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The Bel-1 Protein of Human Foamy Virus Activates Human Immunodeficiency Virus Type 1 Gene Expression via a Novel DNA Target Site

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The Bel-1 protein of human foamy virus can activate transcription directed by the long terminal repeat (LTR) promoter of human immunodeficiency virus type 1 (HIV-1). The target sequence for Bel-1 is shown to lie within the HIV-1 LTR U3 region but does not coincide with any previously described factor-binding site. Gene expression directed by an HIV-1 LTR lacking functional sites for the inducible cellular transcription factor NF-κB was activated over 100-fold by coexpression of Bel-1. These observations suggest that Bel-1 has the potential to significantly enhance the level of HIV-1 gene expression in cells dually infected with HIV-1 and human foamy virus.

Humans are natural hosts to members of all three classes of complex retroviruses (6, 8, 10, 18, 27). The most pathogenic of these is human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS (27). Infection with human T-cell leukemia virus type I (HTLV-I) is also associated with pathogenic consequences and can, in particular, lead to adult T-cell leukemia or tropical spastic parapheresis (10). Although infection with human foamy virus (HFV) has not been shown to lead to any specific disease state, recent data suggest that HFV may induce neurodegenerative effects in the infected host (2, 8, 18).

As predicted by their inclusion in the complex retrovirus family, each of these three human retroviruses has been shown to encode a sequence-specific activator of transcription from the homologous long terminal repeat (LTR) promoter (6). The most unusual of these is clearly the HIV-1 Tat protein, which directly interacts with a structured RNA target sequence, termed TAR, located at the 5′ end of all HIV-1 mRNA species (5). This interaction is believed to activate HIV-1 mRNA synthesis by enhancing the otherwise highly inefficient elongation of initiated viral transcripts. The Tax protein activates HTLV-I LTR-specific transcription via a 21-bp DNA repeat sequence located in the LTR U3 element (3, 28). However, Tax has not been shown to bind DNA directly and is instead believed to activate LTR-specific transcription via a currently unidentified cellular DNA-binding protein (9, 17, 25).

Like HIV-1 and HTLV-I, HFV also encodes a nuclear regulatory protein, termed Bel-1, that potently activates HFV LTR-specific transcription (12, 26, 31). A transcriptional *trans* activator similar to Bel-1, termed Taf, has also been described for the related simian foamy virus (SFV) (18, 19). Although the target sequence for Bel-1 has not been fully defined, it is known to be located within the HFV LTR U3 region (12, 26, 31). Bel-1 therefore shares with Tax the property of being a DNA sequence-specific activator of viral transcription. As these proteins are incapable of cross-transactivating (12), it has been hypothesized that they act via distinct mechanisms. However, it has also been reported that both Tax and Bel-1 can enhance transcription from the HIV-1 LTR (12, 31). In the case of Tax, this is known to result from the activation of the cellular transcription factor NF-κB (13, 29, 30). It has remained unclear whether Bel-1 also induces NF-κB function or whether this HFV regulatory protein instead acts through a novel DNA target site. In this report, we present data that support the latter hypothesis.

To determine the location of the Bel-1 target site within the HIV-1 LTR, we used as a starting point a previously described plasmid, pBC12/HIV/SEAP, in which this LTR is positioned 5′ to the indicator gene for secreted alkaline phosphatase (SEAP) (Fig. 1) (1, 12). The pMκB/SEAP vector is identical to pBC12/HIV/SEAP except that each of the two NF-κB sites located within the LTR has been rendered nonfunctional by the 3-bp substitution mutation described by Nabel and Baltimore (20). The previously described (11) HIV-1 LTR mutant present in pΔTAR/SEAP lacks sequences located 3′ to nucleotide +8 relative to the LTR cap site. This HIV-1 LTR deletion mutant has therefore lost not only the viral TAR element but also the underlying DNA-binding sites for the leader-binding protein (LBP) (32) (Fig. 1).

The level of SEAP expression induced by each of these three reporter constructs was quantitated by transfection (4) into the monkey cell line COS in the presence or absence of expression plasmids (12) that encode the three viral regulatory proteins Tat (pcTat), Tax (pcTax), and Bel-1 (pBel-1) (Table 1). As expected, the wild-type HIV-1 LTR present in the pBC12/HIV/SEAP plasmid was efficiently *trans*-activated by the HIV-1 Tat protein, ~27-fold in this experiment, and was also modestly responsive to both the Tax and Bel-1 proteins (~7-fold and ~5-fold, respectively). Specific inactivation of the HIV-1 LTR NF-κB site, in pMκB/SEAP, rendered the LTR nonresponsive to Tax (Table 1). This substitution mutation also resulted in a ~10-fold drop in the basal activity of the HIV-1 LTR, suggesting the presence of a significant level of functional NF-κB protein in these transfected COS cells (20). Interestingly, the pMκB/SEAP construct displayed a markedly enhanced response to both Tat and Bel-1. The extraordinarily high level of *trans* activation detected with Bel-1, ~120-fold in this experiment,

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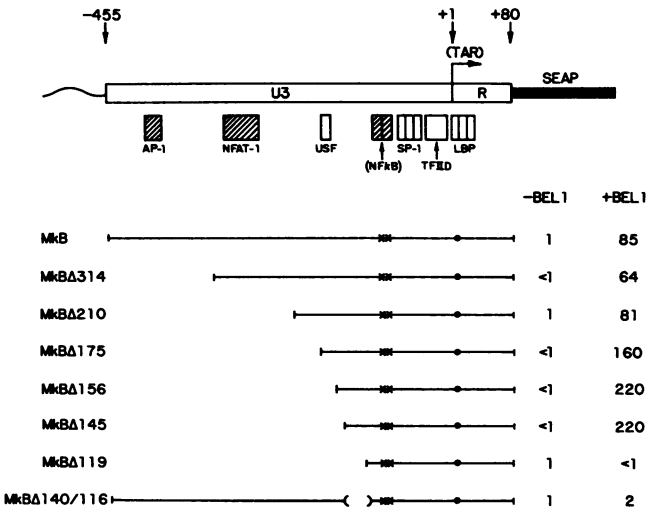

FIG. 1. Deletion mapping of the HIV-1 LTR Bel-1 target site. The upper panel is a schematic representation of known binding sites for cellular regulatory proteins located within the HIV-1 LTR (15, 32). Inducible factors are indicated by hatching. A construct, pBC12/HIV/SEAP, in which the indicated HIV-1 LTR sequence is juxtaposed to the SEAP indicator gene has been described (1, 12). The HIV-1 LTR present in pMκB/SEAP differs from that present in pBC12/HIV/SEAP in that the two NF-κB sites have each been inactivated (x) by a 3-nucleotide substitution mutation (20). The MκB/Δ145 and MκB/Δ119 mutants were constructed by deletion of sequences located 5′ to cleavage sites for the restriction enzymes *Bsp*EI and *Eco*RV, respectively. Other deletion mutants of the HIV-1 LTR present in the pMκB/SEAP plasmid were constructed by the polymerase chain reaction as described previously (12). The name of each construct is derived from the 3′ border of each deletion. The pMκBΔ140/116/SEAP mutant lacks the indicated internal, 24-bp sequence and was also constructed by polymerase chain reaction. Each HIV-1 LTR construct was transfected into COS cells (4) together with the negative control vector pBC12/CMV (−Bel-1) or with the Bel-1 expression vector pBel-1 (+Bel-1) (12). Supernatant media were harvested at 70 h posttransfection, and SEAP activity was quantitated as described previously (1, 12).

appeared to reflect not only the low basal activity of this mutated HIV-1 LTR but also a modest but consistent increase in the absolute level of Bel-1-induced SEAP expression from the MκB LTR compared with the wild-type LTR. As expected, the HIV-1 LTR (pΔTAR/SEAP) lacking sequences 3′ to position +8 relative to the cap site, and hence lacking a functional TAR element (11), remained responsive

TABLE 1. Response of HIV-1 LTR mutants to different retroviral *trans* activators[a]

Construct	SEAP activity			
	Basal	+Tat	+Tax	+Bel-1
pBC12/HIV/SEAP	3.1	83	21	17
pMκB/SEAP	0.3	44	0.4	35
pΔTAR/SEAP	3.6	10	31	22
pΔ119/SEAP	3.3	129	41	6
pΔ140/116/SEAP	2.8	72	15	2.5

[a] Cultures were transfected (4) with equimolar amounts of the indicated HIV-1 LTR-based plasmids and with a negative control vector (pBC12/CMV) or with a vector encoding the indicated retroviral regulatory protein (12). Supernatant media were harvested at 70 h posttransfection, and SEAP activity was quantitated as described previously (1, 12).

TABLE 2. Substitution mutations within the HIV-1 LTR can inhibit Bel-1 *trans* activation[a]

Construct	SEAP activity	
	−Bel-1	+Bel-1
pMκB/SEAP	1	181
pMκBΔ140/116/SEAP	1	3
pMκBS5/SEAP[b]	2	19
pMκBS8/SEAP[b]	1	11

[a] Levels of HIV-1 LTR-dependent SEAP expression, in the presence and absence of Bel-1, were determined after transfection of COS cell cultures as described in the legend to Fig. 1.

[b] The wild-type HIV-1 LTR sequence −126-CTGCTGATATCGAGC was mutated to CTGCTGccggaGAGC in pMκBS5 and to CTGagaccggaGAGC in pMκBS8. Differences from the wild-type sequence are indicated by lowercase letters.

to both Tax and Bel-1 but had lost its ability to effectively respond to the HIV-1 Tat *trans* activator. We therefore conclude that the Bel-1 target site is distinct from the previously identified (32) NF-κB, TAR, and LBP elements and must be located 5′ to HIV-1 LTR position +9.

The HIV-1 LTR contains target sites for several inducible or constitutive DNA-binding proteins (15, 20, 32), and these are schematically represented in Fig. 1. To map the Bel-1 target site within the HIV-1 LTR, we generated a nested set of 5′ deletion mutants that sequentially removed the binding sites for the cellular regulatory proteins AP-1, NFAT-1, and USF (15). These mutations were constructed both in the context of the wild-type LTR and with the LTR bearing mutated NF-κB sites (MκB). As similar results were obtained in each case, only the latter data are presented (Fig. 1). The sequential deletion of HIV-1 LTR sequences between −455 and −145 relative to the cap site had no significant effect on Bel-1 *trans* activation of the HIV-1 LTR. However, deletion of a further 26 bp, in pMκBΔ119/SEAP, precluded *trans* activation of the HIV-1 LTR by Bel-1. Therefore, the HIV-1 LTR U3 region contains a Bel-1 target site that extends 5′ of −119, but not 5′ of −145, relative to the cap site. We also constructed a minimal deletion mutant lacking only sequences between −140 and −116 relative to the cap site (pMκBΔ140/116/SEAP). This mutant was also refractory to *trans* activation by Bel-1, suggesting that the HIV-1 LTR likely contains a single Bel-1 target site. As shown in Table 1, the same deletion mutants in the context of the wild-type HIV-1 LTR (pΔ119/SEAP and pΔ140/116/SEAP) remained fully responsive to both the Tat and Tax *trans* activators but were not affected by coexpression of Bel-1. As predicted from earlier work (15, 32), these deletions also had little or no effect on the basal activity of the HIV-1 LTR promoter element (Table 1). Overall, these results therefore demonstrate that the Bel-1 target site is not only fully distinct from the sequences required for HIV-1 LTR Tax and Tat responsiveness but is also distinct from the known target sites for the cellular factors indicated in Fig. 1.

To more fully define the HIV-1 sequences that mediate *trans* activation by Bel-1, we next introduced substitution mutations between −120 and −116 (pMκBS5/SEAP) or between −123 and −116 (pMκBS8/SEAP) into the pMκB/SEAP plasmid (Table 2). Both of these HIV-1 LTR mutants were severely impaired in their ability to respond to the HFV Bel-1 protein, demonstrating that these DNA sequences form part of the Bel-1 target site. However, the finding that these substitution mutants differ from pΔ140/116 in retaining a low but detectable ability to respond to Bel-1 (Table 2)

suggests that the HIV-1 LTR target sequence for Bel-1 extends somewhat beyond this mutationally defined −123 to −116 sequence.

We have previously shown that the Bel-1 *trans* activator is functional in cells derived from a wide range of species (12). To confirm that Bel-1 is also active in human T cells, we transfected (4) the CD4$^+$ T-cell line Jurkat with an indicator construct, pMκB/CAT, in which an HIV-1 LTR bearing mutated NF-κB sites was juxtaposed to a gene encoding the enzyme chloramphenicol acetyltransferase (CAT). The ability of Tat and Bel-1 to activate this HIV-1 LTR was assessed by cotransfecting Jurkat cells with the Tat expression vector pcTat or the Bel-1 expression vector pBel-1 (12). Relative CAT expression levels were determined at 70 h after transfection as described previously (12, 23). Under conditions in which a Jurkat culture transfected with the negative control vector pBC12/CMV gave a background activity of 530 cpm, the culture transfected with the pMκB/CAT construct induced a basal CAT activity of 2,000 cpm. Cotransfection with pcTat led to an 84-fold increase in CAT enzyme activity (127,000 cpm), while cotransfection with Bel-1 increased relative CAT expression by 23-fold (35,000 cpm). We therefore conclude that Bel-1 can *trans*-activate HIV-1 LTR-dependent gene expression in CD4$^+$ human T cells.

We and others have previously demonstrated that the HFV Bel-1 protein is a modest (four- to eightfold) *trans* activator of the HIV-1 LTR in primate fibroblasts (12, 31). Recently, it was reported that the equivalent regulatory protein of SFV, termed Taf, can also activate gene expression directed either by the HIV-1 LTR or by an LTR derived from a simian immunodeficiency virus (19). Here, we show that the HIV-1 LTR is responsive to Bel-1 in transfected human T cells. We further demonstrate that Bel-1 *trans* activates the HIV-1 LTR via a novel DNA target site located, at least in part, between positions −123 and −116 relative to the LTR cap site. Remarkably, Bel-1 *trans* activation was not only independent of the HIV-1 LTR NF-κB sites but was actually enhanced, in both relative and absolute terms, by inactivation of these sites (Table 1). Given the proximity of the Bel-1 DNA target site mapped in this study to the reiterated NF-κB sites present in the HIV-1 LTR (20, 32) (−109 to −79, Fig. 1) occupation of the latter sites by the relevant DNA-binding proteins may well interfere with *trans* activation by Bel-1. Interestingly, the HIV-1 LTR sequences reported here as being responsive to Bel-1 bear no evident homology to Bel-1 target sequences previously mapped in the HFV LTR (12). It is therefore possible that Bel-1, like Tax (30), may be able to activate gene expression via more than one DNA target site.

The level of activation of the inducible cellular regulatory protein NF-κB has been proposed to be a key determinant of whether HIV-1 establishes a productive or latent infection of susceptible cells in vivo (27). The data presented in this article suggest that Bel-1 might well be able to functionally substitute for NF-κB in cells coinfected with HFV and HIV-1 (Table 1), thus perhaps resulting in activation of HIV-1 replication. In fact, recent data demonstrate significant levels of HFV seropositivity in some regions of Africa that also have high levels of HIV-1 infection (8, 16). It is also of interest that the majority of rhesus macaques, a major experimental animal for the study of simian immunodeficiency virus, are seropositive for SFV (8, 18). Although many foamy virus isolates are tropic for fibroblasts (8, 18), lymphotropic foamy viruses have been isolated from several primate species, including African green monkeys, baboons, gorillas, and chimpanzees (7, 21, 24). Several of these viruses also display the ability to replicate in human T cells (7, 21, 24). Perhaps most striking is the reported simultaneous infection of the human CD4$^+$ T-cell line H9 with HIV-1 and a lymphotropic chimpanzee foamy virus during the reisolation of virus from chimpanzees that had been experimentally challenged with HIV-1 (21). It therefore appears possible that HFV, like HTLV-I and a number of DNA tumor viruses, may have the potential to act as a cofactor in the pathogenesis of HIV-1-induced disease in dually infected humans or primates (22, 27).

After submission of this manuscript, Lee et al. (13) reported the identification of sequences within the HIV-1 LTR that mediate *trans* activation by Bel-1. These data are in close agreement with the observations reported here.

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