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In vitro activation of the HIV-1 enhancer in extracts from cells treated with a phorbol ester tumor promoter

Harald Dinter, Robert Chiu[1], Masayoshi Imagawa[1], Michael Karin[1] and Katherine A.Jones

Molecular Biology and Virology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037 and [1]Department of Pharmacology, M-036H, School of Medicine, University of California, San Diego, La Jolla, CA 92093, USA

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The transition from persistent to lytic infection by the human immunodeficiency virus, HIV, is marked by a burst of viral replication and gene expression that occurs when infected cells are stimulated by physiological inducers or tumor promoters like 12-*O*-tetradecanoyl phorbol acetate (TPA). We report here that the HIV enhancer is activated specifically by TPA in several non-lymphoid cell types, and that this transcriptional regulation can be reproduced in a cell-free system. *In vitro* transcription experiments revealed a 6-fold activation of the HIV promoter in nuclear extracts prepared from TPA-induced HeLa tk$^-$ cells, whereas a control (human α-globin) promoter was transcribed with equal efficiency in either induced or uninduced cell extracts. A corresponding increase in the activity of a cellular DNA-binding protein that interacts with the HIV enhancer was detected in TPA-treated cells with DNase I footprint experiments. This increase occurred in the absence of *de novo* protein synthesis, suggesting a post-transcriptional activation mechanism. Analysis of HIV deletion mutants suggests that the enhancer is the target for the TPA effect both *in vitro* and *in vivo*. The cell-free system described here should facilitate studies on the mechanism of phorbol ester induction of gene-specific transcription factors.

Key words: phorbol ester/tumor promoter/*in vitro* transcription system/inducible transcription factor/HIV enhancer

Introduction

The development of *in vitro* transcription systems has been a crucial step towards understanding the control of eukaryotic transcription initiation. Biochemical analyses of such systems has led to the identification and purification of several DNA-binding proteins that interact with control sequences of eukaryotic promoters and activate RNA synthesis directly *in vitro* (Briggs *et al.*, 1986; Chodosh *et al.*, 1986; Bodner and Karin, 1987; Jones *et al.*, 1987; Lee *et al.*, 1987a,b). In addition to providing detailed information on the events involved in RNA polymerase II promoter recognition, certain regulatory phenomena have been recapitulated in cell-free extracts. These include viral repression (Rio *et al.*, 1980) and *trans*-activation of RNA synthesis (Abmayr *et al.*, 1985; Spangler *et al.*, 1987), cell-cycle control (Heintz and Roeder, 1984) and cell or tissue-specific transcription (Mizushima-Sugano and Roeder, 1986; Gorski *et al.*, 1986; Bodner and Karin, 1987). Other aspects of regulation however, such as distance-independent enhancer function and hormonal signal transduction, have proven more elusive to *in vitro* approaches.

One well-characterized signal transduction mechanism involves the activation of protein kinase C by mitogenic stimuli or phorbol esters such as TPA (Nishizuka, 1984, 1986). The stimulation of specific cellular genes by TPA may contribute to its role in tumor promotion. Known targets for TPA induction can be separated into an early response class that responds in the absence of *de novo* protein synthesis, including proto-oncogenes c-*fos*, c-*myc* and c-*sis* (Kelley *et al.*, 1983; Greenberg and Ziff, 1984; Kruijer *et al.*, 1984; Colamonici *et al.*, 1986), and the early genes of several animal cell viruses (Imbra and Karin, 1986; Wasylyk *et al.*, 1987), and a late-response class, exemplified by the genes encoding interleukin-2 and γ-interferon (Wiskocil *et al.*, 1985; Fujita *et al.*, 1986). The specificity of the response to TPA ultimately lies in the sequence elements controlling the expression of inducible genes, which in several examples have been mapped to discrete enhancer domains. Because eukaryotic promoters generally contain multiple sites of interaction for transcription factors, one or more of which may be the ultimate target of activated protein kinase C (Sen and Baltimore, 1986; Angel *et al.*, 1987; Atchinson and Perry, 1987; Chiu *et al.*, 1987; Lee *et al.*, 1987a,b), we decided to develop an *in vitro* system to monitor the magnitude and specificity of activation of individual *trans*-acting factors by phorbol esters.

Several properties of the HIV-1 enhancer suggest it as a good model for studies on transcription induction. First, expression and replication of the virus are highly inducible upon activation of human T cell lines with diverse exogeneous stimuli such as specific antigens, phytohemagglutinin or phorbol esters (McDougal *et al.*, 1985; Folks *et al.*, 1986; Harada *et al.*, 1986; Zagury *et al.*, 1986). Secondly, although the virus is primarily localized in the immune system through interaction with cell-specific surface antigens, the HIV-1 promoter is active in a variety of distinct cell types *in vivo* (Rosen *et al.*, 1985; Sodroski *et al.*, 1985) and directs accurate RNA initiation in uninfected HeLa cell-free extracts. Moreover, the arrangement of HIV promoter elements appears to be much less complex than those of other TPA-responsive genes. Adjacent to the RNA start site lie three tandem binding sites for the Sp1 transcritpion factor (Jones *et al.*, 1986) and two 13-bp repeats that possess enhancer activity in a variety of cell types (Rosen *et al.*, 1985) and are homologous to the SV40 and immunoglobulin κ gene enhancers. A 50-bp downstream control region contains elements responsive to the viral *trans*-activator protein, tat (Rosen *et al.*, 1985; Cullen, 1986; Peterlin *et al.*, 1986; Wright *et al.*, 1986; Muesing *et al.*, 1987). Thus the known elements required for optimal expression of HIV reside within a relatively compact region of the viral long terminal repeat (LTR).

A complete understanding of the mechanism of HIV promoter function and induction by phorbol esters will require the identification of specific inducible promoter domains and the characterization of cellular proteins that recognize them. This paper describes the development of an *in vitro* system from HeLa cells that reproduces the enhancer-dependent induction of HIV RNA synthesis observed *in vivo* in response to treatment with

phorbol esters. The DNA-binding activity of a protein that recognizes the HIV-1 enhancer is dramatically enhanced in the transcriptionally active extracts from TPA treated cells. Both DNA-binding and transcription induction *in vitro* are unaffected by protein synthesis inhibitors, suggesting a post-translational control of transcription factor activity.

Results

The HIV enhancer is TPA-responsive in non-lymphoid cell lines

As a first step towards developing a simple system to study cell stimulation, we examined whether the induction of HIV expression by TPA occurs by activation of the viral promoter, and, if so, whether it can be observed in non-lymphoid cell lines. Plasmid constructs (designated pLTR-CAT), in which the wild-type LTR or 5′ deletion mutants were linked to the marker gene encoding chloramphenicol acetyltransferase (CAT), were transfected into three distinct cell lines. The cervical carcinoma HeLa S3, a slower-growing variant, HeLa tk$^-$, and the human hepatoma Hep G2 cell lines were chosen for these experiments because a variety of genes have been found to be TPA responsive after transfection into these cell lines (Angel *et al.*, 1986, 1987; Imbra and Karin, 1986, 1987; Chiu *et al.*, 1987; Lee *et al.*, 1987a,b), and because extracts from these cells are transcriptionally active. Enhancement of CAT enzyme was measured after transfection of plasmid DNA into each cell type (Figure 1). TPA induction was carried out as described (Angel *et al.*, 1986; Imbra and Karin, 1986). Briefly, the cells were shocked with glycerol for 4 h after transfection, and then kept in media containing low serum or serum plus TPA (100 ng/ml) for 14 h prior to determination of CAT activity. As shown in Figure 1A, expression of the wild-type HIV promoter was activated 3- to 6-fold by TPA treatment of all three cell lines. Deletion of the distal upstream sequences, including a GA-rich region homologous to the TPA-inducible IL-2 and γ-interferon promoters (Fujita *et al.*, 1986), did not diminish the inducibility of the promoter. Further deletion of one copy of the enhancer repeats decreased basal activity 2- to 5-fold, and reduced induction by a variable extent in each cell line. Deletion to position −76, resulting in the loss of both repeats, further dropped basal expression and abolished the response to TPA. The changes in basal level activity are comparable to those reported previously (Muesing *et al.*, 1987), and the TPA target region is similar to that observed in lymphoid cell lines (Nabel and Baltimore, 1987; Tong *et al.*, 1987).

To establish that the regulation observed in HeLa cells was due to increased RNA synthesis from the HIV promoter, plasmid constructs containing the wild-type LTR fused to the bacterial neomycin resistance gene (Neo) were transfected into HeLa and HeLa tk$^-$ cells, and steady-state RNA levels were examined by Northern blot analysis after stimulation with TPA for 3 h (Figure 1B). The specificity of induction was tested by including in each transfection experiment a reference plasmid construct in HIV RNA the human α-1 globin gene. A significant increase in HIV RNA was detected following TPA treatment of both cell types, which approximated 10-fold in the HeLa S3 cell line (Figure 1B). By contrast, transcription from the α-globin promoter was unaffected by TPA. These results suggest that the HIV promoter is transcriptionally regulated in non-lymphoid cell in a manner that resembles the stimulation of virus production and gene expression in T cells by TPA (McDougal *et al.*, 1985; Folks *et al.*, 1986; Zagury *et al.*, 1986) and that the target for TPA induction in each case is the enhancer.

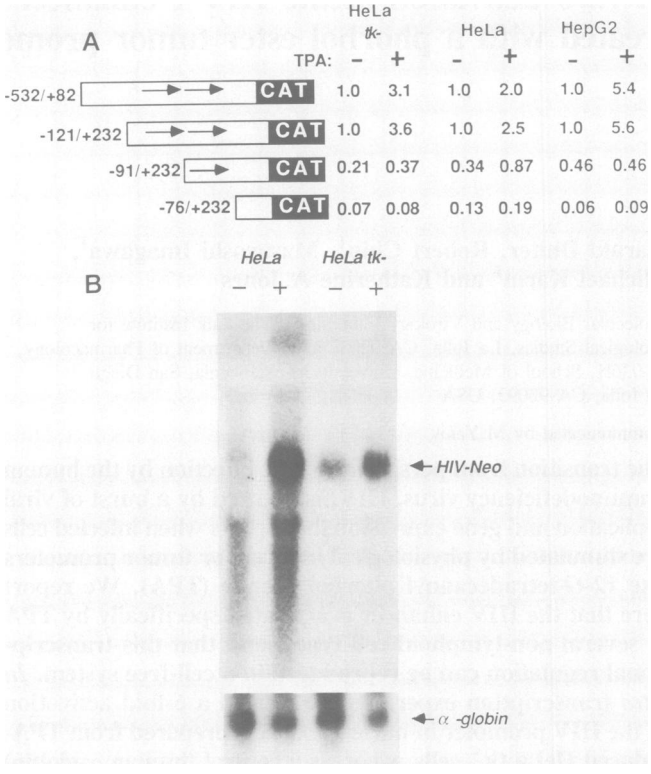

Fig. 1. Activation of the HIV-1 enhancer *in vivo* by phorbol esters. (**A**) The table lists relative levels of CAT enzyme activity and the fold induction observed upon TPA treatment from HIV-1 wild-type and enhancer deletion mutants, which are the averages of two transfection experiments. The various cell lines were transfected as described in Materials and methods. (**B**) TPA induction of steady-state HIV-Neo RNA *in vivo*. Total cellular RNA was isolated from HeLa or HeLa tk$^-$ cells transfected with pLTR-Neo and was analyzed by Northern blot hybridization (40 μg/lane) as described in Materials and methods. Transfected cells were subjected to a glycerol shock 12 h after transfection, then incubated in low-serum-containing medium with TPA (+) or without TPA (−) for 3 h before RNA was extracted. LTR-Neo RNA was detected with a 2.3-kb *Bam*HI to *Hin*dIII fragment from pSV2-Neo; the Neo fragment was then removed by washing and the blot was re-hybridized with the *Pst*I fragment from pα-1 to detect α-1 globin RNA.

TPA regulation of HIV transcription in vitro

To determine whether TPA-mediated changes in the nuclear environment can be reconstituted in cell-free systems, we prepared nuclear extracts from induced and uninduced HeLa tk$^-$ cells. The relative activities of the HIV and α-globin promoters in these extracts were compared by means of *in vitro* transcription experiments in which correctly initiated RNA was detected by primer extension. The results (Figure 2A) show that the HIV promoter is significantly more active in extracts of TPA-treated cells than in uninduced cell extracts. Moreover, the *in vitro* effect was specific for the HIV promoter because the human α-globin promoter was expressed equivalently in the two extracts. These experiments strongly suggest that TPA treatment affects the transcriptional efficiency of the viral promoter directly.

Previous experiments with the SV40, human metallothionein IIA, and immunoglobulin κ genes demonstrated that TPA regulation of these genes was not affected by pre-treatment of the cells with the protein synthesis inhibitor, cycloheximide (Imbra and Karin, 1986, 1987; Sen and Baltimore, 1986). Because these TPA-responsive elements share considerable homology with the HIV enhancer, we determined the effect of cycloheximide on

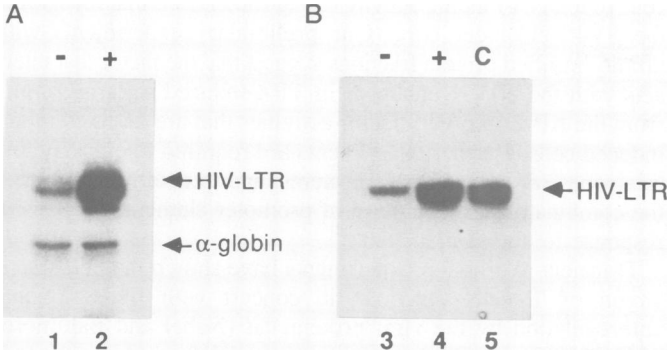

Fig. 2. Phorbol ester regulation of HIV-1 transcriptional activity *in vitro*. (**A**) HIV transcription in extracts derived for TPA-treated (+, **lane 2**) or control (−, **lane 1**) HeLa tk⁻ cells was detected by primer extension. The HIV LTR template contained the entire HIV LTR (−532/+80) fused to the bacterial CAT gene. The human α-1 globin gene (pα-1) was used as an internal control. The positions of accurately initiated HIV and α-globin transcripts are indicated with arrows. (**B**) Transcription from the HIV-1 promoter in extracts from control cells (**lane 3**), TPA-treated cells (**lane 4**), or cells pre-treated with cycloheximide prior to induction with TPA (C, **lane 5**). Nuclear extracts were prepared as described in Materials and methods.

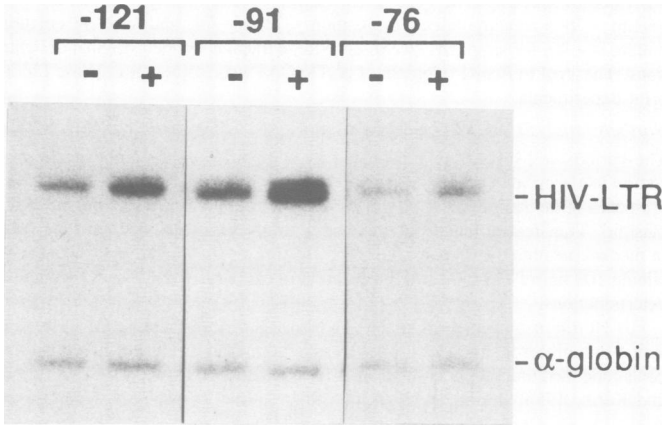

Fig. 3. The HIV-1 enhancer is the target for TPA regulation *in vitro*. Primer extension analysis of HIV deletion mutants in control (−) and TPA-treated (+) HeLa tk⁻ cell extracts. Transcription reactions contained 80 ng of each template and 50 μg of extract in a final volume of 50 μl. Conditions were otherwise as described in Materials and methods.

phorbol ester induction of the HIV promoter *in vitro*. The results (Figure 2B) demonstrate that cycloheximide has no effect on activation of the HIV promoter *in vitro*, and supports the model that protein modification events alter the relative activity of a pre-existing transcription factor.

Is the activation observed *in vitro* an accurate reflection of the regulation observed *in vivo*? The fidelity of the *in vitro* reaction was tested by analyzing the transcriptional properties of HIV enhancer deletion mutants. The data in Figure 3 show that induction of HIV transcription *in vitro* required sequences between −121 and −76, which includes the enhancer domain and its two repeats. Although deletion of one repeat did not notably influence induction under these conditions, the enhancerless construct (−76 deletion mutant) was significantly less inducible by TPA than the wild-type promoter. In addition, a decrease in the basal activity of the −76 template was observed *in vitro*, as had been noted *in vivo* (Figure 1A) (Muesing *et al.*, 1987). In contrast, the 5-fold decrease in activity observed *in vivo* for the −91

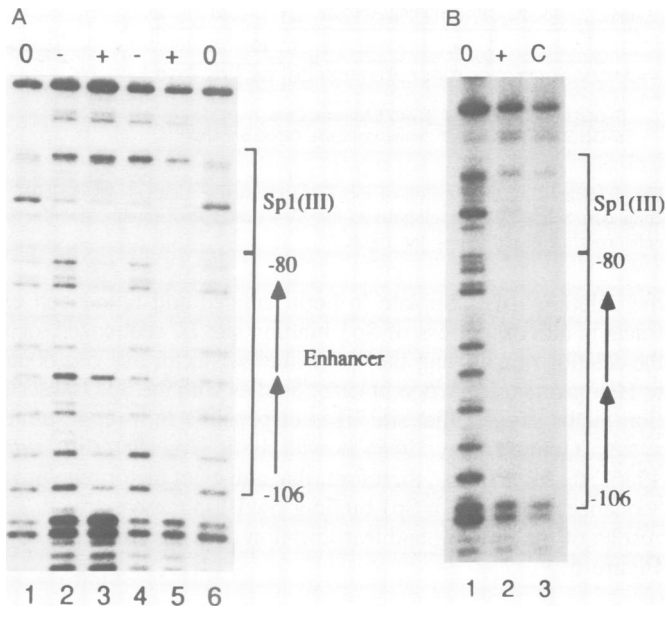

Fig. 4. DNase I footprint analysis of the binding of cellular proteins to the HIV-1 enhancer. (**A**) The autoradiogram displays the footprint pattern observed on the HIV-1 enhancer in the absence of added protein (0, **lanes 1** and **6**) or in the presence of control extract (−, 100 μg, **lane 2**; 180 μg, **lane 4**) or TPA-induced extract (+, 100 μg, **lane 3**; 180 μg, **lane 5**). Brackets also define the footprint boundaries of the distal Sp1 binding site (III) determined previously (Jones *et al.*, 1986). (**B**) DNase I protection pattern observed in the absence of added protein (−, **lane 1**), or with 200 μg of TPA-induced extract (+, **lane 2**), or with 200 μg of extract from cells that had been exposed to cycloheximide (30 μg/ml) 10 min before TPA induction (C, **lane 3**). The pLTR-CAT footprint probe was 5′ end-labelled at an *Ava*I site upstream of the enhancer (−156).

template was not detected *in vitro*. Thus, although subtle aspects of basal enhancer function were not reproduced in these extracts, the general properties of HIV enhancer induction by TPA were reconstituted accurately.

Induction of an HIV enhancer-binding protein

Although the exact mechanism of TPA induction is unknown, a direct stimulation of the activity of protein kinase C could initiate a cascade of protein modification events that differentially control transcription factor activity. The specificity of the effect observed *in vitro* suggests the modification of a factor that interacts specifically with the HIV enhancer domain. To determine whether phorbol ester treatment alters the relative binding affinity of factors that recognize the HIV promoter in HeLa tk⁻ cells, we carried out DNase I footprint experiments. No sequence specific binding was detected in the HIV enhancer region (−106 to −85) in uninduced extracts, whereas this entire region was protected in transcriptionally active nuclear extracts from induced cells (Figure 4A). Both cell extracts otherwise contained equivalent levels of distinct DNA-binding proteins, like Sp1, that recognize other domains of the HIV LTR (Figure 4A, and other data not shown). The relative levels of HIV enhancer binding activity were not diminished in extracts from cycloheximide-treated cells (Figure 4B), providing further evidence that the relative activity of this DNA-binding protein influences the activity of the HIV-1 enhancer. A shortened pattern of footprint protection was observed with the −91 template, which contains only a single copy of the HIV enhancer repeat, whereas no binding of the inducible factor was observed to the −76 template (data not shown). It is not yet clear whether the low activity of

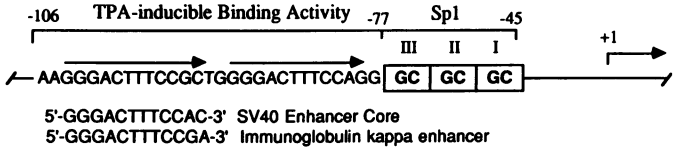

Fig. 5. Schematic localization of the TPA-responsive elements of the HIV-1 enhancer. Sequences homologous to the SV40 early and immunoglobulin κ enhancers are listed below the figure.

the deletion −76 template in both induced and uninduced extracts is due to the loss of Sp1 binding site III sequences, or to the deletion of a binding site for yet another factor that contributes to HIV promoter function *in vitro*. Studies with the Sp1 transcription factor suggest that site III is dispensible in a reconstituted system (Jones *et al.*, 1986) as well as *in vivo* (Peterlin *et al.*, 1986). It seems likely, therefore, that a distinct factor recognizes sequences upstream of Sp1 binding site III in these extracts. The results of the binding and transcription data are summarized in Figure 5.

Discussion

This paper describes a system with which the induction of RNA synthesis by phorbol esters can be monitored *in vitro*. The induction observed is most dramatic upon concurrent treatment of cells with low serum, suggesting a connection between physiological activation and the growth rate of the cell. Using HIV as a model promoter, we demonstrate that enhancer function can be modulated *in vitro* by phorbol ester treatment of cells. The data support a mechanism of post-translational modification of a positive-acting protein that recognizes the HIV-1 enhancer repeats.

Although extensive studies have been carried out on site-specific protein phosphorylation events that occur following protein kinase C activation, little is known about its potential role in the modification of individual transcription factors. The best-characterized example of a 45-kd DNA-binding protein, designated AP-1, which recognizes the TPA-inducible element 5′-TGAG/CTCAG-3′ present in the metallothionein IIA and collagenase promoters, and the SV40 enhancer (Angel *et al.*, 1987; Chiu *et al.*, 1987; Lee *et al.*, 1987a,b). DNA binding experiments suggest that the AP-1 DNA-binding domain is activated in response to TPA in several cell lines (Angel *et al.*, 1987). The observation that distinct elements are also TPA-responsive (Comb *et al.*, 1986; Elsholtz *et al.*, 1986), including the binding sites on SV40 for the AP-2 and AP-3 transcription factors (Chiu *et al.*, 1987), suggests that an entire subset of cellular promoter-specific factors may be modulated by phorbol esters in a similar manner. Nevertheless, recent studies reveal that TPA does not always lead to an increase in the apparent affinity of a particular transcription factor for DNA (Chiu *et al.*, 1987), suggesting that the mechanism of activation may vary among individual cell types and transcription factors. The availability of *in vitro* systems which reproduce the TPA response should allow detailed biochemical studies of the various mechanisms through which these and similar transcription factors become activated.

The TPA-responsive region of the HIV-1 enhancer contains two repeats homologous to the enhancer core regions of the SV40 early and immunoglobulin κ genes (Figure 5). Nabel and Baltimore (1987) recently reported a correlation between induction of a DNA-binding activity (designated NF-κ B) *in vitro* and activation of the HIV-1 enhancer in T lymphoid cells. Similarly, Chiu *et al.* (1987) have observed induction by TPA in HeLa tk^- cells of a DNA-binding activity (designated AP-3) that recognizes the SV40 enhancer core. Our preliminary experiments with affinity-purified protein fractions suggest that the factor that interacts with the HIV-1 enhancer in TPA-induced cells is distinct from the AP-2 and AP-3 factor activities that are detected in uninduced cells. Further experiments are required to elucidate the relatedness of each of these proteins, and to determine whether the combinatorial recognition of promoter elements changes in response to signal induction.

Transient expression experiments have shown that TPA activation of T cells functions in concert with transcriptional autoregulation by the viral protein, tat (Nabel and Baltimore, 1987; Tong *et al.*, 1987), which activates HIV expression through downstream control elements distinct from the enhancer (Rosen *et al.*, 1985). These observations support a model in which activation of the enhancer-binding protein assists in the initial production of the tat protein, leading to a further and permanent increase in viral expression and replication.

Materials and methods

Plasmid DNA constructs

Construction of the wild-type HIV-1 pLTR-CAT and pLTR-Neo plasmids is described by Peterlin *et al.* (1986), and the HIV enhancer deletion mutants are described by Muesing *et al.* (1987). Our sequence analysis suggests that deletion mutant −76 is actually deleted to position −73 or −74, and the −91 deletion mutant is −90, for simplicity, we refer to these mutants by their original designations. The *AvaI* to *Eco*RI fragment of pLTR-CAT was isolated for DNase I footprint experiments.

Northern analysis and CAT enzyme assays

The various cell lines were transfected by a modified calcium-phosphate procedure as described (Imbra and Karin, 1986). Individual transfections included 5 μg each of the indicated test plasmid and the control human α-1 globin promoter plasmid. Transfected cells were shocked with glycerol then incubated for 14 h in media containing low (0.5%) fetal calf serum, or 0.5% fetal calf serum containing 100 ng/ml TPA, prior to preparation of extracts for CAT enzyme activity determinations.

For Northern blot analysis of HIV-1-Neo RNA, transfected cells were subjected to a glycerol shock 12 h after transfection, then incubated in low-serum-containing medium with or without TPA for 3 h before RNA was extracted (Imbra and Karin, 1986).

Preparation of TPA-induced transcription extracts

Nuclear extracts were prepared as described by Angel *et al.* (1986) from HeLa tk^- cells. Cells were kept for 24 h with 0.5% fetal calf serum, and induced for 1−2 h with 100 ng/ml TPA prior to harvesting. All buffers contained 10 mM sodium molybdate (a phosphatase inhibitor), 1 mM dithiothreitol and 0.4 mM phenylmethylsulfonyl fluoride. Ammonium sulfate pellets were desalted by size-exclusion chromatography because prolonged dialysis or repeated freeze-thawing of the extracts significantly diminished the magnitude of the transcriptional induction. These preparations yielded ~1 ml each of control and induced extract (10 mg/ml) from a preparation of $1.1-1.4 \times 10^9$ cells. Although transcriptional induction was also observed with HeLa S3 (spinner) cells the effect was not as pronounced as with HeLa tk^- cells.

In vitro transcription and DNA-binding experiments

For transcription reactions, the protein concentration of each extract was adjusted to 1 mg/ml, and the DNA template concentration was 1.6 μg/ml (unless otherwise described in the figure legends) in a final buffer concentration of 25 mM Tris−HCl, pH 7.9, 6.25 mM $MgCl_2$, 0.5 mM EDTA, 50 mM KCl, 0.5 mM dithiothreitol, 300 μM rNTPs, 2% polyvinyl alcohol and 10% glycerol. Transcription reactions were carried out for 45 min at 30°C, and work-up and primer extension reactions were carried out as described. Single-stranded oligodeoxynucleotides primers complementary to HIV-1 RNA (5′-GAAATAACTCCGAATTCGTCACCC-3′) and to α-globin RNA (5′-GGTTCTTGTCGGCAGGAGAGCACC-3′) were annealed at 55°C for 1 h. DNA-binding and transcription experiments were otherwise performed as described (Jones *et al.*, 1987).

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References

Abmayr,S.M., Feldman,L.D. and Roeder,R.G. (1985) *Cell*, **43**, 821–829.
Angel,P., Poting,A., Mallick,U., Rahmsdorf,H.J., Schorrp,M. and Herrlich,P. (1986) *Mol. Cell. Biol.*, **6**, 1760–1766.
Angel,P., *et al.* (1987) *Cell*, **49**, 729–739.
Atchinson,M.L. and Perry,R.P. (1987) *Cell*, **48**, 121–128.
Bodner,M. and Karin,M. (1987) *Cell*, **50**, 267–275.
Briggs,M., Kadonaga,J.T., Bell,S.P. and Tjian,R. (1986) *Science*, **234**, 47–52.
Chiu,R., Imagawa,M., Imbra,R.J., Bockoven,J.R. and Karin,M. (1987) *Nature*, in press.
Chodosh,L.A., Carthew,R.W. and Sharp,P.A. (1986) *Mol. Cell. Biol.*, **6**, 4723–4733.
Colamonici,O.R., Trepel,J.B., Vidal,C.A. and Neckers,L.M. (1986) *Mol. Cell. Biol.*, **6**, 1847–1850.
Comb,M., Birnberg,N.C., Seasholtz,A., Herbert,E. and Goodman,H.M. (1986) *Nature*, **323**, 353–356.
Cullen,B.R. (1986) *Cell*, **46**, 973–982.
Elsholtz,H.P., Mangalam,H.J., Potter,E., Albert,V.R., Supowit,S., Evans,R.M. and Rosenfeld,M.G. (1986) *Science*, **234**, 1552–1557.
Folks,T., *et al.* (1986) *J. Immunol.*, **136**, 4049–4053.
Fujita,T., Shibuya,H., Ohashi,T., Yamanishi,K. and Taniguchi,T. (1986) *Cell*, **46**, 401–407.
Gorski,K., Carneiro,M. and Schibler,U. (1986) *Cell*, **47**, 767–776.
Greenberg,M.E. and Ziff,E.B. (1984) *Nature*, **311**, 433–435.
Harada,S., Koyanagi,Y., Nakashima,H., Kobayashi,N. and Yamamoto,N. (1986) *Virology*, **154**, 249–258.
Heintz,N. and Roeder,R.G. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2713–2717.
Imbra,R.J. and Karin,M. (1986) *Nature*, **323**, 555–558.
Imbra,R.J. and Karin,M. (1987) *Mol. Cell. Biol.*, **7**, 1358–1363.
Jones,K.A., Kadonaga,J.T., Luciw,P.A. and Tjian,R. (1986) *Science*, **232**, 755–759.
Jones,K.A., Kadonaga,J.T., Rosenfeld,P.J., Kelly,T.J. and Tjian,R. (1987) *Cell*, **48**, 79–89.
Kelley,K., Cocran,B.H., Stiles,C.D. and Leder,P. (1983) *Cell*, **35**, 603–610.
Kruijer,W., Cooper,J.A., Hunter,T. and Verma,I.M. (1984) *Nature*, **312**, 711–716.
Lee,W., Haslinger,A., Karin,M. and Tjian,R. (1987a) *Nature*, **325**, 368–372.
Lee,W., Mitchell,P. and Tjian,R. (1987b) *Cell*, **49**, 741–752.
McDougal,J.S., Mawle,A., Cort,S.P., Nicholson,J.K., Cross,G.D., Scheppler-Campbell,J.A., Hicks,D. and Sligh,J. (1985) *J. Immunol.*, **135**, 3151–3162.
Mizushima-Sugano,J. and Roeder,R.G. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8511–8515.
Muesing,M.A., Smith,D.H. and Capon,D.J. (1987) *Cell*, **48**, 691–701.
Nabel,G. and Baltimore,D. (1987) *Nature*, **326**, 711–713.
Nishizuka,Y. (1984) *Nature*, **308**, 693–698.
Nishizuka,Y. (1986) *Science*, **233**, 305–312.
Peterlin,B.M., Luciw,P.A., Barr,P.J. and Walker,M.D. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 9734–9738.
Rio,D., Robbins,A., Myers,R. and Tjian,R., (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5706–5710.
Rosen,C.A., Sodroski,J.G. and Haseltine,W.A. (1985) *Cell*, **41**, 813–823.
Sen,R. and Baltimore,D. (1986) *Cell*, **41**, 921–928.
Sodroski,J., Patarca,R., Rosen,C., Wong-Staal,F. and Haseltine,W. (1985) *Science*, **229**, 74–77.
Spangler,R., Bruner,M., Dalie,B. and Harter,M.L. (1987) *Science*, **237**, 1044–1046.
Tong-Starksen,S.E., Luciw,P.A. and Peterlin, B.M. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 6845–6849.
Wasylyk,C., Imler,J.L., Perez-Mutul,J. and Wasylyk,B. (1987) *Cell*, **48**, 525–534.
Wiederrecht,G., Shuey,D.J., Kibbe,W.A. and Parker,C.S. (1987) *Cell*, **48**, 507–513.
Wiskocil,R., Weiss,A., Imboden,J., Kamin-Lewis,R. and Stobo,J. (1985) *J. Immunol.*, **134**, 1599–1603.
Wright,C.M., Felber,B.K., Paskalis,H. and Pavlakis,G.N. (1986) *Science*, **234**, 988–992.
Zagury,D., Bernard,J., Leonard,R., Cheynier,R., Feldman,M., Sarin,P. and Gallo,R. (1986) *Science*, **231**, 850–853.

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