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NF-κB-Mediated Activation of the Human Immunodeficiency Virus Enhancer: Site of Transcriptional Initiation Is Independent of the TATA Box

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The activation of the human immunodeficiency virus (HIV) enhancer in T cells can occur through multiple independent pathways. This enhancer is stimulated by NF-κB or through alternative mechanisms, including E1A transactivation, which is dependent on the TATA box. In this report, the role of the TATA box in activation by NF-κB is examined. E1A stimulation of the HIV enhancer requires the presence of the TATA sequence in the sense orientation. Analysis of mutant HIV enhancer plasmids shows that basal mRNA levels are reduced when the TATA sequence is altered but that inducibility of NF-κB and the site of transcriptional initiation are unchanged. These data suggest that transcriptional initiation in this class II promoter is determined by an initiator factor which does not require binding to the TATA sequence. Because κB is found in a variety of viruses, this mechanism may be relevant to the activation of other viral enhancers.

Transcription of human immunodeficiency virus type 1 (HIV-1) in T lymphocytes is regulated by DNA-binding proteins which recognize sites in the regulatory region of the viral long terminal repeat. Stimulation of T cells by phorbol esters or cytokines (tumor necrosis factor alpha and interleukin-1 [18]) results in increased expression of HIV, mediated by the inducible transcription factor NF-κB (16). In addition, the HIV-1 enhancer can be stimulated by several viral transactivators, including herpesvirus ICP0 (17) and adenovirus E1A, which acts through the TATA box (17). Because the HIV enhancer is activated by NF-κB and is also responsive to stimulation by E1A, the possibility of an interaction between these two regulatory elements was raised. We have examined the role of the TATA sequence in activation of the HIV enhancer by NF-κB. Because κB is present in several primate virus enhancers (1, 4, 5, 14), this mechanism has relevance both to TATA box function and also to a basic understanding of virus activation.

To determine the role of the TATA box in activation by NF-κB, Jurkat cells stably expressing the *tat-I* gene, Jurkat *tat-III* (9), were cotransfected by using DEAE-dextran (16) with the HIV enhancer linked to the chloramphenicol acetyltransferase gene (CAT) and with a plasmid which expressed adenovirus E1A or with a control (pUC13). Previous studies have shown that the presence of the *tat-I* gene improves the detection of low levels of HIV CAT activity but does not otherwise affect these regulatory signals compared with those of Jurkat cells (17). Cotransfections were also performed with otherwise identical HIV CAT plasmids with altered TATA box sequences. Twenty-four hours after transfection, cells were incubated with phorbol 12-myristate 13-acetate (PMA) for an additional 20 h. Jurkat *tat-III* cells transfected with HIV CAT showed an 8.7-fold increase in CAT activity in the presence of E1A compared with that of controls (Fig. 1A). When stimulated additionally by PMA, a further approximately ninefold increase in CAT activity was observed, suggesting that activation by NF-κB and E1A was more than additive (Fig. 1A).

When these cells were transfected with an HIV CAT plasmid containing an altered TATA box, basal CAT activity decreased from 7- to 20-fold relative to that of the HIV CAT plasmid in different experiments (Fig. 1B; note scale change). In contrast to stimulation mediated by E1A (17), PMA induced a 14.5-fold increase in CAT activity (Fig. 1B). Similar to results for a κB mutant with an intact TATA box (16), no stimulation was seen after transfection with a plasmid containing mutations in both the κB and the TATA box, demonstrating that the PMA effect was mediated by NF-κB (Fig. 1C) or a related κB-binding protein (3, 7); however, the expression of this double-mutant plasmid is increased by the immediate early transactivator of cytomegalovirus (data not shown). Similar results were observed in Jurkat cells which lack the *tat-I* gene.

To determine whether the orientation of the TATA box was important for enhancer activation, a plasmid containing the reverse complement of the TATAA sequence, TTATA, was transfected into Jurkat *tat-III* cells. The CAT activity of the plasmid with the inverted TATA box was similar to that of the plasmid with the mutant TATA box (TGCGC), 6- to 10-fold less than expression from HIV CAT (wild type, with the sequence TATAA) in resting and stimulated cells. Cotransfection with the E1A expression plasmid did not significantly increase CAT activity (Fig. 2B), suggesting that E1A stimulation mediated by the TATA box is dependent on orientation. Because the TATA sequence in simian virus 40 (SV40) can partially substitute for TATA in the hsp70 promoter (20), we prepared plasmids containing this sequence in both orientations (TATTTAT and ATAAATA, wild type and reverse complement, respectively). When these plasmids were transfected into Jurkat *tat-III* cells and incubated with PMA, CAT activity was induced at levels comparable to those of the mutant TATA plasmid (Fig. 2C and D), showing that the SV40 TATA box did not substitute for the native TATA sequence.

To determine the site of transcriptional initiation after stimulation of the mutant TATA plasmids, S1 nuclease analysis was performed (2). Protection of an expected 60-base-pair fragment was observed in cells stimulated by

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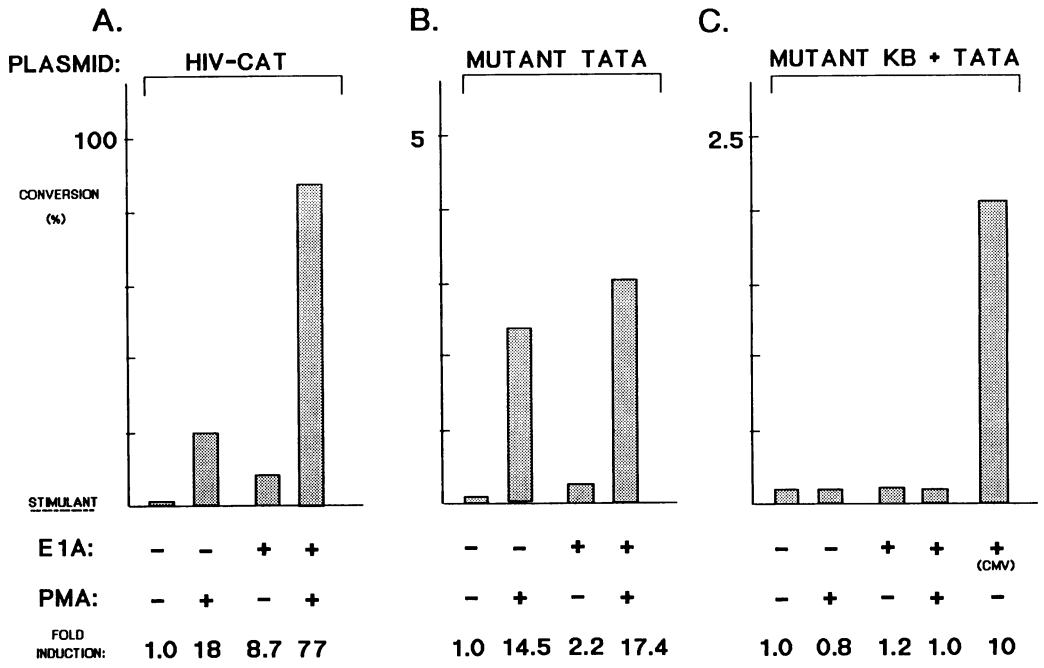

FIG. 1. Activation of HIV CAT mutant plasmids by PMA and E1A. Jurkat *tat-III* cells (10) were transfected with 10 to 20 μg of HIV CAT (A), TATA box mutant (GCGC) (B), or a double mutant with altered κB and TATA (GCGC) box (C) (14). For viral transactivation, cells were cotransfected with 2 μg of E1A (6) or with control plasmids and 5 to 20 μg of the relevant HIV-CAT plasmid. Twenty hours after transfection, cells were maintained in medium alone or incubated with 26 nM PMA for an additional 20 h. Conversion of chloramphenicol to its acetylated forms was assayed (8, 16). The efficiency of transfection was standardized as described elsewhere (16, 17) by cotransfection with the plasmid expressing interleukin-3 and measuring interleukin-3 activity (25). Results are representative of at least three independent transfections. The percent conversions for the uninduced plasmids were as follows: HIV CAT, 1.1%; TATA box mutant, 0.15%; and mutant κB plus TATA box mutant, 0.21%.

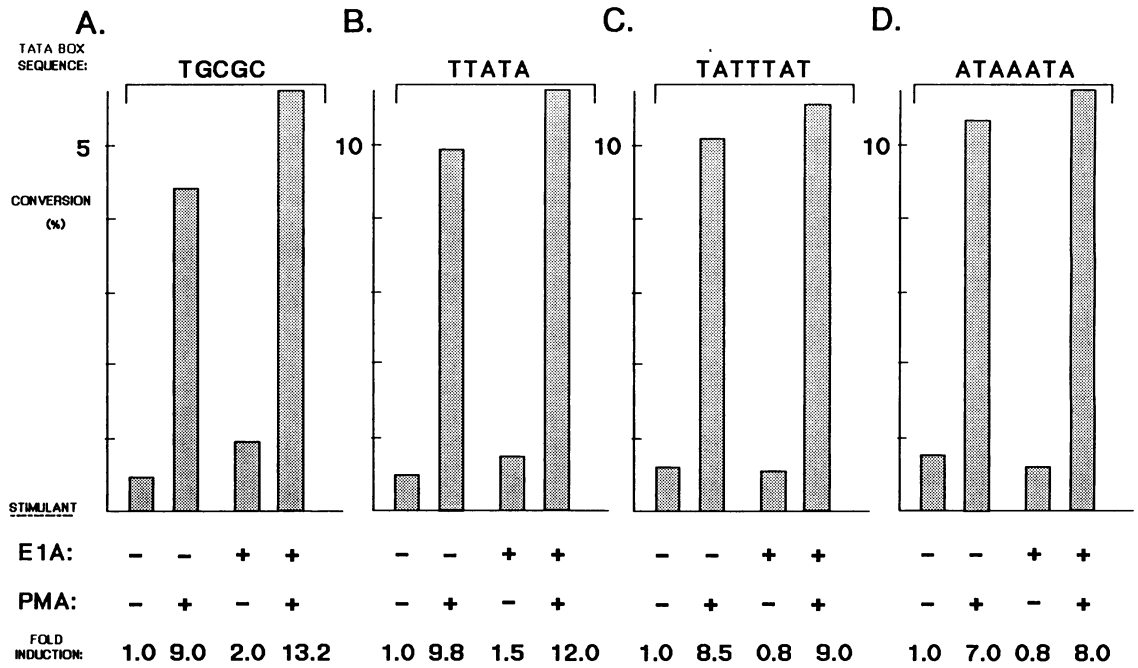

FIG. 2. Stimulation of HIV CAT mutant TATA box plasmids by PMA and E1A. Cells were transfected with HIV CAT plasmids with a mutant TATA box (A), the reverse complement of the TATAA sequence (B), SV40 TATA (sense) (C), or SV40 TATA (reverse complement) (D) and were stimulated as described in the legend to Fig. 1, except that 20 μg containing the TATA box in the inverted orientation (reverse complement) was obtained by a site-directed mutagenesis technique (16). The mutant plasmids containing the SV40 TATA box were constructed by inserting a double-stranded oligonucleotide (TGCAGTATTTATGCAGC) in both orientations into the *Pst*I site created in a previously described mutant TATA box plasmid (17). Basal CAT conversions for each group were as follows: TATA box mutant, 0.31%; TTATA, 0.5%; TATTTAT, 0.6%; and ATAAATA, 0.7%.

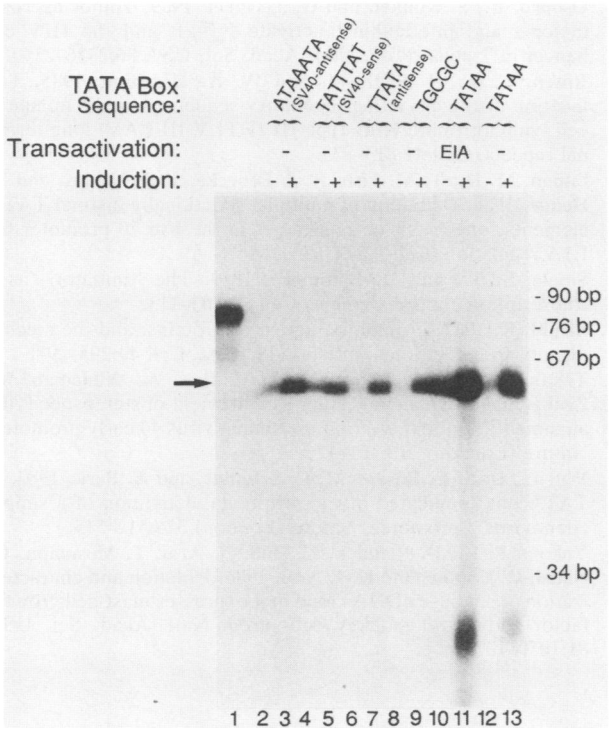

FIG. 3. S1 nuclease analysis of RNA from cells transfected with HIV CAT and TATA box mutant plasmids. Jurkat *tat-III* cells were transfected with HIV CAT (10 μg) or the indicated mutant plasmids (10 μg) with altered TATA boxes. Twenty-four hours later, cells were incubated alone (−) or with 26 nM PMA (+) for an additional 12 h. Total cellular RNA was isolated, and 10 μg was used for S1 nuclease analysis. Quantitation by densitometry revealed the following induction ratios in each group: SV40 ATAAATA, 8.8-fold; SV40 TATTTAT, 5.6-fold; TTATA, 8.4-fold; TGCGC, 10.3-fold; HIV CAT+E1A, 4.5-fold; HIV CAT, 19.5-fold. A single-stranded end-labeled oligonucleotide probe, complementary to positions −17 to +60 of the sense strand of HIV with a 7-base extension at the 3′ end, was used as the probe. The position of the protected 60-base-pair fragment is shown (➨). Undigested probe is shown in lane 1. Size markers (in base pairs [bp]) are indicated at the right.

PMA, whereas protection was markedly reduced in unstimulated cells (Fig. 3, lane 12 versus lane 13). This site, which corresponds to the viral transcriptional initiation site, was used also by HIV CAT plasmids containing the mutant TATA box (Fig. 3, lane 9), the inverted TATA box (Fig. 3, lane 7), and SV40 TATA boxes (Fig. 3, lanes 3 and 5). The position of transcriptional initiation in the HIV enhancer was therefore not dependent on the TATA box sequence. The same initiation site was also utilized when transcription was stimulated by E1A (Fig. 3, lanes 10 and 11).

In this study, we have examined the relationship between activation of the HIV enhancer by NF-κB and the requirement for a specific TATA box sequence. This analysis has shown that the HIV TATA element acts in an orientation-dependent manner to increase mRNA levels. Activation of the HIV enhancer mediated by NF-κB occurred in the absence of a functional TATA box, which is not required to position the transcriptional initiation site. Furthermore, the reverse complement TATAA sequence (TTATA) was not activated by E1A, suggesting that stimulation of this site is also sequence and orientation dependent.

In addition to their role in increasing the efficiency of transcriptional initiation (15, 23), TATA-binding proteins have been sometimes implicated in the determination of transcriptional initiation sites. For example, when the TATA box of the sea urchin H2A histone gene or SV40 early promoter is mutated, these genes remain transcriptionally active, but the site of transcriptional initiation is altered (1, 10, 23). Although a previous study has shown that the TATA box mutation alters the HIV transcriptional initiation site, these studies employed a plasmid containing the SV40 enhancer and replication origin which are known to cause aberrant transcriptional initiation (12). Recently, it has been shown that an initiator site is present in a class II promoter from terminal transferase which lacks a TATA sequence and acts to determine the site of transcription initiation (21). Similar sites have been defined in yeast promoters (22). Our findings suggest the presence of an analogous site in the HIV enhancer because the TATA box is not required for the precise transcriptional initiation, implying that proteins need not bind to TATA to determine the site of initiation. In vitro transcription studies have also demonstrated that the TATA box contributes to basal enhancer function, although it may not determine the initiation site (13). Similar observations have been made for other regulatory regions, including the E1B promoter, in which linker-scanning mutations decreased E1A inducibility but did not alter the transcriptional initiation site (24). Taken together, these findings suggest that the role of TATA-binding proteins in different enhancers is determined by the specific interaction with other DNA-binding proteins. In the HIV enhancer and possibly elsewhere, the TATA box sequence acts as an orientation-dependent element which acts to enhance HIV gene expression but which is not needed to specify the site of transcriptional initiation.

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