, the whole HTV-1 LTR coding region was generated by PCR using pCMVL-tatHA as a template and the upstream primer 5'-CTCGAGTTACTGACAGAGGAGACGAAGAAGA-3' and the downstream primer 5'-ATGGATCCATCAGAAGCTGATACGTGTTACACATCGATGGGTATTCCCGGACCCTGTCG-3' which covered with whole HA epitope. A 400 bp S.pombe actin gene fragment corresponding to nucleotides 1083 to 1484 (as defined in ref. 27) was generated by PCR using 5'-CAACCCCTGACGTCTTT-3' as an upstream primer, 5'-TCAGAAGAATCGATGT-3' as a downstream primer, and a S.pombe genomic DNA library (28) as a template. The actin gene probe was used as a control probe to determine the amount and quality of yeast RNA loaded onto the gels. This fragment hybridizes with the three transcripts of the actin gene which are 1.8, 1.6 and 1.2 kb long.

For promoter extension studies, an antisense oligonucleotide (5'-CAACCGTGTATATCTCACT-3') corresponding to nucleotides +12 to +31 of the CAT coding sequence was labeled at its 5' end using 32P-ATP and T4 polynucleotide kinase and annealed to total yeast RNA. Primer extension was performed as described (20). The reaction mixture was fractionated on a 6% polyacrylamide gel containing 8 M urea. A dideoxy sequencing reaction was run in parallel as a size marker.

RESULTS AND DISCUSSION

Transcription

The transcriptional activity of various HIV-1 LTR promoter deletion constructs (Figure 1) was measured by assaying the chloramphenicol acetyltransferase (CAT) activity in protein extracts prepared from S. pombe transformants. In addition, gene expression was monitored by Northern blot analysis using the CAT gene as a radiolabelled probe. The plasmid containing the whole HIV-1 LTR (pCHIV452) and constructs with 5' promoter deletions extending downstream to -176 (pCHIV278 and pCHIV176) showed about 1% conversion of radiolabelled chloramphenicol (Figure 2A, lanes 1-3). pCHIV117, in which sequences upstream of the two NF-kB binding sites of the HIV-1 LTR promoter have been deleted, showed an about 30-fold increase in CAT activity compared to pCHIV452 (Figure 2A, lane 4). pCHIV80 was at least 15 times less active than pCHIV117 (Figure 2A, lanes 4 and 5). This plasmid (pCHIV80) does no longer contain the two NF-kB binding sites, but still has three copies of the Spl binding site (Figure 1). pCHIV65, which contains the core promoter and one and a half binding sites for transcription factor Spl, did not give any detectable CAT activity (Figure 2A, lane 6).

The results obtained by Northern blot analysis (Figure 2B) were in good agreement with the data obtained in the CAT assays (Figure 2A). Transformants containing pCHIV117 (lane 4) showed significantly higher levels of CAT mRNA (~1.35 kb) than pCHIV452, pCHIV278, or pCHIV176 (Figure 2B, lanes 1-4). The amount of CAT mRNA seen with pCHIV80 (lane 5) was low, similar to that obtained with pCHIV452, pCHIV278, and pCHIV176. No CAT mRNA transcript was observed with pCHIV65. In this case only a high molecular weight band appeared on the Northern blot which is usually seen when the promoter activity of the CAT reporter construct is extremely low (R.Toyama, unpublished data). As a control, the same blot was hybridized to a S.pombe actin gene probe, verifying that equal amounts of RNA were loaded in each lane of the gel.

These data suggest that sequences between nucleotides -176 and -117 of the HIV-1 LTR promoter repress gene expression, and that the region containing the NF-kB sequences (-117 through -81) are required for strong HIV-1 LTR promoter activity in S.pombe. It is unlikely that the construct pCHIV117 results in an artifactual positive cis-element at the junction of vector and -117 site, since an oligonucleotide -112 to -79 is able to efficiently activate a heterologous promoter (see below for details in the activation of heterologous promoter). In mammalian cells, a number of transcription factors including AP1 (-349 to -330), NFAT-1 (-254 to -216) and USF (-173 to -159) recognize sequences within the NRE (18). Deletion of NFAT-1 and USF sequences within the HIV-1 LTR results in an increase in gene expression in mammalian cells (18). In this study, deletion of the segment between -176 to -117 of the HIV-1 LTR allowed NF-kB sequences to activate transcription from the HIV-1 LTR promoter in S.pombe. Interestingly, this sequence element (-176 to -117) contains a binding site for USF (-173 to -159) which has been shown to contribute to the repression of HIV-1 LTR promoter activity in mammalian cells (18). A more detailed analysis may be required to gain insight whether S. pombe contains an USF-like transcription factor and whether the recognition sequence is identical to that found in mammalian cells.

The HIV-1 Tat protein is known to bind to the TAR RNA element (Figure 1) and to transactivate transcription from the HIV-1 LTR promoter (12, 13). Control of transactivation can occur at different levels including increased rate of initiation of transcription or elongation of the short RNAs which truncate within the HIV-1 leader sequence (7, 8, 10, 11, 14-16). We have tested the possibility that Tat may transactivate the HIV-1 LTR promoter in the fission yeast. Initially, the plasmid pCMVL-tatHA encoding the Tat protein tagged with a short sequence encoding a 11 amino acid epitope (HA) of the Haemophilus influenzae haemagglutinin protein (25) to allow its immunological
Detection, was tested for its ability to induce transactivation of the HIV-1 LTR promoter in Hela cells. Transactivation of the HIV-1 LTR by the TatHA fusion protein was compared to that by the Tat protein without the HA epitope. We found that the TatHA fusion protein was able to transactivate the HIV-1 promoter as efficiently as the Tat protein alone (data not shown). Subsequently, the ability of pCMVL-tatHA to induce synthesis of TatHA protein in S. pombe was tested by labelling exponentially growing S. pombe transformants with [35S]-cysteine. In parallel, cells transformed with the vector (pCMVL) alone were processed as a control. Figure 3C shows that antibody 12CA5 was able to immunoprecipitate TatHA protein in cells transformed with pCMVL-tatHA but not in those transformed with pCMVL alone. Our effort to map the subcellular localization of the TatHA fusion protein in S. pombe was not successful because of the high background observed with the 12CA5 monoclonal antibody in the absence of Tat.

The yeast strain [genotype: ura4-294 leu1-32 h-] was cotransformed with pCMVL-tatHA and various HIV-1 LTR promoter deletion constructs described above. The plasmid pCMVL which does not carry the tatHA gene was used as a control. Transformation of S. pombe with pCHIV452, pCHIV117 or pCHIV65 together with pCMVL-tatHA resulted in CAT activities (Figure 3A, lanes 1–3) or CAT mRNA levels (Figure 3B, lanes 1–3) similar to those observed after cotransformation with pCMVL (Figure 3A, B, lanes 4–6). Tat mRNA was found in cells cotransformed with pCMVL-tatHA but not in those cotransformed with pCMVL (Figure 3B). Our data therefore suggest that Tat does not transactivate the HIV-1 LTR promoter in the fission yeast.

**Primer extension**

The 5′ end of the TAR RNA sequence is important for Tat to transactivate the HIV-1 LTR promoter in mammalian cells (10). In order to examine the possibility that the demonstrated lack of Tat transactivation of the HIV-1 LTR in S. pombe may be due to a different site of transcription initiation in S. pombe (resulting in a loss of TAR sequences), primer extension analysis was performed. An antisense oligonucleotide (corresponding to nucleotides +12 to +31 of the CAT coding sequence) was 5′ end labelled, annealed to total RNA prepared from yeast cells cotransformed with pCHIV117 and pCMVL (Figure 4, lane 1) or cotransformed with pCHIV117 and pCMVL-tatHA (Figure 4, lane 2), and extended with reverse transcriptase. The 5′ end analysis showed two major clusters of 5′ ends, each cluster containing 3 to 4 bands (Figure 4). The major 5′ ends were located 142 and 150 nucleotides away from the 5′ end of the primer. The transcription initiation start site(s) of the HIV-1 LTR in S. pombe is therefore very similar to that found in mammalian cells (147 bases away from the 5′ end of the primer). In fact, there are minor transcripts in S. pombe whose synthesis is initiated exactly at the same site as in mammalian cells (Figure 4). Since the transcripts found in S. pombe would allow transactivation of the HIV-1 LTR by Tat in mammalian cells, the observed lack of transactivation by Tat in S. pombe appears not to be due to different points of transcription initiation.

Recent reports suggest that transactivation of the HIV-1 promoter by Tat is partly dependent on the presence of Spl binding sites within the core promoter (17). One possible explanation for the inability of Tat to transactivate transcription from the HIV-1 LTR promoter in S. pombe may therefore be the lack of the Spl transcription factor in the fission yeast. Alternatively, S. pombe may lack a cellular factor(s) (other than Spl) necessary for the Tat transactivation process which has been proposed to exist in mammalian cells (17).

**Activation of a heterologous promoter by NF-kB sequences**

As outlined above, a short segment in the HIV-1 LTR (−117 to −81) containing two NF-kB recognition sequences is responsible for the increase in promoter activity seen after removal of upstream promoter sequences. To further characterize this sequence element we inserted a 34 bp oligonucleotide (5′-GCTACAAGGGACTTTCCGCTGGGGACTTTCCAGG-3′) (−112 to −79; NF-kB sequence shown underlined) upstream of

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**Figure 5.** Transcriptional activity of pCEH core vector constructs containing NF-kB binding sequences. (A) An oligonucleotide corresponding to the NF-kB protein recognition sequence −83 to −105 (Figure 1) or the mutant sequence, NF-kBm, was inserted into the polylinker site upstream of the pCEH core promoter vector. pCEH contains the CGA core promoter directing transcription of the CAT reporter gene. (B) CAT analysis of protein extracts prepared from S. pombe cells transformed with pCEH (lane 1, control), pCEH-NF-kB (lane 2) and pCEH-NF-kBm (lane 3). Conversion rates of [14C]chloramphenicol product (given as% acetylation/30 min; mean values ± S.E.M; n = 3) were: pCEH (1.67 ± 0.24), pCEH + NF-kB (17.6 ± 0.4), pCEH + NF-kBm (6.17 ± 0.55).
the CGα core promoter into the yeast replicating vector pCEH. This URA3 based vector contains the CGα core promoter upstream of the CAT gene flanked by a 5' polylinker region which allows the insertion of enhancer sequences (Figure 5). For comparison, an additional oligonucleotide (5'-GCTACAACTCACTTGCCTGGCTCACCCTTCCAGG-3') containing mutations (shown in bold lefters) in the NF-κB protein recognition sequence which abolishes NF-κB binding in mammalian cells (29) was also cloned into pCEH (Figure 5). Yeast cells were transformed with both plasmids and CAT activities were determined in cell extracts as described. Transformants containing wild type NF-κB sequences displayed (Figure 5). Yeast cells were transformed with both plasmids and NF-κB binding in mammalian cells (29) was also cloned into pCEH. Together with the results presented in Figures 2 and 3, these data suggest the existence of a cis-acting element containing two NF-κB binding site (between -117 to -81) in the HIV-1 LTR which is required for promoter activation in the fission yeast. However, our data do not show that the transcription factor which recognizes this sequence element in S.pombe has a recognition sequence identical to that of NF-κB.

It is unclear whether S.pombe will serve as a useful system for the study of the different functional domains of the HIV-1 LTR promoter, because the cis-acting sequences identified here may interact with yeast factors not relevant to the process in mammalian cells. On the other hand, the inability of Tat to activate the HIV-1 LTR promoter in S.pombe may allow the isolation of genes which are involved in mediating this process in mammalian cells. To test this, we are presently developing a genetic selection system based on cotransformation of S.pombe with mammalian cDNA libraries.

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