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12-*O*-Tetradecanoylphorbol-13-acetate induces the enhancer function of human T-cell leukemia virus type I

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Phorbol esters were employed in studies on the molecular mechanism of the induction of expression of human T-cell leukemia virus type I (HTLV-I) by a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Experiments using the chloramphenicol acetyltransferase (CAT) system showed that CAT expression directed by the long terminal repeat (LTR) of HTLV-I was induced by TPA, but not by 4α-phorbol-12,13-didecanoate, which is not an activator of protein kinase C, and that like other known enhancers, irrespective of its position and orientation, a 230-bp fragment in the U3 region of the HTLV-I LTR confers susceptibility to induction by TPA.

Enhancer; Gene expression; Long terminal repeat; Retrovirus; Tumor promoter

1. INTRODUCTION

Human T-cell leukemia virus type I appears to be the causative agent of adult T-cell leukemia and a member of the family of retroviruses [1,2]. Transcription from the LTR of HTLV-I is found only in cells containing a product of this virus, designated p40 [3–5], and the sequence in the LTR responsible for the p40-dependent induction is within an enhancer element in the U3 region [6–9]. A tumor promoter, TPA, is known to induce the expression of viral antigens of HTLV-I [1], but the molecular mechanism of this induction by TPA is unknown. In the present study, we found that TPA markedly activates gene expression directed by the LTR of HTLV-I and that the enhancer element in the U3 region mediates this activation.

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Abbreviations: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; 4α-PDD, α-phorbol-12,13-didecanoate; HTLV-I, human T-cell leukemia virus type I; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase

2. MATERIALS AND METHODS

2.1. *Cell lines*

The B cell lines LCL-Kan and ARH 77, the T cell line Jurkat, the monocyte line U937 and the erythroleukemia cell line K562 were used. These cell lines did not contain HTLV-I. TL-Oml is a T cell line that carries the HTLV-I proviral genome, but does not express any product of HTLV-I detectable at the mRNA level or protein level [10,11].

2.2. *DNA transfection*

Plasmid DNAs (10 μg) were transfected into cells (5×10^6) by the DEAE-dextran method [9,12]. The transfected cells were cultured in RPMI 1640 medium containing 20% fetal calf serum for 12 h and then cultured for 24 h in the presence or absence of TPA (5 ng/ml).

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2.3. *CAT assay*

The CAT activity was determined as described in [9,13]. Cell extracts prepared by the freeze-thaw method were incubated for 1 h in 150 μl of reaction mixture containing 0.5 mM acetyl CoA and 0.1 μCi [^{14}C]chloramphenicol (54 mCi/mmol, Amersham, Bucks, England). Chloramphenicol and its derivatives were extracted with ethyl acetate and analyzed on silica gel thin layer plates (Merck, Darmstadt, FRG) in 95% chloroform:5% methanol. Then the plates were autoradiographed and the radioactivity of the spots was counted. The CAT activity was expressed as the percentage of [^{14}C]chloramphenicol converted to acetylated forms.

3. RESULTS

To study the mechanism of induction of HTLV-I by TPA, we examined the effect of TPA on expression of the CAT gene under the control of the HTLV-I LTR. For this, we first tested various hematopoietic cell lines for use in the CAT assay. A plasmid pCHL4 (fig.3A), which contains the LTR-directed CAT gene [9], was transfected into cells and 12 h later TPA was added. TPA treatment increased CAT activity in all the cell lines tested, the increase in the K562 cell line being greatest (table 1). Therefore, K562 cells were used in subsequent experiments. The specificity of phorbol esters was examined by comparing TPA effect with that of 4α-PDD, which does not induce viral expression or activate protein kinase C. The CAT activity from pCHL4 was increased 96-fold by treatment with TPA, but was not increased by 4α-PDD (fig.1).

Table 1

Effects of TPA on gene expression driven by the HTLV-I promoter unit in various cell lines

Cell line	CAT activity % conversion		Index
	−TPA	+TPA	
Jurkat	1.8	5.5	3.1
TL-Oml	0.2	0.5	2.5
LCL-Kan	0.4	1.3	3.3
ARH77	18.5	60.3	3.3
U937	0.9	3.4	3.8
K562	0.5	43.1	86.2

pCHL4 was transfected into cells (5×10^6) and after 12 h, TPA was added at a concentration of 5 ng/ml, and cells were cultured for a further 24 h. CAT activity in the extract was assayed and is shown as the percent conversion. The index represents the ratio of the CAT activities in the presence and absence of TPA

The effects of TPA on several other viral transcriptional units were also examined. Increase in CAT activity on addition of TPA was demonstrated with a CAT construct (pRSVcat) containing the promoter unit of Rous sarcoma virus. Plasmids containing the SV40 (pSEHL4) and polyoma virus (pWEN·CAT) enhancers showed similar significant increases in activity on TPA treatment as reported in [14,15]. Slight increase was observed with a plasmid (pMEHL4) containing the murine sarcoma virus (MSV) enhancer (fig.2).

We have reported that two regulatory elements in the HTLV-I LTR, a 230-bp enhancer element in the U3 region and a 300-bp element in the R region, are responsible for maximum gene expression [9]. We thus addressed the question of which element was affected by TPA. CAT plasmids containing the two elements in different positions and orientations were tested for responsiveness to TPA (fig.3A). The CAT activity of the enhancerless pSV1Ccat was not affected by TPA, but the CAT activities of plasmids containing the U3 enhancer element were increased 20–50-fold irrespective of the position and orientation of the fragment (fig.3B). The activity of pdERH-1 containing the R region was increased only 2-fold by TPA. These

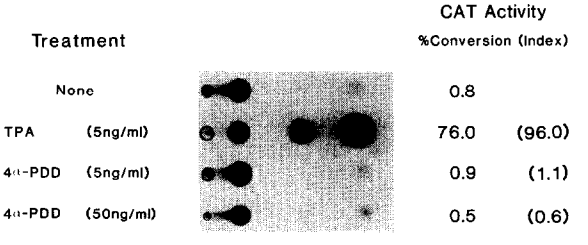

Fig.1. The absence of effect of 4α-PDD on the promoter unit of HTLV-I LTR. K562 cells were transfected with pCHL4 and 12 h later TPA or 4α-PDD was added at the indicated concentrations. The CAT activity in cell extracts was assayed. The percentage conversion and the index described as in the legend of table 1 are shown.

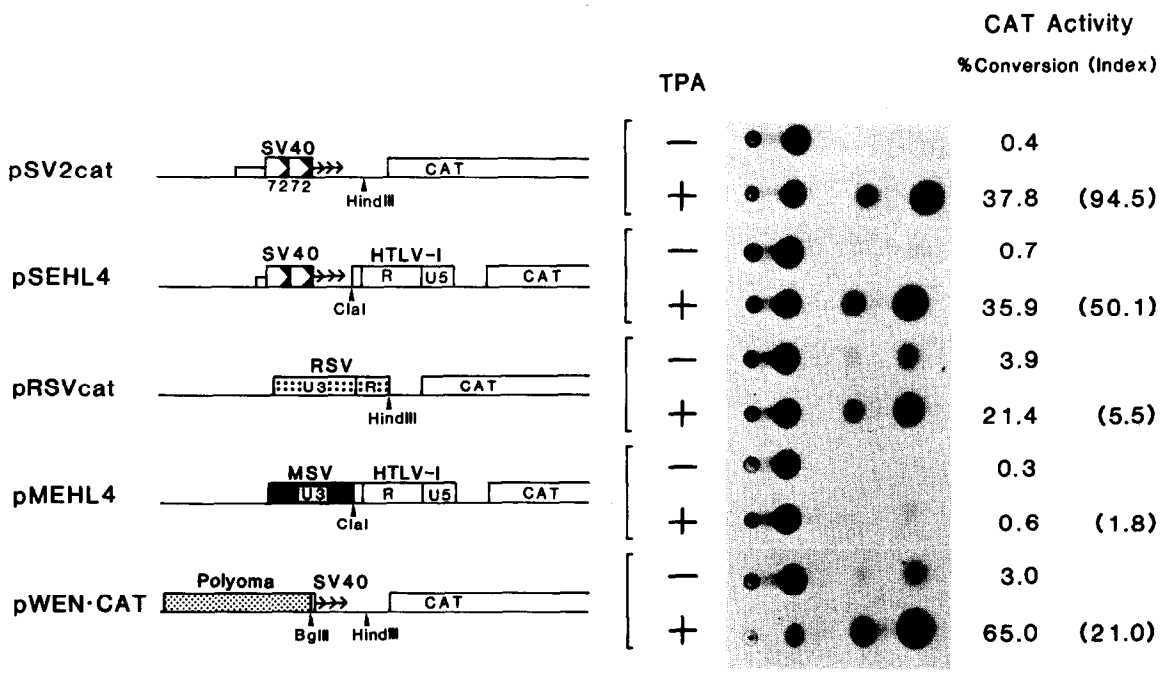

Fig.2. Effect of TPA on several viral promoter units. CAT constructs containing enhancer-promoter units from SV40, RSV, MSV and polyoma virus were introduced into K562 cells. TPA was added at a concentration of 5 ng/ml 12 h after transfection. The CAT assay and the presentation of data are as described in the legend of table 1.

data strongly indicate that the enhancer element in the U3 region of the HTLV-I LTR mediates induction of gene expression from the LTR by TPA.

4. DISCUSSION

We demonstrated that a 230-bp fragment in the U3 region mediates activation by TPA of gene expression directed by the HTLV-I LTR in the cell lines tested. K562 cells showed a greatly enhanced activity on treatment with TPA. We do not know why their response differed quantitatively from those of other cells, but it might be due to abnormal expression of a protooncogene c-*abl* in K562 cells [16]. The domain responsible for TPA enhancement appeared to be a TPA-inducible enhancer, indicating that the TPA-inducible enhancement occurs at a transcriptional level and that there is a cellular factor that interacts with the enhancer element in the 230-bp fragment. The interaction may result in the enhancement of gene expression. The mechanisms involved in the activation of the HTLV-I enhancer by TPA are not understood. It is, however, likely that TPA modulates the so-called *trans*-acting transcriptional factor(s). TPA may induce expression of the factor(s), or alternatively may convert it from an inactive to an active state. Protein kinase C may be involved in this modulation of the factor(s), since the activation was observed with TPA, but not with 4α-PDD, which does not activate protein kinase C. TPA is known to activate enhancers of SV40 and polyoma virus in a similar manner, apparently modifying the *trans*-acting factor post-transcriptionally [14,15].

p40, a product of the pX region of HTLV-I, *trans*-activates gene expression directed by its own LTR, and the 21-bp motif in the U3 region of the LTR has been shown to be responsible for this p40-induced activation [8]. An intriguing question is whether the 21-bp motif can also confer TPA inducibility. Our preliminary results suggested that this is not the case. These observations suggested that multiple elements in the enhancer are involved in activation of gene expression of HTLV-I. The consensus sequences responsible for TPA responsiveness, $^{C}_{G}$TGACT$^{C}_{A}$A and CTGACTCA, have

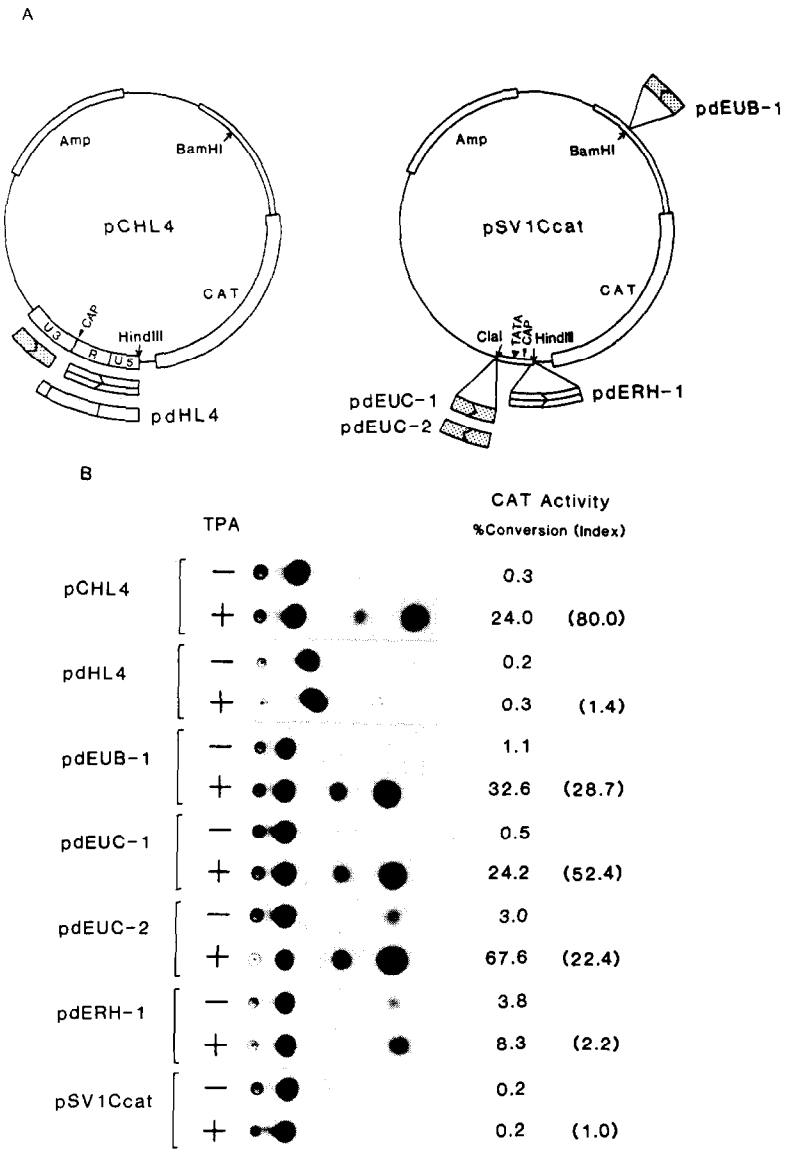

Fig.3. (A) Schematic representation of CAT constructs. Details of the constructions of these plasmids have been described [9]. (B) Ability of the enhancer fragment of HTLV-I to confer TPA-dependent activation. Each plasmid (10 μg) was transfected into K562 cells and 12 h later TPA (5 ng/ml) was added. Conditions for the CAT assay and representation of data are as described in the legend of table 1.

very recently been reported [17,18]. Although we found, in the previous study, that a pentanucleotide, CTGAC, which is also included in the TPA-consensus sequences, is present in severa enhancers including the 230-bp enhancer of the HTLV-I LTR [Saito, S. et al., submitted], the HTLV-I enhancer does not contain the sequence entirely homologous to the consensus sequences.

Our results also suggested that the mechanism of enhancement of gene expression by TPA could be different from that by p40. This notion indicates the possibility that, even in the absence of p40, expression of HTLV-I genes including pX genes may be induced by exogenous stimuli. This possibility could explain the initial step of expression and replication of this virus.

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