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# Alterations in Binding Characteristics of the Human Immunodeficiency Virus Enhancer Factor

FOON WU, JOSEPH GARCIA, RONALD MITSUYASU, AND RICHARD GAYNOR*

*Division of Hematology-Oncology, Department of Medicine, University of California-Los Angeles School of Medicine, and Jonsson Cancer Center, Los Angeles, California 90024*

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**Five regions of the human immunodeficiency virus (HIV) long terminal repeat (LTR) serve as binding sites for cellular proteins as demonstrated by DNase I footprinting. These include the negative regulatory, enhancer, SP1, TATA, and untranslated regions. The HIV enhancer region contains two direct repeats of a sequence, GGGACTTTCC, which is also found in the enhancer sequences of simian virus 40, cytomegalovirus, and the immunoglobulin kappa gene. To further characterize binding to the enhancer sequences in the HIV LTR, DNase I footprinting was performed using extracts prepared from several different cell lines. Extracts prepared from lymphoid cells gave altered binding over the enhancer region as compared with extracts prepared from either monocytes or HeLa cells. This altered binding in extracts prepared from lymphoid cells resulted in protection of both direct repeats in the HIV LTR in contrast to complete protection of only one direct repeat with HeLa cell extracts. When HeLa cells were treated with phorbol esters in either the presence or absence of the protein synthesis inhibitor cycloheximide, the binding characteristics over the enhancer element became similar to those seen in extracts prepared from lymphoid cells. These results suggest that phorbol esters may induce posttranslational modifications of cellular transcription factors that alter their DNA-binding characteristics.**

The human immunodeficiency virus (HIV) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (3, 12, 29). The virus has been shown to infect both lymphoid and nonlymphoid cell lines which possess the T4 receptor (6, 23, 30–32). In addition, the virus expresses its gene products when transfected into a number of cell lines including HeLa cells (28). These results imply that the virus is able to use general cellular transcription factors for its gene expression.

Genetic analysis of the HIV long terminal repeat (LTR) has revealed several *cis*-acting regions which are important for gene expression of the virus (21, 35, 38, 44, 57). These include a negative regulatory region, an enhancer element, SP1-binding sites, and the TAR element, which includes a portion of the untranslated region of DNA (21, 35, 38, 44, 57). Experiments indicate that both the enhancer and the untranslated region are important for complete *tat*-induced *trans*-activation (38, 44). In addition, mutagenesis studies and DNase I footprinting studies using purified SP1 protein indicate that SP1 binding sites are important for in vitro transcriptional regulation of the HIV LTR (21).

To identify cellular proteins involved in the genetic regulation of HIV, DNase I footprinting of the HIV LTR was performed, using partially purified HeLa cell extracts and oligonucleotide affinity column-purified SP1 (J. Garcia, F. Wu, R. Mitsuyasu, and R. B. Gaynor, EMBO J., in press). Five regions of the HIV LTR, including the negative regulatory, SP1, enhancer, TATA, and untranslated regions, gave DNase I protection. Mutagenesis of several of these regions, followed by assays of gene expression, revealed their importance in transcriptional regulation (21, 34, 35, 44; Garcia et al., in press).

The HIV enhancer region has been found to induce transcription from heterologous promoters in an orientation-independent manner (44). The HIV enhancer region contains two direct repeats of the sequence GGGACTTTCC (35). This sequence is also present in the enhancer elements of simian virus 40 (SV40) (19, 54), the immunoglobulin kappa gene (39, 48), and cytomegalovirus (5). In SV40, this sequence, located in the B domain of the enhancer, has been shown to be a part of an important binding domain required for transcriptional regulation (56, 58). Altered binding was seen over this sequence in extracts prepared from B-lymphoid cells as compared to HeLa cells (7). In the kappa enhancer, this sequence has shown binding in gel retardation assays using extracts prepared from mature B cells, but not other cells (48). However, binding to this region of the kappa enhancer could be detected by pretreating pre-B cells with lipopolysaccharide in the presence or absence of cycloheximide, by treating T cells with phytohemagglutinin (PHA) or the phorbol ester phorbol 12-myristate 13-acetate (TPA), or by treating HeLa cells with TPA (1, 49). It was found that the factor which binds to the HIV enhancer region was not detectable in unstimulated T cells, but could be induced by treatment of a T-cell line (Jurkat) with PHA and phorbol esters (35). HIV gene expression was also found to increase in these activated T cells, possibly due to an increase in the level of this enhancer-binding factor (35).

Since a variety of enhancer-binding proteins show tissue-specific patterns of regulation (2, 8, 14, 24–26, 33, 42, 46, 47, 50, 51), we investigated the binding to the HIV LTR enhancer region by using DNase I footprinting with HeLa, monocyte, and T-cell extracts. The binding to the enhancer region was altered in extracts from T-lymphoid cells as compared with HeLa cells such that both direct repeats were completely protected in lymphoid extracts whereas only one repeat was completely protected in HeLa cell extracts. Addition of TPA to HeLa cells resulted in the appearance of binding to the enhancer region similar to that seen in lymphoid cellular extracts. The ability of phorbol esters to induce this change in DNase I protection patterns of the enhancer-binding factor in the presence of cycloheximide

* Corresponding author.

suggests that this effect is mediated by a posttranslational modification.

## MATERIALS AND METHODS

**Cell lines and tissue culture conditions.** HeLa cells were maintained in suspension culture in minimal essential medium with 5% newborn calf serum. H9, a human T4-positive lymphoid cell line (12); U937, a human monocyte cell line (52); and SLB cells, a human T-cell lymphotropic virus type I (HTLV-I)-transformed T4-positive lymphoid cell line (24), were grown in RPMI with 10% fetal calf serum. HIV infection of cells was performed by coculture of irradiated HIV-infected H9 cells with either H9 or U937 cells. Uninfected cells were added twice weekly to the infected cell cultures. Reverse transcriptase and Western blot (immunoblot) analyses with antibody to the HIV gp24 protein were performed. For treatment of HeLa cells with cycloheximide and TPA, the cells were treated with cycloheximide (25 μg/ml) for 1 h before the addition of 100 ng of TPA per ml. Cells were harvested 1 h postaddition of TPA.

**Preparation of cellular extracts.** For all extracts, a minimum of 7 ml of packed cell volume was used. Nuclear extracts were prepared as described (9). Extracts were dialyzed versus a buffer containing 20 mM Tris (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, and 20% glycerol. This extract was then loaded onto a heparin-agarose column, washed with 5 column volumes of this same buffer, and then eluted with 0.5 M KCl. This extract was dialyzed into 20 mM Tris (pH 7.9)–100 mM KCl–0.2 mM dithiothreitol–0.2 mM phenylmethylsulfonyl fluoride and used in DNase I footprinting assays (11).

**Transfections and CAT assays.** To make the HIV LTRCAT constructs used in transfections, the HIV LTR was first cut in the upstream region with either *Hae*III (−70) or *Ava*I (−160) (the numbers in parentheses indicate the position from the cap site [45]). Each fragment was then cut with *Hin*dIII (+80) and cloned into a plasmid containing the Rous sarcoma virus (RSV) LTR fused to the chloramphenicol acetyltransferase (CAT) gene from which the RSV sequence was removed by cutting with *Nru*I and *Hin*dIII. To make the expression vector for *tat*, a fragment from a pUC19 derivative containing nucleotides 5792 to 6087 of the AIDS-associated retrovirus type 2 genome was inserted into the *Hin*dIII-*Bgl*II sites of the vector RSV-beta globin (15), thus replacing the globin-coding sequence with the *tat* coding sequence as described (Garcia et al., in press).

For each transfection, 5 μg of each HIV LTR3CAT construct and 5 μg of either RSV-beta globin or RSV-*tat* were used. Transfections onto 70% confluent HeLa cells by the calcium phosphate method were performed, and the HeLa cells were maintained in Dulbecco modified minimal essential medium with 5% newborn calf serum. At 24 h posttransfection, 100 ng of TPA per ml was added to the medium, and the cells were harvested 20 h later. CAT assays were performed as described (16), and each reaction was harvested at 10 and 50 min to assure linear conversion of the chloramphenicol with each extract. The conversion of chloramphenicol in each assay was determined by scintillation counting of both unacetylated and acetylated chloramphenicol.

**DNase I footprinting.** To make the HIV LTR constructs used for DNase I footprinting, the LTR was first cut in the upstream region at either *Ava*I (−160) or *Tha*I (−218); the numbers in parentheses indicate the position from the cap site (45). These fragments were then cut with *Hin*dIII (+80), treated with T4 polymerase, cloned into the *Sma*I site of pUC19, and screened for orientation. Each clone was digested with *Eco*RI or *Hin*dIII, treated with alkaline phosphatase, and end labeled with $\gamma$-$^{32}$P to generate labeled fragments used in footprinting. Fragments were gel isolated (*Eco*RI-*Hae*II for the coding strand or *Hin*dIII-*Pvu*I for the noncoding strand), electroeluted, and used for DNase I footprinting assays. End-labeled probe (1 to 5 ng) was added to each 50-μl reaction along with extract (0 to 200 μg), poly(dI-dC) (3 μg), and final concentrations of 10 mM Tris (pH 7.4), 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol. The DNA and extract were allowed to bind for 30 min at room temperature, and then the reaction volume was increased to 100 μl and final concentrations of 0.4 to 2.0 μg of DNase I per ml, 5 mM MgCl, and 2.5 mM $CaCl_2$ were added. The reaction was stopped after 30 s with phenol-chloroform and then ethanol precipitated and loaded on a 10% 8 M urea sequencing gel. G+A and C+T Maxam-Gilbert sequencing reactions were performed for each probe. All gels were then subjected to autoradiography.

## RESULTS

**Identification of DNA-binding regions in the HIV LTR.** To identify DNA-binding proteins in the HIV LTR, we performed DNase I footprinting of the HIV LTR, using partially purified extracts prepared from HeLa, monocyte, and lymphoid cells. Each experiment was repeated several times with each extract to rule out variations seen with the DNase I footprinting technique. By use of a clone extending from −218 to +80 in the HIV LTR (−218 LTR3) and partially purified HeLa cell extract, three DNase I-protected regions were seen on the coding strand (Fig. 1A). No other protected regions were seen in the HIV LTR, either upstream or downstream of the −218 to +80 fragment (data not shown). These protected areas on the coding strand of the HIV LTR correspond to a negative regulatory element (44) between −173 and −159, an enhancer element (34, 35) and a portion of the third SP1-binding site (21) between −97 and −78, and the TATA and untranslated region (34, 44) between −42 and +28 (Fig. 1A). Deletion analysis of the HIV LTR revealed that the region between −42 and +28 consists of at least two protein-binding domains, one domain over the TATA sequence extending from −42 to −13 and the other domain extending from −13 to +28 (Garcia et al., in press). On the HIV LTR noncoding strand, there were no differences in the extent of the binding domains, except that binding over the untranslated region extended to +52 rather than +28 (Garcia et al., in press). The binding between −13 and +52 occurs in a region previously referred to as the TAR region. This region is necessary, but not sufficient, for complete *tat*-induced activation, which also requires the presence of upstream regulatory elements (38, 44). The factor or factors binding to this region have been referred to as the TAR factor(s) (Fig. 1B) on the basis of earlier terminology, but additional cellular binding proteins are required for complete *tat*-induced activation.

Protection over all three SP1-binding sites between −82 and −44 in the HIV LTR which was previously detected by DNase I footprinting using oligonucleotide affinity column-purified SP1 (21) was not detected with these partially purified extracts. However, oligonucleotide competition studies using an oligonucleotide made to the high-affinity SP1-binding site in SV40 (22) indicated that the third SP1-binding site, which has the highest affinity of the three SP1

sites, was protected with our extracts (Garcia et al., in press). Mutagenesis of several of these regions has confirmed their importance in both basal and *tat*-induced transcription of the HIV LTR (21, 34, 35, 44; Garcia et al., in press). A summary of the binding domains in the HIV LTR for cellular proteins is shown in Fig. 1B.

When another HIV LTR clone extending from −160 to +80 (−160 LTR3) was used for DNase I footprinting on the coding strand, there was protection over the untranslated

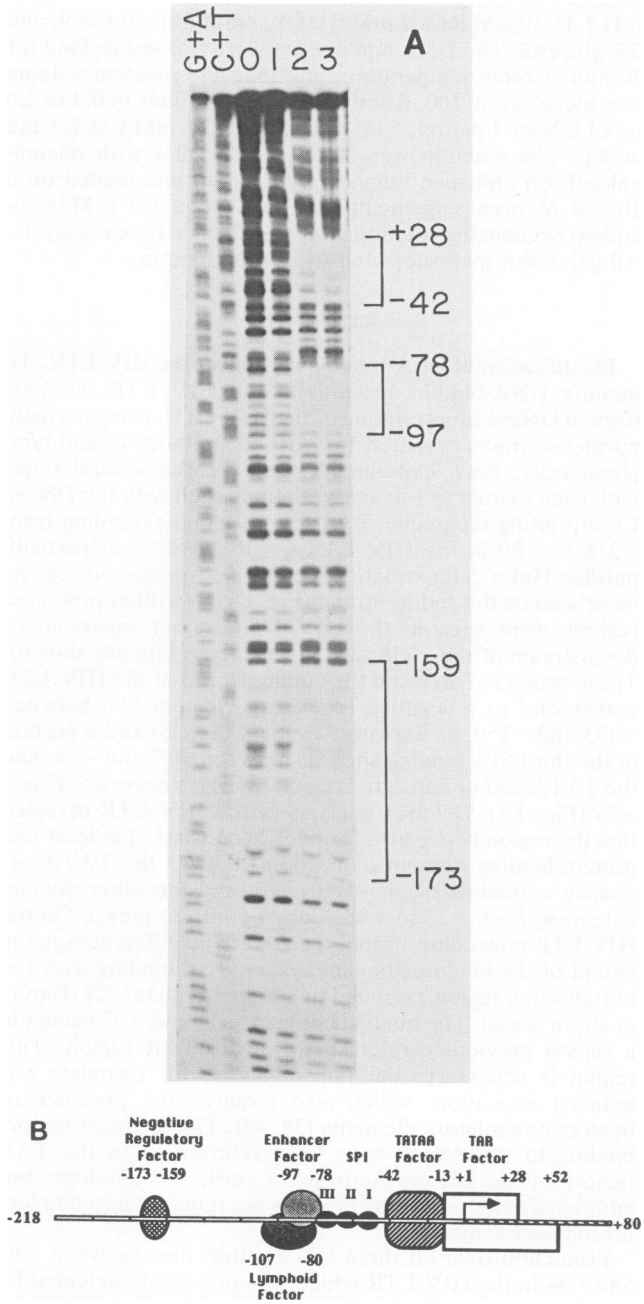


FIG. 1. DNase I footprinting of the HIV LTR. (A) HeLa cell extracts were used for DNase I footprinting of the HIV LTR fragment extending from −218 to +80. Lane 0 contains no extract, lane 1 has 25 μg, lane 2 has 50 μg, and lane 3 has 200 μg. G+A and C+T, Maxam-Gilbert sequencing lanes. (B) Schematic illustration of HIV LTR binding domains.

TATA region between −42 and +28, but a decrease was seen in the amount of clearing over the enhancer region between −97 and −78 (Fig. 2A). Complete clearing of this enhancer region did not occur with 200 μg of partially purified HeLa cell extract per reaction (Fig. 2A, lane 3), whereas in the presence of the negative regulatory element, clearing occurred with 50 μg of partially purified HeLa cell extract (Fig. 1A, lane 2). These data suggested that the region between −173 and −159 may influence protection patterns over the enhancer region. Because HeLa cell extracts gave altered binding characteristics over the enhancer region in response to different fragments (−218 LTR3 compared to −160 LTR3), we used each of these fragments to examine enhancer factor binding in extracts prepared from different cell lines.

**Alterations of binding characteristics of the HIV enhancer factor.** Studies have shown that the HIV enhancer-binding protein is important in transcriptional regulation of the HIV LTR (35). A number of enhancer-binding factors have been shown to have tissue-specific binding alterations (2, 8, 14, 24–26, 33, 42, 46, 47, 50, 51). Since HIV infects a variety of cells including both T-lymphoid cells and monocytes (6, 23, 30–32), we studied potential tissue-specific alterations in the HIV enhancer-binding factors. Extracts were prepared from both HIV-infected and uninfected H9 T-lymphoid cells and HIV-infected and uninfected U937 monocyte cells. The DNase I protection patterns in extracts prepared from these cell lines were compared with the protection patterns obtained with HeLa cell extract.

When the −160 LTR3 fragment was used (Fig. 2A), there was a difference in DNase I protection over the enhancer region between extracts from H9 cells and extracts from either U937 cells or HeLa cells. There was only partial protection over the enhancer region when up to 200 μg of HeLa cell extract was added (Fig. 2A, lanes 1 through 3), and only moderate protection over this region in infected (lanes 13 through 15) or uninfected (lanes 10 through 12) U937 extracts. In contrast, with both infected (Fig. 2A, lanes 7 through 9) and uninfected (lanes 4 through 6) H9 extracts, there was complete protection over the enhancer region when 50 μg of extract was added. The amount of protection over the region between −42 and +28 was similar in all of the cell extracts tested (Fig. 2A). Thus, the enhancer-binding factor in T-lymphoid cell extracts completely protects the enhancer region in the absence of the negative regulatory element, in contrast to results found in both HeLa and monocyte extracts. There was no difference in binding characteristics of our extracts in the presence of HIV infection (Fig. 2A).

There was no difference in protection of the −218 LTR3 fragment in the protected regions between −173 and −159 and between −42 and +28 for either HeLa, H9, or monocyte extracts (data not shown). However, in the enhancer region, there was protection between −97 and −78 in HeLa cell extract (Fig. 2B, lanes 1 and 2) and U937 cell extract (data not shown) as compared to the protection seen between −107 and −80 with H9 cell extract (Fig. 2B, lanes 3 and 4). In both HeLa cell and monocyte extracts, there was complete protection over only one direct repeat with the sequence GGGACTTTCC when the −218 LTR3 fragment was used. In T-cell extracts, there was complete protection over both direct repeats containing this sequence when either the −218 LTR3 or the −160 LTR3 fragment was used. Thus, in the enhancer region there are changes in both the extent of protection (−107 to −80 with lymphoid extract compared to −97 to −78 with HeLa extract, using the −218 LTR3

fragment) and amount of protection at low protein concentrations (complete protection with lymphoid extracts as compared with partial protection with HeLa extracts, using the −160 LTR3 fragment). The HIV enhancer region sequences protected in lymphoid extracts, compared with HeLa cell extracts, are shown in Fig. 2C.

**Effect of phorbol esters on binding to the HIV enhancer region.** The presence of TPA has also been shown to induce binding to the sequence GGGACTTTCC in the kappa enhancer in both HeLa and T cells (49). In addition, TPA has been shown to induce transcription of a number of genes (16, 20, 27, 41, 53), including those of HIV (18, 35). To study the effect of TPA on binding of cellular proteins to the HIV LTR, extracts were made from TPA-treated HeLa cells. HeLa cells were also treated with TPA for 1 h after a 1-h pretreatment with cycloheximide. Extracts were prepared from these cells, and their pattern of DNase I protection was compared with those of untreated HeLa cells or an HTLV-I-transformed T-cell line, SLB. Extracts prepared from HeLa cells treated with phorbol esters, in either the presence or absence of cycloheximide, exhibited an alteration in binding over the −160 LTR3 fragment enhancer region as compared with untreated HeLa cells (Fig. 3A). There was only partial protection over the enhancer element in extracts prepared from HeLa cells (Fig. 3A, lanes 1 to 3) even at 150 μg of added protein. Complete protection at lower protein concentrations was seen with extracts prepared from SLB cells (Fig. 3A, lanes 4 through 6) and with extracts prepared from HeLa cells treated with TPA in the presence or absence of cycloheximide (lanes 7 through 12).

When the −218 LTR3 fragment was used, HeLa cell extracts prepared in the presence of TPA with or without cycloheximide resulted in complete protection over the enhancer region from −107 to −80 (Fig. 3B, lanes 9, 10, 12, and 13). This was the same pattern seen with extracts prepared from two T-cell lines, SLB (Fig. 3B, lanes 5

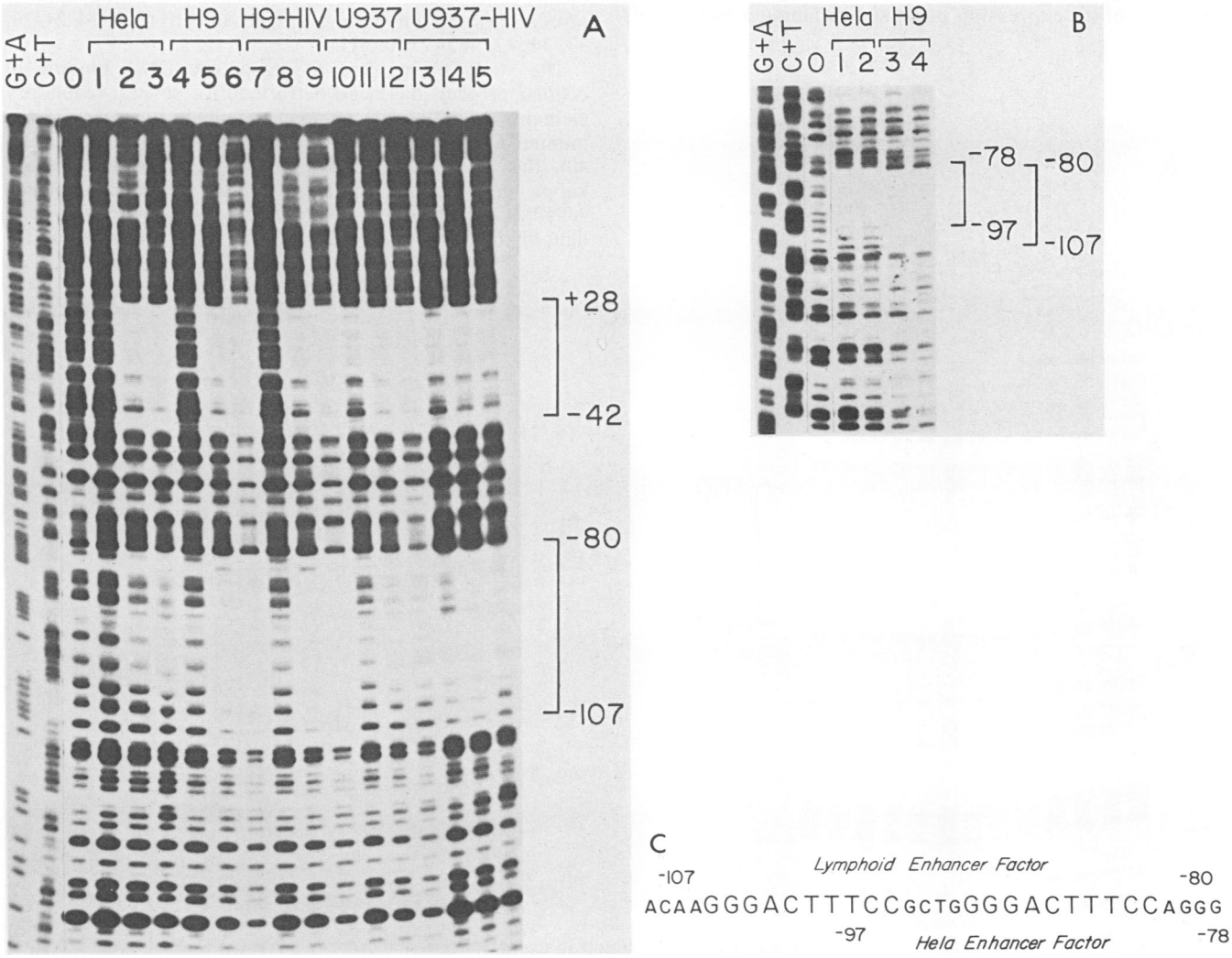


FIG. 2. Comparison of different cellular extracts on DNase I footprinting of the HIV LTR. (A) HeLa cell extracts (lanes 1 to 3), uninfected (lanes 4 to 6) and HIV-infected (lanes 7 to 9) H9 extracts, and uninfected (lanes 10 to 13) and HIV-infected (lanes 13 to 15) U937 extracts were used for DNase I footprinting of the −160 to +80 HIV LTR fragment. Lane 0 contains no extract; lanes 1, 4, 7, 10, and 13 have 25 μg; lanes 2, 5, 8, 11, and 14 have 50 μg; and lanes 3, 6, 9, 12, and 15 have 200 μg. G+A and C+T, Maxam-Gilbert sequencing lanes. (B) HeLa (lanes 1 and 2) and H9 (lanes 3 and 4) extracts were used for DNase I footprinting of the −218 to +80 HIV LTR fragment. Only the portion of the gel containing the enhancer region is shown. Lane 0 contains no extract, lanes 1 and 3 have 100 μg, and lanes 2 and 4 have 200 μg. (C) HIV LTR sequences protected by extracts prepared from both lymphoid and HeLa cells.

through 7) and H9 (Fig. 2). In contrast, protection using untreated HeLa cell extracts extended from −97 to −78 (Fig. 3A, lanes 3 and 4). TPA did not induce a change in the extent of protection of the other domains, nor did cycloheximide alone cause a change in the binding over the enhancer region (data not shown). Thus, the treatment of HeLa cells with TPA, in the presence or absence of cycloheximide, resulted in characteristics of binding to the HIV enhancer region similar to those seen in extracts prepared from lymphoid cells.

**Effect of phorbol esters on gene expression of the HIV LTR.** To test the effect of TPA on the ability to alter gene expression from the HIV LTR, we performed transfection assays of two HIV LTR fragments fused to the CAT gene, −160 LTR3CAT and −70 LTR3CAT. The −160 LTR3CAT construct contains four HIV LTR binding domains (enhancer, SP1, TATA, and untranslated), while the −70 LTR3CAT construct lacks the enhancer sequences and third SP1-binding site. These constructs were transfected either in the presence of a control plasmid (RSV-beta globin) or in the presence of an expression plasmid containing a portion of the *tat* gene (RSV-*tat*). To one set of these transfections no TPA was added, and to the other 100 ng of TPA per ml was added at 24 h posttransfection. Transfections were harvested at 44 h. CAT conversion in the presence of *tat* for −160 LTR3CAT was 22% and increased to 84% in the presence of TPA. However, for the −70 LTR3CAT construct there was minimal change in CAT conversion in the presence of TPA. Thus, TPA increases the level of gene expression from the HIV LTR in the presence of the enhancer region but not in its absence.

## DISCUSSION

Both viral and cellular genes which are linked to enhancer elements frequently exhibit transcriptional activity in a highly cell-type-specific manner (2, 8, 14, 24, 25, 42). Cellular proteins have been shown by both in vitro and in vivo transcriptional and DNA-binding assays to mediate this tissue-specific enhancer function (26, 33, 34, 46, 47, 50, 51, 55). Thus, it is likely that the cell specificity found in some enhancer elements is dependent on *trans*-acting cellular proteins present only in certain types of cells (2, 8, 14, 24–26, 33, 34, 42, 46, 47, 50, 51).

The study of in vitro tissue-specific DNA binding of cellular proteins has been performed for several regulatory elements including the octamer sequence upstream of the immunoglobulin heavy- and light-chain genes (4, 10, 17, 37, 40), the SV40 enhancer (7, 56), and the immunoglobulin kappa gene enhancer (1, 49). The octamer sequence ATTTGCAT is found upstream of both the heavy- and light-chain promoters (4, 10, 17, 37, 40) and is required for

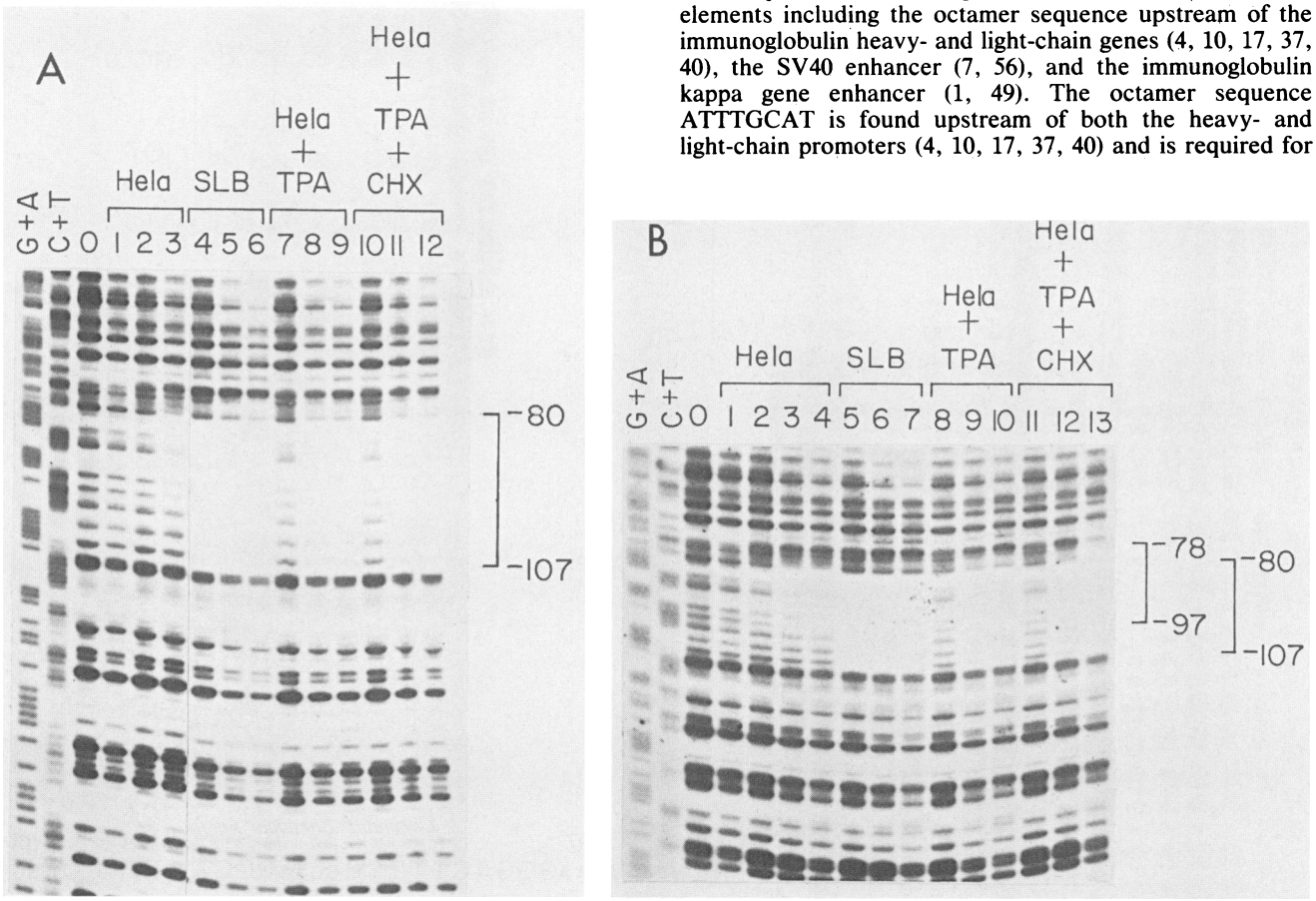


FIG. 3. DNase I footprinting of the HIV LTR with HeLa cell extracts in the presence and absence of TPA. (A) Extracts from untreated HeLa cells (lanes 1 to 3), SLB cell extracts (lanes 4 to 6), extracts from HeLa cells treated with TPA (lanes 7 to 9), or extracts from HeLa cells treated with both TPA and cycloheximide (lanes 10 to 12) were used for DNase I footprinting of the HIV LTR fragment from −160 to +80. Only the portion of the gel containing the enhancer region is shown. Lane 0 contains no added extract; lanes 1, 4, 7, and 10 have 25 μg; lanes 2, 5, 8, and 11 have 50 μg; and lanes 3, 6, 9, and 12 have 150 μg. G+A and C+T, Maxam-Gilbert sequencing lanes. (B) The same cell extracts were also used for DNase I footprinting of the HIV LTR fragment from −218 to +80. Only the portion of the gel containing the enhancer region is shown. Lane 0 contains no added extract; lane 1 has 15 μg; lanes 2, 5, 8, and 11 have 25 μg; lanes 3, 6, 9, and 12 have 50 μg; and lanes 4, 7, 10, and 13 have 200 μg. G+A and C+T, Maxam-Gilbert sequencing lanes.

transcriptional regulation of immunoglobulin genes. Two species of binding to this sequence were detected by gel retardation assays using the octamer sequence (26, 57). One form was found in all cell lines tested (50, 51), and another form was restrieted to cells of lymphoid lineage (26, 51). The SV40 enhancer consists of multiple sequence motifs which bind cellular proteins (56, 58). Both HeLa and B-lymphoid extracts bind to specific and overlapping elements of the SV40 enhancer (7). Several sequence elements of the SV40 enhancer are recognized by using B-cell extracts, but not when using HeLa extracts. For instance, the octamer motif is not protected by HeLa cell extracts, but is protected by B-cell extracts; the domain containing the sequence GG GACTTTCC exhibits altered protection characteristics with B-cell extracts as compared with HeLa cell extracts (7). It has been shown for the kappa enhancer that this latter sequence is recognized with extracts prepared from B cells, but not other cell lines (48), unless stimulated by agents such as TPA, PHA, or lipopolysaccharide (1, 49). These results suggest that different or posttranslationally modified cellular proteins can bind to a single regulatory sequence.

In addition to altering binding of cellular proteins to DNA regulatory sequences (49), phorbol esters have been shown to induce transcription from a number of different genes (27) including c-*fos* (17, 41) and both the SV40 (20) and polyomavirus (53) enhancers. This increase in transcription may involve the induction or modification of a cellular transcription factor by protein kinase C, through which phorbol esters likely exert their effects (36). However, with the c-*fos* gene, this increase in transcription with TPA is not accompanied by an increase in binding to the enhancer domain required for growth factor stimulation (41). Treatment with epidermal growth factor does induce binding to this region (41). These data suggest that the effect of phorbol esters on the c-*fos* gene, in contrast to results with the kappa enhancer (49), does not involve a mechanism of increased binding of this factor to the c-*fos* enhancer element.

A previous study showed that TPA and PHA treatment induced HIV gene expression 50-fold in Jurkat cells (35). This induction was additive to the induction seen with the *tat* protein. No enhancer-binding protein was detected by gel retardation assays in nonstimulated Jurkat cells, but when these cells were activated there was the appearance of a factor that bound to the HIV enhancer region. Our ability to detect altered binding to the HIV enhancer region in unstimulated cell lines by using DNase I footprinting as compared to gel retardation assays is likely due to the difference in the amount of protein used. For the gel retardation assays, only 5 to 10 μg of crude nuclear extract was used (35), whereas in our DNase I footprinting experiments from 50 to 150 μg of partially purified extract was used. Thus, it is likely that this large amount of added protein increased our ability to detect this enhancer-binding protein. The altered HIV enhancer binding found in lymphoid cells is not restricted to activated lymphoid cells such as the HTLV-I-transformed T-cell line SLB (3, 24), but also occurs in H9 cells (12) and in extracts prepared from calf thymus (unpublished data). Thus, this alteration in binding appears to be present in a variety of nonstimulated and stimulated lymphoid cell sources.

The level of induction of the HIV LTR by TPA in several different experiments was only three- to fourfold. This was less than that seen in Jurkat cells which were activated by a combination of TPA and PHA (35), but this effect was likely due to the addition of both substances. It has been shown for cellular genes such as c-*fos* that the effect of TPA occurs maximally after 15 min of stimulation and decreases rapidly by 2 h (16, 41). Thus, transient gene expression assays of cells harvested 20 h after TPA treatment may not accurately measure the immediate effect of TPA treatment. In addition, HeLa cell extracts treated with TPA showed an increase in binding to the NF-kappa B region of the kappa enhancer at 1 to 2 h posttreatment, but extracts prepared at 8 h after treatment failed to show this increase (49). Our TPA-treated HeLa cell extracts are now being tested to determine whether the altered binding to the HIV LTR seen at 1 to 2 h posttreatment correlates with an increase in HIV LTR in vitro transcription.

The alteration of factor binding to the HIV enhancer region in response to TPA could be due to changes in the concentration or binding activity of a factor or to the induction of a novel cellular protein. Changes in the HIV enhancer factor with TPA and cycloheximide suggest that this occurs in the absence of de novo protein synthesis, perhaps by a posttranslation modification. This modification may alter the binding of the factor or affect its interaction with other cellular proteins. The enhancer-binding factor in untreated HeLa cells may be a completely different factor from the one that binds to the HIV enhancer in TPA-treated HeLa cells and lymphoid cells. Also, the enhancer factor in TPA-treated HeLa cells may be different from the enhancer factor in lymphoid cells. However, it is interesting that the binding of the enhancer factor in the presence of TPA results in protection patterns similar to those found in extracts prepared from lymphoid cells. The different binding to the enhancer region seen in extracts prepared from lymphoid cells may be one factor in the T-cell tropism of HIV. These results are consistent with a model whereby transcription factor binding could be altered in different cell types by posttranslational modifications of common cellular transcription factors. Both the cell type and the state of cellular differentiation (43) are likely to be important in determining the level and activity of specific DNA-binding proteins. Characterization of these proteins in a number of different tissues by immunoprecipitation using specific antibodies and subsequent peptide mapping will be required to determine the role of posttranslational modification in cell-specific gene regulation.

TABLE 1. CAT conversion of HIV LTRCAT constructs in the presence and absence of TPA[a]

| LTR construct | % CAT conversion | | | |
|---|---|---|---|---|
| | − TPA | | + TPA | |
| | − *tat* | + *tat* | − *tat* | + *tat* |
| −70 LTR3CAT | 0.2 | 1.0 | 0.2 | 0.9 |
| −160 LTR3CAT | 0.4 | 22.0 | 1.4 | 84.0 |

[a] Constructs were transfected in the presence (+ *tat*) or absence (− *tat*) of an expression plasmid containing the *tat* gene. See the text.

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## LITERATURE CITED


1. **Atchison, M. L., and R. P. Perry.** 1987. The role of the kappa enhancer and its binding factor NF-kappa B in the developmental regulation of kappa gene transcription. Cell **48**:121–128.
2. **Banerji, J., L. Olsen, and W. Schaffner.** 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33**:729–740.
3. **Barre-Sinoussi, T., J. C. Chermann, F. Reye, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauquet, C. Alxer-Blin, F. Vezinet-Brun, C. Rouzious, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome. Science **220**:868–871.
4. **Bergman, Y., D. Rice, R. Grosschedel, and D. Baltimore.** 1985. Two regulatory elements for immunoglobulin kappa light chain gene expression. Proc. Natl. Acad. Sci. USA **81**:7041–7045.
5. **Boshart, M., F. Werber, G. John, Porsch-Hasler, B. Fleckenstein, and W. Schaffner.** 1985. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell **41**:521–530.
6. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–766.
7. **Davidson, I., C. Fromental, P. Augereau, A. G. Wildeman, M. Zenke, and P. Chambon.** 1986. Cell type specific protein binding to the enhancer of simian virus 40 in nuclear extracts. Nature (London) **323**:544–548.
8. **DeVilliers, J., and W. Schaffner.** 1981. A small segment of polyoma virus DNA enhances the expression of a cloned beta globin gene over a distance of 1400 bp. Nucleic Acids Res. **9**:6251–6264.
9. **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.
10. **Foster, J., J. Stafford, and C. Queen.** 1985. An immunoglobulin promoter displays cell-type specificity independently of the enhancer. Nature (London) **315**:423–425.
11. **Galas, D. J., and A. Schmidt.** 1978. DNAase footprinting, a simple method for the detection of protein DNA binding specificity. Nucleic Acids Res. **5**:3157–3170.
12. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplen, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**:500–503.
13. **Gazallo, L., and M. D. Dodon.** 1987. Direct activation of resting T lymphocytes by human T-lymphotropic virus type I. Nature (London) **326**:714–717.
14. **Gillies, S. D., S. L. Morrisson, V. T. Oi, and S. Tonagawa.** 1983. A tissue-specific enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell **33**:717–728.
15. **Gorman, C. M., G. Merlino, M. Willingham, J. Paston, and B. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eucaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79**:6777–6781.
16. **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.
17. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature (London) **311**:433–438.
18. **Harada, S., Y. Koyanagi, H. Nakashima, W. Kobayashi, and N. Yamamoto.** 1986. Tumor promoter, TPA, enhances replication of HLVIII/LAV. Virology **154**:249–258.
19. **Herr, W., and Y. Gluzmann.** 1985. Duplications of a mutated simian virus enhancer restore its activity. Nature (London) **313**:711–714.
20. **Imbra, R. J., and M. Karin.** 1986. Phorbol ester induces the transcriptional stimulatory activity of the SV40 enhancer. Nature (London) **323**:555–558.
21. **Jones, K. A., J. T. Kadonaga, P. A. Luciw, and R. Tjian.** 1986. Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. Science **232**:755–759.
22. **Kadonaga, J. T., and R. Tjian.** 1986. Affinity purification of sequence specific DNA binding proteins. Proc. Natl. Acad. Sci. USA **85**:5889–5893.
23. **Klatzman, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.
24. **Koeffler, H. P., I. S. Y. Chen, and D. Golde.** 1984. Characterization of a novel HTLV-infected cell line. Blood **64**:482–490.
25. **Laimens, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem receptors from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA **79**:6453–6457.
26. **Landolfi, W. F., J. D. Capra, and P. W. Tucker.** 1986. Interaction of cell-type specific nuclear proteins with immunoglobulin VH promoter region sequence. Nature (London) **323**:548–551.
27. **Lau, L., and D. Nathans.** 1985. Identification of a set of genes expressed during the G0/G1 transition of cultured mouse cells. EMBO J. **4**:3145–3151.
28. **Levy, J. A., C. Cheng-Mayer, D. Dina, and P. A. Luciw.** 1985. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. Science **232**:998–1001.
29. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshino.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
30. **Levy, J. A., I. Shimabukuno, T. McHugh, C. Casavant, D. P. Stites, and L. S. Oshino.** 1985. AIDS-associated retrovirus (ARV) can productively infect other cells besides human T helper cells. Virology **147**:441–448.
31. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
32. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Maule, and J. K. A. Nicholson.** 1986. Binding of the HTLVIII/LAV to T4+ T cells by a complex of the 110 K viral protein and the T4 molecule. Science **231**:382–385.
33. **Mercola, M., J. Goverman, C. Mirell, and K. Calame.** 1985. Immunoglobulin heavy chain enhancer requires one or more tissue specific factors. Science **227**:266–270.
34. **Muesing, M. A., D. H. Smith, and D. J. Capon.** 1987. Regulation of mRNA accumulation by an HIV trans-activator protein. Cell **48**:691–701.
35. **Nabel, G., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326**:711–713.
36. **Nishizuka, Y.** 1986. Studies and perspectives of protein kinase C. Science **233**:305–312.
37. **Parslow, T. G., D. L. Blair, W. J. Murphy, and D. J. Granner.** 1984. Chromatin changes accompany immunoglobulin kappa gene activation. A potential control region within the gene. Proc. Natl. Acad. Sci. USA **81**:2650–2654.
38. **Peterlin, B. M., P. A. Luciw, P. J. Barr, and M. D. Walker.** 1986. Elevated levels of mRNA can account for the trans-activation of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **83**:9734–9738.
39. **Picard, D., and W. Schaffner.** 1984. A lymphocyte specific enhancer in the mouse immunoglobulin kappa gene. Nature (London) **307**:80–82.
40. **Picard, D., and W. Schaffner.** 1985. Cell type preference of immunoglobulin kappa and lambda gene promoters. EMBO J. **4**:2831–2838.
41. **Prywes, R., and R. G. Roeder.** 1986. Inducible binding of factor to the c-fos enhancer. Cell **47**:777–784.

42. **Queen, C., and D. Baltimore.** 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell **33**;741–748.
43. **Reichel, R., I. Kovesdi, and J. R. Nevins.** 1987. Developmental control of a promoter-specific factor that is also regulated by the EIA gene product. Cell **48**:501–506.
44. **Rosen, C. A., J. G. Sodroski, and W. A. Haseltine.** 1986. Location of *cis*-acting regulatory sequences in the human T-lymphotrophic virus type III (HTLV-III/LAV) long terminal repeat. Cell **41**:813–823.
45. **Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw.** 1984. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science **224**:506–508.
46. **Sassone-Corsi, P., J. P. Dougherty, B. Wasytyk, and P. Chambon.** 1985. A trans-acting factor is responsible for the simian virus 40 enhancer activity *in vitro*. Nature (London) **313**:458–463.
47. **Scholer, H. R., and P. Gruss.** 1984. Specific interaction between enhancer-containing molecules and cellular components. Cell **336**:403–411.
48. **Sen, R., and D. Baltimore.** 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell **46**:705–716.
49. **Sen, R., and D. Baltimore.** 1986. Inducibility of kappa immunoglobulin enhancer-binding protein NF-kappa B by a post-translational mechanism. Cell **47**:921–925.
50. **Singh, H., R. Sen, D. Baltimore, and P. A. Sharp.** 1986. A nuclear factor that binds a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature (London) **319**:154–158.
51. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323**:640–643.
52. **Sundröm, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U937). Int. J. Cancer **17**:565–571.
53. **Wasylyk, C., J. M. Imler, J. Perez-Mutul, and B. Wasylyk.** 1987. The C-Ha-ras oncogene and a tumor promoter activate the polyoma virus enhancer. Cell **48**:525–534.
54. **Weiher, H., M. Koenig, and P. Gruss.** 1983. Multiple point mutations affecting the SV40 enhancer. Science **219**:626–631.
55. **Wildeman, A. G., P. Sassone-Corssi, T. Grundstrom, M. Zenke, and P. Chambon.** 1984. Stimulation of in vitro transcription from the SV40 early promoter by the enhancer involves a specific trans-acting factor. EMBO J. **3**:3129–3133.
56. **Wildeman, A. G., M. Zenke, C. Schatz, M. Wintzerith, T. Grundström, H. Matthes, K. Takahashi, and P. Chambon.** 1986. Specific protein binding to the simian virus 40 enhancer in vitro. Mol. Cell. Biol. **6**:2098–2105.
57. **Wright, C. M., B. K. Felber, H. Pavlakis, and G. N. Pavlakis.** 1986. Expression and characterization of the trans-activator of HTLVIII/LAV virus. Science **234**:988–992.
58. **Zenke, M. T., T. Grundström, H. Mathës, M. Wintzerith, C. Schatz, A. Wildeman, and P. Chambon.** 1986. Multiple sequence motifs are involved in SV40 enhancer function. EMBO J. **5**:387–397.