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Direct evidence that the kinase activity of protein kinase C is involved in transcriptional activation through a TPA-responsive element

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In order to examine the involvement of protein kinase C (PKC) in the transcriptional activation of genes by TPA (12-*O*-tetradecanoyl phorbol 13-acetate) we have constructed a series of PKC expression plasmids. Transient expression of an active fragment of PKC in rat fibroblasts resulted in the transcriptional activation of a TRE (TPA-responsive element)-CAT chimeric gene which contains various repetitions of collagenase TREs. These provide the first direct evidence that kinase activity of PKC is involved in TPA-induced transcriptional activation through TRE.

Phorbol ester; Tumor-promoter-responsive element; Protein kinase C

1. INTRODUCTION

Transcription of certain genes is activated by treatment of cells with tumor-promoting phorbol esters, such as TPA (12-*O*-tetradecanoyl phorbol 13-acetate). Such TPA-inducible genes, including the collagenase and metallothionein IIA genes, share a common *cis*-acting element, i.e. TRE (TPA-responsive element) [1–3]. Several lines of evidence suggest that the nuclear oncoproteins FOS and AP-1 are involved in TPA-inducible transcriptional activation through a direct interaction between the FOS/AP-1 complex and TRE [4–8]. Since protein kinase C (PKC) is a major cellular receptor for phorbol esters, it has been suggested that PKC mediates many of the biological effects of the phorbol ester and is also involved in transcriptional activation [9,10]. However, until now direct evidence showing the involvement of PKC in TPA-induced transcriptional activation has not been available.

To examine the involvement of PKC in transcriptional activation through TRE, we introduced PKCα cDNA constructs into rat fibroblast 3Y1 cells [11] and then analyzed the transcriptional activation of TRE.

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Abbreviations: PKC, protein kinase C; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate; TRE, TPA-responsive element; CAT, chloramphenicol transferase; FCS, fetal calf serum; IFN, interferon

2. MATERIALS AND METHODS

2.1. *Plasmid construction*

The construction of pSRD-α_w and pIFNCAT has been described previously [12,13]. pSRDα_c is of the same construction as pSRD-α_w except that the sequence for the regulatory domain (Ala-7 to Gly-297) is replaced by the sequence GGSSRVDL. The reporter plasmids, pTREx1/IFNCAT, pTREx2/IFNCAT, pTREx3/IFNCAT and pTREx6/IFNCAT, respectively contain one, two, three and six copies of a synthetic human collagenase TRE motif (col-TRE) (5′-AAGCATGAGTCAGACA-3′) in tandem upstream of the IFN promoter.

2.2. *Cell culture, transfection, and CAT assay*

Rat fibroblast 3Y1 cells were transfected with the expression plasmid (10 μg/10 cm dish) and the reporter plasmid (2 μg/10 cm dish). Transfections were carried out as in [12] ex-

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cept that, following transfection, cells were cultured in a low-serum medium (0.5% FCS) for 48 h. CAT activities were determined on protein equivalents (60 μg) as described [13].

3. RESULTS AND DISCUSSION

3.1. *Transient expression of an active fragment of PKC induces transcriptional activation of the TRE-CAT chimeric gene*

Fig.1A shows the expression plasmids for PKC cDNA. Transfection of these PKC cDNA constructs into COS cells allowed characterization of the enzymological properties of the encoded proteins, the 80 kDa wild-type PKCα_w and the 45 kDa PKCα_c [12]. PKCα_w showed protein kinase activity in the presence of TPA [12]; PKCα_c exhibited kinase activity even in the absence of TPA (not shown). After co-transfection of these PKC expression plasmids with the reporter CAT plasmid (fig.1B) into 3Y1 cells, transcriptional activation by TRE was analyzed in terms of CAT expression (fig.2). Co-transfection resulted in increased CAT expression in the case of PKCα_c (fig.2, lanes 2,3) whereas no stimulation was observed for PKCα_w (fig.2, lane 4).

The degree of CAT expression increased in proportion to the copy number of TRE (fig.3). These results indicate that such activation is mediated by TRE, possibly through the binding of the FOS/AP-1 complex to TRE.

3.2. *The C-terminal protein kinase domain of PKCα is sufficient for TRE-mediated transcriptional activation*

The observation that TRE CAT expression is induced by the active fragment of PKCα and not authentic PKCα in the absence of TPA indicates that the protein kinase activity of PKC is required and sufficient for TRE-mediated transcriptional activation. The regulatory domain of PKC, which contains a potential DNA-binding sequence (zinc-finger motif) [12,14,15], is dispensable to transcriptional activation. It has been demonstrated in vitro that calpain (calcium-dependent protease) cleaves PKC at the D3 region (fig.1A), generating the N-terminal regulatory domain and a 45 kDa fragment which corresponds to the PKCα_c used here [16,17]. It may be possible that an active fragment such as PKCα_c is generated and functions in intact cells.

One interesting point revealed by the present work is that the amount of active PKCα is a major limiting factor in the signaling pathway from TPA to TRE in 3Y1 cells. We and others have recently reported data obtained in cDNA cloning and subsequent expression studies that demonstrate the presence of at least four PKC types [14,15,18–20],

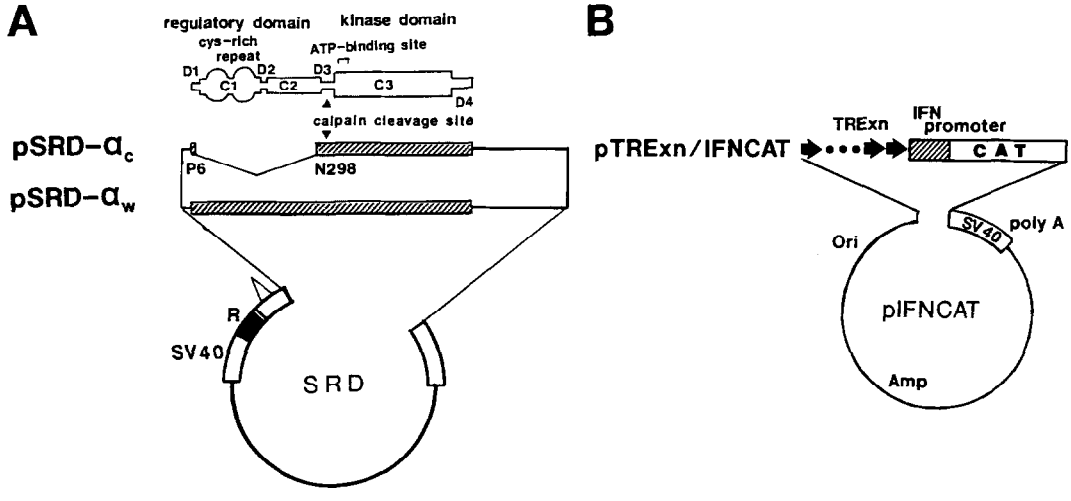

Fig.1. Structures of PKC expression and reporter plasmids. (A) PKC expression plasmids. pSRD-α_w and pSRD-α_c encode the 80 kDa wild-type PKCα and the 45 kDa C-terminal protein kinase domain of PKCα, respectively. (B) Reporter plasmids. n indicates the repetition of the TRE motif upstream of the IFN promoter.

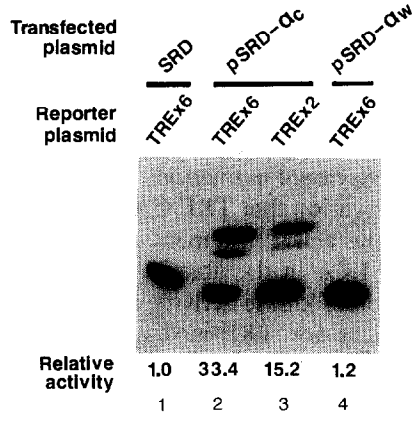

Fig.2. Activation of TRE-CAT expression by transient expression of an active fragment of PKCα. An expression plasmid for PKCα and a reporter plasmid were co-transfected into rat fibroblast 3Y1 cells and the CAT activities were determined. Relative activities are shown taking the level of SRD-transfected cells as standard.

as well as an additional PKC-related enzyme, nPKC [12], each of which acts as a phorbol ester receptor. These PKC types are expressed in a tissue- or cell-type-specific manner. Previous experiments have indicated that the level of TPA-induced activation of TRE depends on the cell type [1,2]. This may be explained at least partly on the basis of differences in the amount and type of PKC.

The experimental system employed here should permit further scrutiny of the potential substrates of PKC as well as investigation of the possible differences in action of multiple PKC molecular types on TRE-mediated transcriptional activation through TPA signals and physiological stimuli.

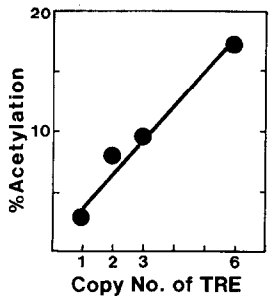

Fig.3. TRE-dependent CAT expression induced by the active fragment of PKCα. Each reporter plasmid which contains various numbers of the TRE motif was co-transfected with pSRD-α_c into 3Y1 cells and CAT activities were analyzed.

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