Purification of the pets Factor

A NUCLEAR PROTEIN THAT BINDS TO THE INDUCIBLE TG-RICH ELEMENT OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 2 ENHANCER*

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The peri-ets (pets) site is a TG-rich element found immediately adjacent to two binding sites for the ets family member Elf-1 in the human immunodeficiency virus type 2 (HIV-2) enhancer. Enhancer activation in response to T cell stimulation by phorbol myristate acetate, phytohemagglutinin, soluble or cross-linked antibodies to the T cell receptor, or antigen is mediated through this site in conjunction with its two adjacent Elf-1 binding sites, PuB1 and PuB2, and a κB site. Site-specific mutation of the pets element significantly reduces inducible activation of this enhancer but does not affect its transactivation by HIV-2 tat or other viral transactivators. Similar TG-rich sequences adjacent to ets-binding sites have also been found to be functionally important in the human T-cell leukemia virus type I and murine Moloney leukemia virus enhancers. As the cellular factor binding to the pets site plays a significant role in regulating the HIV-2 enhancer in both T cells and monocytes, we have purified this protein from bovine spleens and demonstrate that it is 43 kDa in size. In addition, using glycerol gradient centrifugation, Southwestern blotting, electrophoretic mobility shift assays employing purified protein eluted from a gel, and a new in solution UV-cross-linking competitive assay, we show that the dominant protein binding to the pets site is 43 kDa in size. These results indicate that a nuclear protein of 43 kDa binds specifically to the pets site of the HIV-2 enhancer and may mediate transcriptional activation of this important human pathogen in response to T cell stimulation. As retroviruses generally expropriate important human regulatory proteins for their own use, the 43-kDa pets factor is also likely to play a significant role in signal transduction in T cells and in other cellular processes.

Like HIV-1, HIV-2 can cause AIDS, but typically the asymptomatic period following HIV-2 infection is much longer than that following HIV-1 infection. Genetically, HIV-2 is quite different from HIV-1 and only shares ~40% nucleic acid sequence similarity (1, 2). Different transcriptional enhancer elements which respond to T cell stimulation in the enhancers of these two viruses may explain, in part, the clinical differences observed between persons infected with HIV-1 and HIV-2. Unlike HIV-1, in which the two κB sites play the dominant role in regulating inducible enhancer function in activated T cells (3–6), HIV-2 enhancer activation in T cells is regulated by at least four distinct cis-acting elements: two purine-rich sites (PuB1 and PuB2), which bind the ets proto-oncogene family member Elf-1; the peri-ets, or pets site, which binds a protein described below; and a single κB site, which binds the well described components of NF-κB (3, 7–11). A fifth element, the peri-κB site, mediates HIV-2 enhancer induction in mononuclear cells but not in T cells (12). While these five sites are not present in HIV-1, they are conserved in a wide range of HIV-2 isolates. Mutation of any of these elements markedly diminishes the response of the enhancer to cellular activation but does not affect the response to tat or to other viral transactivating proteins. Therefore, these elements specifically mediate enhancer stimulation in activated T cells or monocytes and serve as the final common mediators in signal transduction pathways.

The pets site is a TG-rich element (TTGTCAGGG) found between the two Elf-1 binding sites in the HIV-2 enhancer (Fig. 1). We have demonstrated the functional importance of this site in the activation of the HIV-2 enhancer in both T cells and monocytes (8, 9). The pets element mediates activation whether the enhancer is stimulated by phorbol myristate acetate alone, phytohemagglutinin alone, phorbol myristate acetate plus phytohemagglutinin, soluble antibodies to the T cell receptor, immobilized antibodies to the T cell receptor, or by antigen (3, 7–11). We and others have also demonstrated that a similar TG-rich pets-like site, again adjacent to an Elf-1 binding site, plays a significant role in mediating activation of the human T cell leukemia virus type 1 (HTLV-1) enhancer in stimulated T cells (13, 14). TG-rich sites adjacent to the ets-binding sites are also found in murine retroviruses, and alteration of these pets-like sites can change the type of malignancy seen in mice following infection (15). Therefore pets-like elements adjacent to ets-binding sites appear to play an important role in the enhancers of retroviruses and serve as a final common mediator for signal transduction pathways in monocytes and especially T cells.

In this report, we describe the biochemical purification of the pets factor and show that it is a 43-kDa protein. This conclusion is based on glycerol gradient sedimentation, Southwestern
HIV-2

then clarified by centrifugation at 12,000 × g for 20 min. Proteins in the supernatant were precipitated by the gradual addition of ammonium sulfate to 50% saturation. The precipitate was collected by centrifugation at 10,000 × g for 30 min and stored in aliquots at −70°C.

Chromatographic Purification—The following steps were performed at 4°C: for each column run, the ammonium sulfate precipitate from half of a spleen was resuspended in buffer A (20 mM Tris-HCl, pH 7.0, 100 mM KCl, 1 mM EDTA, 0.01% Nonidet P-40, 10% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) and dialyzed overnight against 100 times its volume of buffer A. The dialysate was centrifuged at 10,000 × g for 60 min, and successively filtered through 2.7- and 1.6-μm glass fiber filters (Whatman) to clarify the protein solution before loading onto a column packed with 250 ml of Q-Sepharose Fast Flow resin (Pharmacia) according to the manufacturer’s guidelines. Following loading, the column was washed with 2 liters of buffer A or until the absorbance at 280 nm returned to baseline. Proteins bound to the column were eluted with 10 bed volumes of a 100 mM KCl stepwise gradient. The binding activity, as measured by EMSA, typically eluted at 400–500 mM KCl. Positive fractions were then pooled and dialyzed against buffer A before loading onto a 40-ml heparin-agarose (Bio-Rad) column. The procedures for subsequent washing and elution were the same as for the Q-Sepharose chromatography. The binding activity eluted around 300 mM KCl. DNA affinity chromatography was performed using a DNA-Sepharose column and the method of Kadonaga and Tjian (16), with the pets oligonucleotide used in EMSAs. The positive fractions eluted from the heparin column were pooled, dialyzed against buffer A, and loaded onto a 2-ml DNA affinity column at gravity flow. During the first pass over the DNA affinity column, 12 μg of calf thymus DNA was added as a nonspecific competitor to the mg protein applied to the column. The column was washed with 20 ml of buffer A and bound proteins were eluted with 10 ml of buffer A containing 1 mM KCl. The eluate was diluted to 100 mM KCl in buffer A and reapplied to a newly prepared 1-ml DNA affinity column. To precipitate the eluted proteins, trichloroacetic acid was added to 10% final concentration in the presence of 200 μM sodium deoxycholate. After incubation on ice for 60 min, the precipitate was collected by centrifugation at 13,000 × g for 10 min and washed once with acetone before it was resolubilized in 100 μl of 3 mM guanidine HCl (or 5 mM urea for SDS-PAGE). 0.05 mM Tris, pH 8.5, 25 mM DTT. 5 μl were analyzed on SDS-PAGE, and the remainder was injected onto a C4 reverse phase column (Vydac). Proteins were eluted using a 0 to 100% acetonitrile gradient in 0.1% trifluoroacetic acid and the 220-nm absorbance peaks were collected and analyzed by silver staining after SDS-PAGE.

Glycerol Gradient Sedimentation—Density gradient centrifugation was performed according to the method of Martin and Ames (17), with the following modifications. After 2 successive rounds of DNA affinity chromatography, 100 μl of the eluate was mixed with 50 μg each of lysostaphin, bovine serum albumin, ovalbumin, and alcohol dehydrogenase and layered onto a 7-ml 10–25% glycerol gradient in buffer A without DTT. After 21 h of centrifugation at 45,000 rpm in a Beckman SW-41 rotor, a Beckman fractionator was used to collect 40 aliquots. Each fraction was assayed for binding activity (EMSA) and silver staining after SDS-PAGE. The specific binding activity was measured by counting the 32P cpm in the EMSA shifted bands using a β-counter (Betagen). SDS-Polyacrylamide Gel Electrophoresis—SDS-PAGE was performed as described by Laemmli (18). Sample buffer contained 50 mM Tris, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol. Prestained molecular mass standards were purchased from Life Technologies, Inc. Silver staining was performed as described by Wray et al. (19).

DNase I Protection Assay—Jurkat nuclear extract was prepared by a modification of the method of Dignam et al. (20). Purified pets factor was prepared as described above. A radiolabeled probe corresponding to nucleotides −107 to −189 (Fig. 1) was prepared, and DNase I protection assays were performed as described previously (3, 8, 21, 22). Purified proteins were concentrated in Centricon (Amicon) before use in DNase I footprints.

Electrophoretic Mobility Shift Assays—Oligonucleotides corresponding to both strands of the HIV-2 sequence from −162 to −131 with the PuB2 site mutated (Fig. 1) were synthesized with an Applied Biosystems 380B synthesizer. The sequence of the sites was determined by sequence analysis. Each strand was 5′-CTCAGCATCTTACGACTGGCGGTCTTACTA-3′. Equimolar amounts of the two strands were mixed, boiled for 1 min in 0.5 M NaCl, and allowed to cool gradually. The double-stranded oligonucleotide was then radiolabeled with T4 polynucleotide kinase in the presence of [γ-32P]ATP.
M urea and 50 mM DTT for 60 min at room temperature. The blot was reblocked as indicated in Fig. 2. Glycerol gradient analysis of the pets factor. 5 μl of each fraction was tested for pets binding activity using EMSA as described under “Experimental Procedures.” The arrow indicates the pets complex as seen in EMSA. 5 μl of each fraction was also separated on an 8% SDS-PAGE and stained with silver (not shown) to identify the fractions containing the internal standards as indicated. In lane 14, the peak of the pets-site binding activity corresponds with the ovalbumin peak (43 kDa).

Purification of the pets Factor

western hybridization, polymerase chain reaction primers flanking the multimeric insert were synthesized and used to amplify from a linearized 8x-pets construct using standard polymerase chain reaction procedures but with the inclusion of 5 μl of [α-32P]dCTP (Amersham, 6000 Ci/mmol, 10 mCi/ml). Unincorporated nucleotides were removed after polymerase chain reaction, using Ultrafree-MC filter units (Millipore).

RESULTS

Determination of the Molecular Mass of the pets Factor—Bovine spleen nuclear extracts were found to have a high level of specific binding activity for the HIV-2 pets site, and was chosen as a starting material for purification because of the large quantity that was readily available. To estimate the native molecular mass of the pets factor, purified pets factor (see below) was applied to a glycerol gradient and protein complexes were separated on the basis of their sedimentation coefficients using ultracentrifugation. Individual fractions were collected and assayed for sequence-specific DNA binding activity using EMSA. In our glycerol gradient sedimentation experiments, we have included four internal standards in each tube: alcohol dehydrogenase (150 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), and lysozyme (17 kDa). The location of these standards in the glycerol gradient fractions was determined by SDS-PAGE of 10 μl of each fraction followed by silver staining. The fraction that contained the peak DNA binding activity was also the fraction with the peak ovalbumin concentration (Fig. 2, lane 14).

As determination of molecular mass by glycerol gradient ultracentrifugation may be misleading due to aggregation of the protein of interest with itself or co-purified proteins, we used crude spleen extracts in UV cross-linking studies to find out the molecular mass of the pets factor under conditions where all nuclear proteins are present. Proteins which are capable of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe were capa ble of close contact with the bromouridine pets probe.
cross-linked to the $^{32}$P-labeled probe with UV irradiation. The protein-DNA complexes were then separated by SDS-PAGE to estimate the molecular mass of the protein-DNA complex. Since many nuclear proteins in the crude extract will exhibit nonspecific affinity for the labeled DNA, we improved the specificity of this often used technique by including poly(dI-dC) at 1 μg reaction and oligonucleotide competitors as indicated in Fig. 3. As expected, crude Jurkat nuclear extracts contain numerous proteins that cross-link to the pets probe (lane 3). Inclusion of 500 μ excess of an unlabeled pets oligonucleotide competed out two bands: one indicated by the arrow in Fig. 3, and the other at 70 kDa (lane 4). However, inclusion of a 500 μ excess of an oligonucleotide with an unrelated mutant HIV-2 kB (lane 6) or wild-type HIV-2 kB (lane 7) oligonucleotide did not compete away the two bands. The mutant pets oligonucleotide, in which only three nucleotides have been changed from the wild-type sequence, only weakly competed with the pets probe for binding (lane 5). In lane 8, purified bovine pets factor (after one round of DNA affinity chromatography) was used in the cross-linking reaction and a more highly purified fraction (after two rounds of DNA affinity chromatography) of bovine pets factor was used in lane 9. The cross-linking bands created by the purified pets factor appear to be identical in mobility to the two bands that competed out specifically in the experiments using crude extracts. As the effect of the cross-linked pets probe on protein migration in SDS-PAGE was not known, we exploited the affinity for DNA of two commercially available enzymes to estimate this effect. DNA polymerase I (Klenow fragment, New England Biolabs) and T₄ DNA polymerase (Boehringer Mannheim) were cross-linked to the pets site probe (lanes 1 and 2). From these two DNA-binding proteins, the effect of a cross-linked pets probe on protein mobility in SDS-PAGE was estimated to be an addition of about 10 kDa to the proteins apparent molecular mass, assuming that the dynamics of DNA-protein cross-linking did not vary considerably. From these estimates, the dominant band from the purified protein represents a protein of about 43 kDa. A lighter band at 70 kDa capable of specific binding was observed in the experiments using crude spleen nuclear extracts. This band was also seen in lanes using highly purified pets factor (Fig. 3, lanes 8 and 9). As Southwestern blotting experiments using purified pets factor (Fig. 6C) did not show any additional bands other than the 43-kDa protein, this suggests that either some other protein may be cross-linked to the pets probe through its affinity for the 43-kDa protein or that two or more proteins that do not bind to the pets site by themselves dimerize to bind to the pets site. The improvements we made to the commonly used UV cross-linking assay gives this technique the additional ability to test the sequence specificity of proteins binding to DNA, as well as a means to estimate the effect of a particular DNA probe in mobility of protein-DNA complexes through SDS-PAGE.

**Purification of the pets Factor**—The pets factor was purified from bovine spleen using a five-step purification scheme (Fig. 4). Initial studies done with EMSA using nuclear extracts from mice and bovine heart, lung, thymus, and brain tissues indicated that a high pets-site specific binding activity was detected in spleen extracts (not shown). We therefore obtained spleens from freshly slaughtered cows and processed them as described under “Experimental Procedures.” A Q-Sepharose Fast Flow column was chosen as the first chromatography step because of its high binding capacity and high flow rates. For each chromatography procedure used, a KCl salt gradient was used to elute the bound proteins from the column. Two μl from each fraction was used in EMSA to test for the presence of the pets factor. Two rounds of DNA-affinity chromatography purified the pets factor more than 3000-fold over crude extract with an overall yield of 4.7% (Table I). Using approximately 6000 times less protein than that used with crude Jurkat nuclear extracts, the purified pets extracts bound to the HIV-2 pets site in DNase I footprinting assays (Fig. 5, lane A). Of note, the pets footprint using either crude or purified extract extended somewhat more 5’ than in our previous experiments (Fig. 1 and Ref.

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**Table I**

| Purification step | Total protein mg | Total activity 10^6 cpm | Specific activity 10^6 cpm/mg | Purification fold | Yield % |
|------------------|------------------|------------------------|-------------------------------|------------------|--------|
| Nuclear extract  | 212              | 452                    | 0.21                          | 1                | 100    |
| Q-Sepharose anion-exchange | 136       | 142                    | 1.04                          | 4.9              | 157    |
| Heparin-agarose affinity | 16.8  | 30.8                   | 1.8                           | 8.45             | 34     |
| DNA-affinity     | 0.003            | 215                    | 716.6                         | 3364             | 4.7    |

a Determined using protein assay kit (Bio-Rad) with bovine serum albumin as standard.
b Determined by cpm counting of shifted complex on EMSA gel.
c Represents the fraction that is loaded onto the Q-Sepharose column.
d Represents two successive rounds over a DNA affinity column.
e Estimated from Coomassie staining intensity relative to bovine serum albumin standards.
8). The 3’ extension of the footprint obtained with the crude Jurkat extract (lane J) corresponds to the PuB2 site, which our previous work has shown binds Elf-1 (7, 9). Silver staining of the highly purified extract shows only one dark staining band at 43 kDa (Fig. 7A, lanes 3–6), although other much lighter staining bands were also detected.

The 43-kDa Polypeptide Is Sufficient for Binding to the pets Site—To determine whether or not the purified 43-kDa protein itself was capable of binding to the pets site, we performed Southwestern blotting experiments. Our initial concatemeric pets site probe generated by ligation of oligonucleotides containing a single pets-binding site yielded inconsistent results in Southwestern blotting experiments and we were unable to detect any definite binding. In contrast to Southwestern experiments using a probe generated through ligation in vitro of 1 × oligonucleotides, we found that an 8 × concatemeric probe with the binding sites all cloned in the same orientation gave more well defined bands and much more consistent results from one experiment to another. This may be because a “flip-flop” orientation of the binding sites can lead to conflicts in protein-DNA interactions. Southwestern experiments performed using this probe and crude Jurkat nuclear extracts (Fig. 6A), or crude spleen extracts (Fig. 6B), demonstrate that the molecular mass of the protein binding specifically to the pets site in the presence of increasing amounts of a nonspecific competitor is a 43-kDa protein. Southwestern blotting of highly purified pets factor also shows binding to a protein of 43 kDa (Fig. 6C). Thus, both bovine and human pets factor are 43 kDa and are likely to be the same protein. To further purify the 43-kDa protein and confirm its ability to bind to the pets site, we injected proteins eluted from the final DNA affinity column onto an HPLC reverse phase column. The protein eluted (peak absorbance) from the reverse phase column was separated by SDS-PAGE and the band corresponding to 43 kDa was extracted from the gel slice. After a denaturation-renaturation step, this 43-kDa protein was shown to bind to the pets probe (Fig. 7B, lane 1) and can be competed out by the addition of unlabeled pets oligonucleotide but not by the unrelated HIV-2 kB oligonucleotide (lane 2 versus lane 3).

DISCUSSION

In this report, we describe the purification of a 43-kDa cellular factor that binds to an element of the HIV-2 enhancer that we have previously shown to be functionally important in mediating transcriptional activation in response to T cell stimulation (8, 10, 11). We have also shown using glycerol gradient ultracentrifugation, DNase I footprinting, UV cross-linking, and Southwestern blotting that, in both crude bovine spleen

Fig. 5. Purified pets factor protects the HIV-2 pets site in DNase footprinting studies. The lane labeled “G” is a G+A ladder generated from the probe, which corresponds to nucleotides −107 to −189 (Fig. 1). The lane labeled “F” shows the result of DNase digestion when no nuclear protein is present. The lane labeled “A” shows the footprint obtained when purified pets factor is added. The lane labeled “J” demonstrates the footprint obtained when crude Jurkat T cell nuclear extract is added. Approximately 6000 times more protein is added in lane J than is added in lane A. The radiolabeled probe used was labeled on the non-coding strand and, therefore, the more 5’ the sequence, the closer to the top of the gel it is found. The pets footprint is indicated by the brackets.

Fig. 6. Southwestern hybridization performed with crude Jurkat nuclear extracts (A), bovine spleen extracts (B), or purified pets factor (C). In A and B, 200 μg of protein was separated by SDS-PAGE for each lane, electroblotted, and hybridized to probe as described. The hybridization was performed with the addition of 30 μg (lanes 2) and 300 μg (lanes 3) of calf thymus DNA as a nonspecific competitor in the 5 ml of hybridization buffer used for each lane. The 43-kDa polypeptide binds specifically to the pets probe. Although other dark bands of lower molecular mass are also seen initially in the bovine spleen extracts, after purification only the 43-kDa band remains (C).
extracts and Jurkat nuclear extracts, the dominant protein binding to the pets site in vitro is 43 kDa in molecular mass. Several proteins of other apparent molecular masses were seen in our less purified fractions, but at much lower concentrations, and it is possible that these may eventually turn out to contribute to the regulation of gene expression mediated by the pets element. The data we present here, based on renaturation after protein elution from SDS-PAGE, DNase I footprinting, Southwestern hybridization, glycerol gradient ultracentrifugation, reverse phase HPLC, and UV cross-linking, taken together point to the 43-kDa polypeptide as the pets-site specific DNA-binding protein. This 43-kDa protein is also seen using purification schemes that slightly differ from the scheme described above. The use of a Superose FPLC (Pharmacia) or a Mono-Q column (Pharmacia) also results in the purification of a 43-kDa protein.2

In this work, we have made improvements to the widely used UV cross-linking and Southwestern blotting techniques. To our knowledge, UV cross-linking studies so far described have not included the use of competitors to test for DNA binding specificity. Traditional UV cross-linking studies also do not include an internal control to estimate the effect of a particular bound probe on protein mobility in SDS-PAGE. We describe here improvements to the method which we have used successfully for our studies on the HIV-2 pets site. During the course of our Southwestern blotting experiments, we noticed that the previously described means of generating an effective concatemeric probe for hybridization to immobilized proteins separated by SDS-PAGE produced inconsistent results. We find that concatenation through ligation of single copy oligonucleotides to each other often did not produce a satisfactory probe because of the conflicting flip-flop orientation of the binding site. While it is possible to synthesize a multimeric oligonucleotide, this alternative is not cost effective. For our Southwestern blotting experiments, we have constructed clones containing the pets-binding site in a head-to-tail orientation, so that all binding sites are in the same orientation. The insert can be excised and end-labeled, or used as a template for polymerase chain reaction-mediated labeling reactions, but probes generated from random-primed labeling gave rise to much higher backgrounds, presumably due to the heterogeneity of labeled probe. We have also found that probes up to 16x-pets proved to be extremely sensitive for Southwestern blotting, but an 8x-pets probe was sufficient for most experiments. In addition, we have also used another multimeric construct containing a different DNA-binding site to successfully show the applicability of this approach to other DNA-binding proteins as well.3

Using the improvements described above, we demonstrate that the bovine protein binding specifically to the pets site appears to have the same 43-kDa molecular mass as the human counterpart. The pets site consists of a TG-rich element found between two Elf-1 binding sites on the HIV-2 enhancer. This arrangement of a TG-rich element immediately adjacent to ets-binding sites is also seen in the HTLV-1, MLV, and human T-cell receptor β-chain (TCRβ) enhancers (13, 15, 25–27). We have previously shown that when the HIV-2 pets site is mutated, enhancer activation in response to T cell stimulation is greatly reduced (8). The HIV-2 pets site appears to be a common element in signal transduction, as this site mediates activation whether the enhancer is stimulated by phorbol myristate acetate alone, phytohemagglutinin alone, phorbol myristate acetate plus phytohemagglutinin, soluble antibodies to the T cell receptor, immobilized antibodies to the T cell receptor, or by antigen (8, 10, 11). Not only is this site important for transcriptional activation of the HIV-2 enhancer, but mutation of the TG-rich sequence of the HTLV-1 enhancer also markedly inhibits inducible enhancer function (13). An intact TG-rich sequence found adjacent to the ets-binding site is again required for transcriptional activation of both the MLV and TCRβ enhancers (25). It has been shown that factors (termed core binding factors) binding to this TG-rich element cooperate in vivo to regulate transcription from the MLV and TCRβ enhancers (25). Furthermore, point mutations at the site where core binding factors bind to the MLV enhancer cause a shift in disease specificity of the virus, resulting in erythroid leukemia rather than T cell leukemia (15). These observations suggest that the pets/ets motif, which has been conserved among widely divergent retroviruses, is likely to be broadly important. As the pets factor binds DNA constitutively (8), it may need to interact with other proteins or be post-translationally modified in order to regulate transcription. Elf-1, an ets family member which binds both immediately upstream and downstream of the pets site in the HIV-2 enhancer, is very similar to the Drosophila developmental factor E74 (7, 8). Perhaps the interaction of

3 N. Clark and D. Markovitz, unpublished observations.
Elf-1 with the pets factor will prove necessary for regulating transcriptional activation of the HIV-2 enhancer. Indeed, many ets family members, including Elf-1, require cofactors in order to bind DNA or to activate transcription (25, 26, 28–30).

While the HIV-2 enhancer arrangement is similar to the MLV enhancer with regards to ets sites found immediately adjacent to a TG-rich sequence, the factors previously described to bind to the MLV TG-rich sequence appear to be distinct from the HIV-2 pets factor, as they are much smaller than 43 kDa, ranging from 19 to 35 kDa (26, 27). Experiments to test the requirement of the HIV-2 pets element for the 43-kDa factor in mediating enhancer activation, either alone or in combination with Elf-1 or other proteins, will be facilitated by the identification and/or cloning of this purified pets factor. The elucidation of the mechanism by which the pets factor mediates HIV-2 transcriptional activation will provide us with a better understanding of this important human pathogen and of signal transduction in T cells. While previous experience suggests that more than one cloned protein is likely to bind to a given enhancer site in vitro (8, 9), the studies presented here show that the dominant protein recognizing the pets site in crude and highly purified nuclear extracts is 43 kDa in size. Therefore, this is likely to be the size of the biologically relevant pets factor.

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