Activation of the Human T-Cell Leukemia Virus Type I Enhancer Is Mediated by Binding Sites for Elf-1 and the *pets* Factor

NINA M. CLARK, MICHAEL J. SMITH, JOHN M. HILFINGER, AND DAVID M. MARKOVITZ*

Department of Internal Medicine, Division of Infectious Diseases, University of Michigan Medical Center, Ann Arbor, Michigan 48109-0680

Received 15 March 1993/Accepted 16 June 1993

Infection with human T-cell leukemia virus type I (HTLV-I) is associated with adult T-cell lymphoma/leukemia. This disease occurs in only a small minority of people infected with HTLV-I and manifests itself many years after infection. Therefore, it appears that a fine balance exists between HTLV-I and the host T-cell factors with which it interacts. HTLV-I encodes a transactivating protein, Tax, which activates viral transcription via cellular mechanisms which are incompletely understood. As viral gene expression is negligible during latency, it is doubtful that Tax controls the initial transition to the replicative state. Tax-independent cellular factors which control HTLV-I transcription, and presumably latency, have received little study. Recently, the product of the chicken proto-oncogene *ets-1* has been shown to bind to the HTLV-I enhancer and modestly activate transcription in certain cell types (S. C. Gitlin, R. Bosselut, A. Gégonne, J. Ghysdael, and J. N. Brady, *J. Virol.* 65:5513-5523, 1991). However, the functional significance of the *ets*-binding site in the intact enhancer has not previously been shown. We now demonstrate that site-specific mutation of the purine-rich *ets*-binding site significantly diminishes inducible enhancer function, but not Tax response, in the human Jurkat T-cell line. Similarly, mutation of the *peri-ets* (*pets*) site, not previously noted in the HTLV-I enhancer, markedly inhibits inducible enhancer function but not Tax response. Further, we show that the predominant protein binding the purine-rich HTLV-I enhancer element in human T cells is not *ets-1* but Elf-1, a member of the *ets* family which is very similar to the *Drosophila* morphogen E74. Regulation of HTLV-I through Elf-1/*pets* enhancer motifs resembles that seen with human immunodeficiency virus type 2 (D. M. Markovitz, M. Smith, J. Hilfinger, M. C. Hannibal, B. Petryniak, and G. J. Nabel, *J. Virol.* 66:5479-5484, 1992; J. M. Leiden, C.-W. Wang, B. Petryniak, M. Smith, D. M. Markovitz, G. J. Nabel, and C. B. Thompson, *J. Virol.* 66:5890-5897, 1992), another human pathogenic retrovirus with a relatively long incubation period.

Human T-cell leukemia virus type I (HTLV-I) is a human pathogenic retrovirus which infects CD4⁺ T lymphocytes (47). The virus is transmitted through sexual, blood, and perinatal contact, and infection is endemic in Japan, the Caribbean, Africa, and the southeastern United States (3, 20). Infection with HTLV-I is associated with adult T-cell lymphoma/leukemia, an often fatal malignancy of mature T cells (3, 20, 39, 40, 48, 49, 58, 63). Forty to sixty percent of all newly diagnosed non-Hodgkin lymphomas of adults are associated with HTLV-I in certain endemic regions (3). It has also been suggested that HTLV-I is associated with some cases of the more indolent cutaneous T-cell lymphoma/leukemia mycosis fungoides (15). HTLV-I may also have an indirect causative role in certain cases of B-cell chronic lymphocytic leukemia (27). In addition to these associations with malignancy, HTLV-I infection has been strongly linked to the demyelinating disease tropical spastic paraparesis (9, 38).

Adult T-cell lymphoma/leukemia occurs in a small minority of people approximately 30 to 40 years following infection with HTLV-I. The pathophysiology underlying this long asymptomatic period, and the subsequent emergence of disease in a minority of infected individuals, remains poorly understood. The mechanism by which the transactivating protein Tax stimulates HTLV-I has been the subject of some

investigation (see below), but this virally encoded protein, which appears to be silent during latency (36), seems unlikely to serve as the major mechanism for the initial transition from latency to replication. At this time, little is known about how Tax-independent cellular factors might interact with HTLV-I and regulate transcription of the viral genome in response to cellular stimuli.

Members of the *ets* proto-oncogene family are involved in the life cycle of several retroviruses (1, 11, 14, 22, 34, 45, 54, 59). It appears that virally encoded *ets* proteins are involved in oncogenesis (1, 34, 45) and that cellular *ets* proteins are involved in viral enhancer regulation (11, 14, 22, 54, 59). *ets* proteins often require cofactors to bind to DNA and activate transcription (23, 57, 62). Recently, data which suggest that *ets* proteins contribute to regulation of the HTLV-I enhancer have been presented (4, 10). In cotransfection experiments in human epithelial (HeLa) and rodent (NIH 3T3) cells, the chicken *c-ets-1* protein was shown to modestly activate the HTLV-I enhancer (4). Studies using DNase footprinting demonstrated that recombinant chicken *c-ets-1* binds to the HTLV-I enhancer in vitro (10). While DNase protection was observed in a small region centered at -295 (designated ERR-2), the larger and more stable footprint was found between positions -162 and -118 (ERR-1), a region which contains two *ets*-binding motifs designated ERE-A and ERE-B (Fig. 1). While these studies suggested a role for the *ets*-binding motifs in the regulation of HTLV-I, they did not show that *ets-1* binds to these sites in vivo, and mutagenesis

* Corresponding author.

HTLV I

FIG. 1. Enhancer region of HTLV-I. Relevant sites within the long terminal repeat are identified. The sequence of the pets site, verified in multiple sequencing reactions, differs slightly from the previously published sequence in which the G at position -146 on the coding (upper) strand is shown as AA (4, 8). Altered bases within the mutant plasmids used in this study are shown above the wild-type sequence. TRE, Tax-responsive element (21-bp repeat element). Identification of the PuB1, PuB2, and pets sites is described in the text. The pets and PuB2 sequences are found in the noncoding (lower) strand.

experiments using the native enhancer/promoter were not presented to confirm the importance of these regions. Further, enhancer function was not investigated in human cells of the T-cell lineage, the cell type predominately infected in vivo (47).

We have recently demonstrated that the enhancer of human immunodeficiency virus type 2 (HIV-2) functions quite differently from that of HIV-1 (25, 29–31). This finding may be clinically significant, as HIV-2 appears to have an incubation period longer than that of HIV-1 (19, 28). Induction of the HIV-2 enhancer is mediated by at least four distinct *cis*-acting elements: two ets-binding motifs, designated PuB1 and PuB2, pets (peri-ets), which lies between PuB1 and PuB2, and a single κ B site (29, 31). Mutation of PuB1 and PuB2 is sufficient to eliminate enhancer induction in activated T cells (25). In vitro, PuB1 and PuB2 both bind Elf-1, a human ets family member with marked similarity to the *Drosophila* development factor E74 (25, 31, 56, 59). While human ets-1 binds the PuB2 site in vitro (31), it appears from supershift electrophoretic mobility assays (EMSAs) that Elf-1 is the predominant protein binding PuB2 (and PuB1) in vivo (25). The nuclear protein which recognizes the pets site, and may cooperate with Elf-1 to activate transcription, has not yet been characterized.

Inspection of the ERR-1 region of HTLV-I revealed that, analogous to HIV-2, a pets motif is located between the two ets-binding motifs (Fig. 1). The pets motif lies in a region previously identified as binding a 36-kDa cellular protein (TIF-1) and capable of conferring Tax responsiveness on a minimal promoter (32, 33). This same region of the enhancer has also been reported to bind an NF- κ B-like nuclear factor (37) and Sp1 (8). Therefore, to ascertain the importance of these elements in the intact enhancer, we performed a mutational analysis of the HTLV-I enhancer in the Jurkat human T-cell line. Site-specific mutations were introduced into the ets (PuB1 or PuB2) and/or pets motifs, and the effects of these mutations were assessed with transfection experiments using reporter gene (chloramphenicol acetyltransferase [CAT]) assays. We show that although these

mutations do not affect activation by Tax, they significantly diminish induction of the enhancer by T-cell stimulants. Using EMSAs, we demonstrate that the pets motif of HTLV-I binds a distinct nuclear protein. Further, we show by using supershift assays that the predominant human T-cell protein which recognizes the ets motifs in HTLV-I is Elf-1. Therefore, as in HIV-2, the pets- and Elf-1-binding motifs play a significant role in regulation of the HTLV-I enhancer.

MATERIALS AND METHODS

Plasmids. The Tax-expressing plasmid and a control plasmid expressing a frameshift mutant of Tax (26) were gifts from Gary Nabel. HTLV-I/CAT (pU3R-I) was constructed by J. Sodroski (53). HTLV-I Δ PuB1/CAT, Δ PuB2/CAT, Δ PuB1 + Δ PuB2/CAT, Δ pets/CAT, Δ pets + Δ PuB1/CAT, and Δ pets + Δ PuB2/CAT were constructed by using the gap-heteroduplex site-specific mutagenesis protocol as previously described (35). The HTLV-I enhancer region and mutants were sequenced by the dideoxy method (kit from U.S. Biochemical). Construction of the plasmids expressing truncated human ets-1 (bp 864 to 1347) and Elf-1 has been described elsewhere (56).

Cell transfections and CAT assays. The Jurkat T-cell leukemia line was maintained in RPMI with 10% fetal bovine serum prior to transfection and 5% fetal bovine serum after transfection. Cells were grown under standard conditions. Jurkat cells (10^7) were transfected with 5 μ g of the indicated plasmid, using the DEAE-dextran method (44). Twenty hours after transfection, cells were incubated for an additional 20 h with 16 nM phorbol myristate acetate (PMA) and 10 nM forskolin. In the cotransfection experiments, 5 μ g of reporter plasmid was transfected along with 5 μ g of the Tax-expressing plasmid or the frameshift mutant of Tax (26), and cells were harvested 40 h later. Cell extracts were prepared and CAT activity was determined according to standard methods (6, 12, 13). Transfection efficiencies were normalized according to protein concentration.

EMSAs. Jurkat nuclear extracts were prepared by a modification of the method of Dignam et al. (7), using cells which were either unstimulated or treated for 4 h with PMA (16 nM) or PMA and forskolin (10 nM). Human T cells were isolated from the peripheral blood mononuclear cells of healthy donors as described previously (56). The T cells were cultured in RPMI with 10% fetal bovine serum and were activated by treatment for 6 to 8 h with PMA (10 ng/ml) and ionomycin (0.4 μ g/ml). Nuclear extracts were then prepared as described previously (56). Protein concentrations were determined by using a commercially available kit (Bio-Rad, Richmond, Calif.). Recombinant Elf-1 and ets-1 proteins were prepared in rabbit reticulocyte lysates from RNA transcribed in vitro (Promega) or from lysates of *Escherichia coli* expressing the recombinant protein (17, 56, 59). The truncated ets-1 protein, amino acids 325 to 441, includes the DNA-binding domain (59).

For the HTLV-I probes, oligonucleotides corresponding to both strands of the HTLV-I sequence from positions -164 to -121 (Fig. 1), either wild type or mutated, were synthesized with an Applied Biosystems 300B synthesizer. For the HIV-2 PuB2 probe, oligonucleotides corresponding to both strands of the HIV-2 sequence from positions -162 to -131 (31) were synthesized in the same way. Both the sense and antisense strands were radiolabeled with polynucleotide kinase in the presence of [γ - 32 P]ATP, and equimolar amounts of the two strands were combined by incubation at

FIG. 2. Site-specific mutational analysis of PMA/forskolin-responsive elements in the HTLV-I enhancer. Mutated sites are illustrated in Fig. 1. Results shown are the average of two experiments and are representative of three to eight independent experiments.

80°C and then slow cooling in 500 mM NaCl. The double-stranded probe was then purified with a Sephadex G-50 column.

The binding reaction for the EMSAs (29, 30, 51) using the pets probe contained 10 µg of T-cell nuclear protein, 10,000 cpm of radiolabeled oligonucleotide probe, and 1 µg of poly(dI-dC) in 1 mM Tris (pH 7.5)–1 mM NaCl–40% glycerol in a final reaction volume of 20 µl. Following incubation at room temperature for 15 min, DNA-protein complexes were run on a 5% polyacrylamide gel at room temperature in 1× Tris-glycine-EDTA buffer at 100 V for ~2.5 h. Certain binding reactions using the PuB1-plus-PuB2 (Δpets) probe were performed with a Ficoll buffer (25), 10 µg of T-cell nuclear extract or 1 µl of recombinant protein, 10,000 cpm of radiolabeled probe, and 250 to 1,000 ng of poly(dI-dC) in a 20-µl total volume with a final concentration of 75 mM KCl. For shift-shift (supershift) EMSA, antibody (25, 56) was incubated with the binding reaction mixture at 4°C for 15 min prior to addition of the radiolabeled probe. The reaction products were run on a 5% polyacrylamide gel at 4°C in 0.25× Tris-borate-EDTA buffer at 110 V for ~3 h. Antibody directed against ets-1 was a gift from Takis Papas.

RESULTS

Mutation of the PuB2 or pets motif disrupts enhancer function. Site-specific mutations were introduced into the pets, PuB1, or PuB2 site (Fig. 1), singly or in combination. These mutants or the wild-type HTLV-I/CAT construct were transfected into the Jurkat T-cell line, and certain samples were stimulated with PMA (a protein kinase C stimulant) and forskolin (a cyclic AMP [cAMP] stimulant) 20 h later, 24 h prior to harvesting. Previous studies have shown that the combination of these two stimulants of cellular activation cascades readily activates the HTLV-I enhancer (36, 42, 61). Mutation of either the pets or PuB2 site, but not the PuB1 site, markedly inhibited enhancer induction (Fig. 2). The lack of function of the PuB1 site is consistent with the observation that this element may not represent a canonical ets-binding site (59). Mutation of PuB1 plus PuB2 or PuB1 plus pets did not affect enhancer function signifi-

FIG. 3. Mutation of the PuB1, PuB2, or pets site(s) does not inhibit Tax response. Mutated sites are illustrated in Fig. 1. Results shown are the average of two experiments and representative of three to six independent experiments.

cantly more than did mutation of PuB2 or pets alone (Fig. 2). However, mutation of the pets site in conjunction with mutation of PuB2 lowered basal enhancer function, leading to a decreased level of induced enhancer activity (Fig. 2). In other experiments designed to maximize the measurement of basal enhancer activity, mutation of the pets site alone reduced uninduced enhancer function by five to eightfold (results not shown). Therefore, the PuB2 and pets sites each contribute significantly to the function of the intact enhancer.

Mutation of the PuB1, PuB2, or pets site does not inhibit the response to Tax. We next investigated whether the PuB1, PuB2, or pets site(s) is involved in the response to Tax. The HTLV-I/CAT construct or the mutant plasmids were cotransfected, with either a Tax-expressing plasmid or a control, into the Jurkat T-cell line. Tax activated the wild-type enhancer 81-fold, and mutation of the PuB1, PuB2, and pets sites alone or in combination did not inhibit this response (Fig. 3). If anything, mutants exhibiting lower basal activity were stimulated slightly more by Tax than was the native enhancer. These findings suggest that the PuB1, PuB2, and pets sites do not play a significant role in the response of the intact HTLV-I enhancer to Tax.

The pets site binds a distinct nuclear protein. Using DNase footprinting, we have previously demonstrated that the pets site of HIV-2, when adjacent to an intact Elf-1-binding site, can bind a nuclear factor. As it was not clear that an isolated pets site can bind a nuclear factor, and because the pets site of HTLV-I varies slightly from that of HIV-2 (31), we investigated binding to the isolated HTLV-I pets site by using EMSA with Jurkat nuclear extracts (Fig. 4). A pets band was seen in unstimulated extracts, nuclear extracts from cells stimulated with PMA (Fig. 4, lane 1 versus lane 2), and extracts from cells stimulated with PMA plus forskolin (not shown). These findings suggest that transcriptional activation is mediated through the pets site by posttranslational modification of the pets-binding factor, perhaps as a result of interactions with another nuclear protein(s). Consistent with this line of reasoning, the pets band, which is competed for by an unlabeled excess of the pets probe (lanes 3 and 4) but not by unrelated oligonucleotides (lanes 7 to 10),

FIG. 4. A nuclear factor from T cells binds to the pets site. For EMSA, nuclear extracts were prepared from Jurkat T cells which were either activated with PMA (+) or unstimulated (-). Extracts were incubated with a radiolabeled double-stranded oligonucleotide probe containing the pets site alone (lanes 1 and 2) or in the presence of the indicated amount of the unlabeled pets (lanes 3 and 4), PuB1-plus-PuB2 (Δ pets) HTLV-I oligonucleotide (lanes 5 and 6), interleukin-2 (IL-2) octamer (lanes 7 and 8), and κ B (lanes 9 and 10) competitors. The arrow indicates the pets complex.

can be partially competed for by an oligonucleotide containing the PuB1 and PuB2 sites but an inactive pets site (lanes 5 and 6). Therefore, it appears that the pets factor binds a distinct motif but may need to be complexed with the protein which recognizes the PuB1 and PuB2 sites in order to bind DNA most efficiently.

Elf-1 is the predominant protein binding the PuB1 and PuB2 sites in Jurkat and peripheral blood T cells. While chicken ets-1 has recently been shown to bind the PuB1 and PuB2 regions of HTLV-I in vitro, we reasoned that, by analogy to HIV-2, the dominant human protein binding these sites in vivo might be Elf-1. Using recombinant, truncated human ets-1, which has previously been shown to bind DNA with greater avidity than the full-length protein does (31, 59), we found that human ets-1, like chicken ets-1, can bind to the PuB1/PuB2 region of the HTLV-I enhancer in vitro (Fig. 5A). Similarly, we found that recombinant Elf-1 binds the PuB1/PuB2 region (Fig. 5B). We next used antibodies to ets-1 and Elf-1 in supershift EMSA to investigate whether either of these proteins bound to this purine-rich region of the HTLV-I enhancer in Jurkat or peripheral blood T cells. Antibodies to Elf-1 (Fig. 6A, lane 2; Fig. 6B, lane 4), but not ets-1 antibodies (Fig. 6A, lane 3; Fig. 6B, lane 5) or preimmune serum (not shown), caused a supershift with extracts from Jurkat cells. This was true whether a probe containing PuB1 and PuB2 with the pets site mutated (Fig. 6A) or a wild-type probe (Fig. 6B) was used. When nuclear extracts from peripheral blood T cells were used, antibodies to Elf-1, but not ets-1, again caused a supershift (Fig. 6B). The Elf-1 antiserum used in these experiments is quite specific and does not recognize ets-1 (55). Therefore, we conclude that Elf-1, or a very similar protein, is likely to be the major cellular factor which binds this purine-rich region of the HTLV-I enhancer in vivo.

DISCUSSION

We have shown that the function of the intact HTLV-I enhancer is dependent on the pets and PuB2 motifs. In this respect, HTLV-I resembles HIV-2, another human pathogenic retrovirus with a relatively long asymptomatic interval

FIG. 5. Human ets-1 (A) and Elf-1 (B) bind PuB1 and PuB2 in vitro. The probe is an oligonucleotide corresponding to PuB1 plus PuB2 with a mutated pets site between them (Δ pets). (A) Protein is either bacterial control extract (lane 1) or recombinant truncated ets-1 produced in bacteria (lanes 2 to 4). The competitor added in lane 3 is 100 ng of unlabeled PuB1 plus PuB2 oligonucleotide; the competitor added in lane 4 is 100 ng of the unrelated Δ CD3R (29) oligonucleotide. The arrow indicates the ets-1 complex. (B) Protein is either bacterial control extract (lane 1) or recombinant Elf-1 produced in bacterial cells (lanes 2 to 4). Anti-Elf-1 (lane 3), preimmune serum (lane 4), or no antibody (Ab) (lanes 1 and 2) was added to the reactions. The black arrow indicates the Elf-1 complex; the white arrow indicates the supershifted Elf-1 complex.

following infection (25, 29, 31). In contrast to previous studies, we have used site-directed mutation of the native enhancer, rather than the deletion mutants or chimeric promoter/enhancer constructs, to define functional elements in T cells. Mutation of either the pets or PuB2 site, but not the PuB1 site, of the HTLV-I enhancer significantly diminishes inducible enhancer activity. Mutation of the pets site in combination with PuB2 has a particularly marked effect on both basal and induced expression. The lack of response mediated through the PuB1 site is consistent with the fact that although ets-1 protects this site in *in vitro* DNase footprinting studies (10), PuB1 is not a canonical binding site for ets family members (59). While we have not formally demonstrated that the effect of mutating pets and PuB2 is mediated at the RNA level, analogy to our HIV-2 data would strongly suggest that this is the case (31). The effect on enhancer function appears to be specific for T-cell activation pathways, as mutation of these enhancer elements has little effect on the response to Tax. This finding is consistent with the data of Bosselut et al. (4), who found that the ERR-1 region did not confer Tax responsiveness on a minimal HTLV-I promoter, but differs from the findings of Marriott et al. (32). Neither of these groups examined the significance of these enhancer elements in the intact HTLV-I enhancer/promoter, however.

Tax effect is thought to be mediated, at least in part, by cAMP-responsive DNA-binding proteins (18, 36, 41, 52). While modest induction of the HTLV-I enhancer is seen with PMA alone, and forskolin alone does not stimulate the enhancer (5), forskolin acts synergistically with PMA to markedly activate the enhancer. It is the PMA-plus-forskolin induction, rather than the induction with PMA alone, which

FIG. 6. Elf-1 is the predominant protein which binds PuB1 and PuB2 in vivo. (A) The probe is an oligonucleotide corresponding to PuB1 plus PuB2, with a mutated pets site between them (lanes 1 to 3), or the PuB2 site (31) from HIV-2 (lanes 4 and 5), used as a positive control for ets-1 binding. Protein is from either Jurkat nuclear extracts (lanes 1 to 3) or bacterial cells producing cloned full-length ets-1 used as a positive control (lanes 4 and 5). Anti-Elf-1 (lane 2), anti-ets-1 (lanes 3 and 5), or no antibody (Ab) (lanes 1 and 4) was added to the binding reaction. Black arrows indicate the supershifted Elf-1 complex; the white arrow indicates the supershifted ets-1 complex. (B) The probe is an oligonucleotide corresponding to the wild-type HTLV-I sequence (−160 to −120) which contains PuB1, PuB2, and pets. Protein is either from Jurkat (lanes 1 to 5) or peripheral blood T-cell (lanes 6 to 8) nuclear extracts. The competitor added in lanes 2 and 3 is unlabeled wild-type oligonucleotide. Anti-Elf-1 (lanes 4 and 7), anti-ets-1 (lanes 5 and 8), or no antibody (Ab) (lanes 1 to 3 and 6) was added to the reactions. The black arrow indicates the Elf-1 complex; the white arrow indicates the supershifted Elf-1 complex.

is most clearly diminished by mutation of the PuB2 or pets site (Fig. 2) (5). As these elements do not mediate Tax response, it appears that the Tax effect and the forskolin (cAMP) effect can be separated. This calls to mind the findings of Niki et al., who showed that Tax and forskolin activate the 21-bp core repeat elements by separate mechanisms (36). These observations are consistent with the idea that the response to Tax is complex and cannot be explained simply as the result of the stimulation of cAMP-responsive proteins.

It has previously been demonstrated that chicken ets-1 can bind to the PuB1/pets/PuB2 (ERR-1) region of the HTLV-I enhancer (10). In addition, transfection experiments have shown that under certain circumstances, chicken ets-1 (and ets-2) can modestly (three- to fourfold) activate the intact HTLV-I promoter in cotransfection assays (4). However, the presence of a pets site suggested, by analogy with HIV-2 (25, 31), that Elf-1, not ets-1, might be the nuclear factor mediating activation through the purine-rich elements. In addition, ets-1 expression decreases with T-cell activation whereas Elf-1 expression is unchanged (2, 56), further suggesting that Elf-1 might be the response protein which recognizes PuB1 and PuB2 (ERR-1) in vivo. We have now shown that Elf-1 binds to PuB1 and PuB2 in vitro and that Elf-1 binding (or that of a closely related factor) predominates in nuclear extracts from a human T-cell line (Jurkat) or from peripheral blood T cells. While binding does not always equate with function, these data suggest that Elf-1, or a closely related ets family member, might mediate HTLV-I enhancer activation in vivo.

In addition to HTLV-I and HIV-2, ets-binding motifs have been identified in other mammalian type C retroviruses (11, 14, 54). However, not all such motifs are functional, as demonstrated above (PuB1) and in HIV-1, which contains a purine-rich region which resembles an ets-binding site (50) but is not functional (30). DNA binding and transcriptional activation by specific ets proteins may be influenced by subtle DNA sequence differences (59), inherent differences in the ets proteins, or interactions with other transcription factors, such as the pets factor. Recent findings suggest that cofactors are often necessary for binding and maximal transcriptional activation by ets proteins (23, 56, 57, 59, 62). Indeed, neither human Elf-1 nor human ets-1 alone appears sufficient to consistently activate the native HTLV-I enhancer in cotransfection experiments (5). Therefore, cofactors such as the pets factor likely cooperate with Elf-1 to activate transcription. The pets factor, which binds to a site between the PuB1 and PuB2 elements of HTLV-I and HIV-2 and mediates transcriptional activation, has not yet been fully characterized. While Elf-1 often requires AP-1 interaction to function (24), and while the pets site resembles an AP-1 site, preliminary data from supershift EMSAs indicate that Jun proteins may not be present in the pets complex (5). Although Numata et al. (37) described an NF- κ B-like activity in the pets/PuB2 region, our data show pets factor binding to be constitutive (unlike NF- κ B), and binding is not competed for by κ B. Gégonne et al. have recently shown that recombinant Sp1 can bind to the pets region of ERR-1 and can function synergistically with ets-1 to activate an ERR-1/heterologous promoter construct in *Drosophila* cells (8). However, EMSAs using nuclear extracts from human T cells or monocytes have shown that an oligonucleotide containing the Sp1-binding site does not compete for pets binding (5, 16). Therefore, it appears unlikely that Sp1 is the protein which binds the pets site in vivo. The pets-binding site is also in the same region as the binding site for TIF-1 (32, 33), but it is not yet clear whether TIF-1 and the pets factor are related proteins. The pets factor might also be related to other recently described proteins which bind TG-rich regions of T-cell receptor and murine leukemia virus enhancers (21, 43, 46, 60). Similar to Elf-1 (29, 31), the pets factor binds DNA constitutively, indicating that posttranslational modifications or interactions with other factors must occur to stimulate transcription. Further studies are necessary to characterize the pets factor and its interaction with Elf-1 and other ets proteins. It appears that interactions between these and other cellular proteins regulate HTLV-I transcription and may modulate the pathogenic potential of this human retrovirus.

ACKNOWLEDGMENTS

We thank Gary Nabel and Bronia Petryniak for helpful discussions, Takis Papas for anti-ets-1 antibodies, Jeffrey Leiden and Craig Thompson for plasmids and anti-Elf-1 antibodies, and Mara Van Dusen for preparation of the manuscript.

This work was supported by grants to D.M.M. from the National Institutes of Health (CA01479 and AI30924) and from the Life and Health Insurance Medical Research Fund. N.M.C. was supported by National Institutes of Health training grant T32AI07360.

REFERENCES

- Ben-David, Y., E. B. Giddens, K. Letwin, and A. Bernstein. 1991. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the *ets* gene family, *Fli-1*, closely linked to *c-ets-1*. *Genes Dev.* 5:908-918.
- Bhat, N. K., C. B. Thompson, T. Lindsten, C. H. June, S. Fujiwara, S. Koizumi, R. J. Fisher, and T. S. Papas. 1990.

- Reciprocal expression of human *ETS1* and *ETS2* genes during T-cell activation: regulatory role for the protooncogene *ETS1*. *Proc. Natl. Acad. Sci. USA* 87:3723-3727.
3. Blattner, W. A. 1989. Human T-lymphotropic viruses and diseases of long latency. *Ann. Intern. Med.* 111:4-6.
 4. Bosselut, R., J. F. Duvall, A. Gégonne, M. Bailly, A. Hémar, J. Brady, and J. Ghysdael. 1990. The product of the *c-ets-1* proto-oncogene and the related *Ets2* protein act as transcriptional activators of the long terminal repeat of human T cell leukemia virus HTLV-I. *EMBO J.* 9:3137-3144.
 5. Clark, N. M., M. J. Smith, J. M. Hilfinger, and D. M. Markovitz. Unpublished data.
 6. Davis, M. G., S. C. Kenney, J. Kamine, J. S. Pagano, and E.-S. Huang. 1987. Immediate-early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 84:8642-8646.
 7. Dignam, J. D., R. M. Lebowitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* 11:1475-1489.
 8. Gégonne, A., R. Bosselut, R.-A. Bailly, and J. Ghysdael. 1993. Synergistic activation of the HTLV-I LTR *ets*-responsive region by transcription factors *ets-1* and *Sp1*. *EMBO J.* 12:1169-1178.
 9. Gessain, A., J. C. Vernant, L. Maurs, F. Barin, O. Gout, A. Calender, and G. De Thé. 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* ii:407-410.
 10. Gitlin, S. C., R. Bosselut, A. Gégonne, J. Ghysdael, and J. N. Brady. 1991. Sequence-specific interaction of the *Ets1* protein with the long terminal repeat of the human T-lymphotropic virus type I. *J. Virol.* 65:5513-5523.
 11. Golemis, E. A., N. A. Speck, and N. Hopkins. 1990. Alignment of U3 region sequences of mammalian type C viruses: identification of highly conserved motifs and implications for enhancer design. *J. Virol.* 64:534-542.
 12. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044-1051.
 13. Graham, F. L., and A. J. van der Erb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *J. Virol.* 52:456-467.
 14. Gunther, C. V., J. A. Nye, R. S. Bryner, and B. J. Graves. 1990. Sequence-specific DNA binding of the proto-oncoprotein *ets-1* defines a transcriptional activator sequence within the long terminal repeat of the Moloney murine sarcoma virus. *Genes Dev.* 4:667-679.
 15. Hall, W. W., C. R. Liu, O. Schneewind, H. Takahashi, M. H. Kaplan, G. Róupe, and A. Vahlne. 1991. Deleted HTLV-I provirus in blood and cutaneous lesions of patients with mycosis fungoides. *Science* 253:317-320.
 16. Hilfinger, J. M., N. Clark, M. Smith, K. Robinson, and D. M. Markovitz. 1993. Differential regulation of the human immunodeficiency virus type 2 enhancer in monocytes at various stages of differentiation. *J. Virol.* 67:4448-4453.
 17. Ho, I.-C., N. K. Bhat, L. R. Gottschalk, T. Lindsten, C. B. Thompson, T. S. Papas, and J. M. Leiden. 1990. Sequence-specific binding of human *Ets-1* to the T cell receptor alpha gene enhancer. *Science* 250:814-818.
 18. Jeang, K.-T., I. Boros, J. Brady, M. Radonovich, and G. Khoury. 1988. Characterization of cellular factors that interact with the human T-cell leukemia virus type I p40^x-responsive 21-base-pair sequence. *J. Virol.* 62:4499-4509.
 19. Kanki, P. 1989. Clinical significance of HIV-2 infection in West Africa, p. 95-108. *In* P. Volberding and M. A. Jacobson (ed.), *AIDS clinical review*. Marcel Dekker, Inc., New York.
 20. Kim, J. H., and D. T. Durack. 1988. Manifestations of human T-lymphotropic virus type I infection. *Am. J. Med.* 84:919-928.
 21. Kingsley, C., and A. Winoto. 1992. Cloning of GT box-binding proteins: a novel *Sp1* multigene family regulating T-cell receptor gene expression. *Mol. Cell. Biol.* 12:4251-4261.
 22. Klemsz, M. J., S. R. McKercher, A. Celada, C. Van Beveren, and R. A. Maki. 1990. The macrophage and B cell-specific transcription factor PU.1 is related to the *ets* oncogene. *Cell* 61:113-124.
 23. LaMarco, K., C. C. Thompson, B. P. Byers, E. M. Walton, and S. L. McKnight. 1991. Identification of *ets*- and *notch*-related subunits in GA binding protein. *Science* 253:789-792.
 24. Leiden, J. M. Personal communication.
 25. Leiden, J. M., C.-Y. Wang, B. Petryniak, D. M. Markovitz, G. J. Nabel, and C. B. Thompson. 1992. A novel *Ets*-related transcription factor, *Elf-1*, binds to human immunodeficiency virus type 2 regulatory elements that are required for inducible *trans* activation in T cells. *J. Virol.* 66:5890-5897.
 26. Leung, K., and G. J. Nabel. 1988. HTLV-I transactivator induces interleukin-2 receptor expression through an NF- κ B-like factor. *Nature (London)* 333:776-778.
 27. Mann, D. L., P. DeSantis, G. Mark, A. Pfeifer, M. Newman, N. Gibbs, M. Popovic, M. G. Sarngadharan, R. C. Gallo, J. Clark, and W. Blattner. 1987. HTLV-I-associated B-cell CLL: indirect role for retrovirus in leukemogenesis. *Science* 236:1103-1106.
 28. Markovitz, D. M. 1993. Infection with the human immunodeficiency virus type 2. *Ann. Intern. Med.* 118:211-218.
 29. Markovitz, D. M., M. Hannibal, V. L. Perez, C. Gauntt, T. M. Folks, and G. J. Nabel. 1990. Differential regulation of human immunodeficiency viruses (HIVs): a specific regulatory element in HIV-2 responds to stimulation of the T cell antigen receptor. *Proc. Natl. Acad. Sci. USA* 87:9098-9102.
 30. Markovitz, D. M., M. C. Hannibal, M. Smith, R. Cossman, and G. J. Nabel. 1992. Activation of the human immunodeficiency virus type 1 enhancer is not dependent on NFAT-1. *J. Virol.* 66:3961-3965.
 31. Markovitz, D. M., M. Smith, J. Hilfinger, M. C. Hannibal, B. Petryniak, and G. J. Nabel. 1992. Activation of the human immunodeficiency virus type 2 enhancer is dependent on purine box and κ B regulatory elements. *J. Virol.* 66:5479-5484.
 32. Marriott, S. J., I. Boros, J. F. Duvall, and J. N. Brady. 1989. Indirect binding of human T-cell leukemia virus type I tax₁ to a responsive element in the viral long terminal repeat. *Mol. Cell. Biol.* 9:4152-4160.
 33. Marriott, S. J., P. F. Lindholm, K. M. Brown, S. D. Gitlin, J. F. Duvall, M. F. Radonovich, and J. N. Brady. 1990. A 36-kilodalton cellular transcription factor mediates an indirect interaction of human T-cell leukemia/lymphoma virus type I TAX₁ with a responsive element in the viral long terminal repeat. *Mol. Cell. Biol.* 10:4192-4201.
 34. Metz, T., and T. Graf. 1991. Fusion of the nuclear oncoproteins v-Myb and v-Ets is required for the leukemogenicity of E26 virus. *Cell* 66:95-105.
 35. Nabel, G. J., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* 326:711-713.
 36. Niki, M., K. Ohtani, M. Nakamura, and K. Sugamura. 1992. Multistep regulation of enhancer activity of the 21-base-pair element of human T-cell leukemia virus type I. *J. Virol.* 66:4348-4357.
 37. Numata, N., K. Ohtani, M. Niki, M. Nakamura, and K. Sugamura. 1991. Synergism between two distinct elements of the HTLV-I enhancer during activation by the trans-activator of HTLV-I. *New Biol.* 3:896-906.
 38. Osame, M., K. Usuku, S. Izumo, N. Ijichi, H. Amitani, A. Igata, M. Matsumoto, and M. Tara. 1986. HTLV-I associated myelopathy, a new clinical entity. *Lancet* i:1031-1032.
 39. Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* 77:7415-7419.
 40. Poiesz, B. J., F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, and R. C. Gallo. 1981. Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sézary T-cell leukaemia. *Nature (London)* 294:268-271.
 41. Poteat, H. T., F. Y. Chen, P. Kadison, J. G. Sodroski, and W. A. Haseltine. 1990. Protein kinase A-dependent binding of a nuclear factor to the 21-base-pair repeat of the human T-cell leukemia virus type I long terminal repeat. *J. Virol.* 64:1264-1270.
 42. Poteat, H. T., P. Kadison, K. McGuire, L. Park, R. E. Park,

- J. G. Sodroski, and W. A. Haseltine. 1989. Response of the human T-cell leukemia virus type I long terminal repeat to cyclic AMP. *J. Virol.* **63**:1604–1611.
43. Prosser, H. M., D. Wotton, A. Gégonne, J. Ghysdael, S. Wang, N. A. Speck, and M. J. Owen. 1992. A phorbol ester response element within the human T-cell receptor β -chain enhancer. *Proc. Natl. Acad. Sci. USA* **89**:9934–9938.
 44. Queen, C., and D. Baltimore. 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. *Cell* **33**:741–748.
 45. Ravel-Chapuis, P., D. Leprince, B. Pain, R. Li, C. Domenget, D. Stehelin, J. Samarut, and P. Jurdic. 1991. *v-myb* and *v-ets* cooperate for the mitogenic stimulation of primary fibroblasts by avian E26 retrovirus. *J. Virol.* **65**:3928–3931.
 46. Redondo, J. M., J. L. Pfohl, C. Hernandez-Munain, S. Wang, N. A. Speck, and M. S. Krangel. 1992. Indistinguishable nuclear factor binding to functional core sites of the T-cell receptor δ and murine leukemia virus enhancers. *Mol. Cell. Biol.* **12**:4817–4823.
 47. Richardson, J. H., A. J. Edwards, J. K. Cruickshank, P. Rudge, and A. G. Dalgleish. 1990. In vivo cellular tropism of human T-cell leukemia virus type I. *J. Virol.* **64**:5682–5687.
 48. Rosenblatt, J. D., I. S. Y. Chen, and W. Wachsman. 1988. Infection with HTLV-I and HTLV-II: evolving concepts. *Semin. Hematol.* **25**:230–246.
 49. Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida. 1983. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA* **80**:3618–3622.
 50. Shaw, J.-P., P. J. Utz, D. B. Durand, J. J. Toole, E. A. Emmel, and G. R. Crabtree. 1988. Identification of a putative regulator of early T cell activation genes. *Science* **241**:202.
 51. Singh, H., R. Sen, D. Baltimore, and P. Sharp. 1986. A nuclear factor that binds to a conserved motif in transcriptional control elements of immunoglobulin genes. *Nature (London)* **319**:154–158.
 52. Smith, M. R., and W. C. Greene. 1991. Molecular biology of the type I human T-cell leukemia virus (HTLV-I) and adult T-cell leukemia. *J. Clin. Invest.* **87**:761–766.
 53. Sodroski, J. G., C. A. Rosen, and W. A. Haseltine. 1984. *Trans*-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. *Science* **225**:381–385.
 54. Speck, N. A., B. Renjifo, and N. Hopkins. 1990. Point mutations in the Moloney murine leukemia virus enhancer identify a lymphoid-specific viral core motif and 1,3-phorbol myristate acetate-inducible element. *J. Virol.* **64**:543–550.
 55. Thompson, C. B. Personal communication.
 56. Thompson, C. B., C.-Y. Wang, I.-C. Ho, P. R. Bohjanen, B. Petryniak, C. H. June, S. Miesfeldt, L. Zhang, G. J. Nabel, B. Karpinski, and J. M. Leiden. 1992. *cis*-acting sequences required for inducible interleukin-2 enhancer function bind a novel Ets-related protein, Elf-1. *Mol. Cell. Biol.* **12**:1043–1053.
 57. Thompson, C. C., T. A. Brown, and S. L. McKnight. 1991. Convergence of Ets- and notch-related structural motifs in a heteromeric DNA binding complex. *Science* **253**:762–768.
 58. Uchiyama, T., J. Yodoi, K. Sagawa, K. Takatsuki, and H. Uchino. 1977. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* **50**:481–492.
 59. Wang, C.-Y., B. Petryniak, I.-C. Ho, C. B. Thompson, and J. M. Leiden. 1992. Evolutionarily conserved sub-families of ets proteins display distinct DNA binding specificities. *J. Exp. Med.* **175**:1391–1399.
 60. Wang, S., and N. A. Speck. 1992. Purification of core-binding factor, a protein that binds the conserved core site in murine leukemia virus enhancers. *Mol. Cell. Biol.* **12**:89–102.
 61. Wano, Y., M. Feinberg, J. B. Hosking, H. Bogerd, and W. C. Greene. 1988. Stable expression of the *tax* gene of type I human T-cell leukemia virus in human T cells activates specific cellular genes involved in growth. *Proc. Natl. Acad. Sci. USA* **85**:9733–9737.
 62. Wasylyk, B., C. Wasylyk, P. Flores, A. Begue, D. Leprince, and D. Stehelin. 1990. The *c-ets* proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature (London)* **346**:191–193.
 63. Yoshida, M., I. Miyoshi, and Y. Hinuma. 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. Natl. Acad. Sci. USA* **79**:2031–2035.