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Repression of 5-aminolevulinate synthase gene by the potent tumor promoter, TPA, involves multiple signal transduction pathways

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Abstract

The potent tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces activator protein-1 (AP-1) transcription factors, early response genes involved in a diverse set of transcriptional regulatory processes, and protein kinase C (PKC) activity. This work was designed to explore the signal transduction pathways involved in TPA regulation of 5-aminolevulinate synthase (ALAS) gene expression, the mitochondrial matrix enzyme that catalyzes the first and rate-limiting step of heme biosynthesis. We have previously reported that TPA causes repression of ALAS gene, but the signaling pathways mediating this effect remain elusive. The present study investigates the role of different cascades often implicated in the propagation of phorbol ester signaling. To explore this, we combined the transient overexpression of regulatory proteins involved in these pathways and the use of small cell permeant inhibitors in human hepatoma HepG2 cells. In these experimental conditions, we analyzed TPA action upon endogenous ALAS mRNA levels, as well as the promoter activity of a fusion reporter construct, harboring the TPA-responsive region of ALAS gene driving chloramphenicol acetyl transferase gene expression. We demonstrated that the participation of α isoform of PKC, phosphatidylinositol 3-kinase (PI3K), extracellular-signal regulated kinase (ERK1/2), and c-Jun N-terminal kinase (JNK) is crucial for the end point response. Remarkably, in this case, ERK activation is achieved in a Ras/Raf/MEK-independent manner. We also propose that $p90^{RSK}$ would be a convergent point between PI3K and ERK pathways. Furthermore, we elucidated the crosstalk among the components of the cascades taking part in TPA-mediated ALAS repression. Finally, by overexpression of a constitutively active $p90^{RSK}$ and the coactivator, cAMP-response element protein (CREB)-binding protein (CBP), we reinforced our previous model, that implies competition between AP-1 and CREB for CBP.

Keywords: Regulation of gene expression; Signal transduction; Tumor promoter; Protein phosphorylation; 12-*O*-Tetradecanoylphorbol-13-acetate; 5-Aminolevulinate synthase; Extracellular-signal regulated kinase; c-Jun N-terminal kinase; Phosphatidyl inositol 3-kinase; Protein kinase C

12-*O*-Tetradecanoylphorbol-13-acetate (TPA),[2] a potent tumor promoter phorbol ester, affects a number of cellular functions including gene expression and protein and DNA synthesis. At the cellular level, TPA has been demonstrated to modulate the growth, differentiation, survival, function, and metabolism of a variety of primary cell cultures and cell lines. The broad range of these phorbol ester-mediated biological effects suggests a

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[2] *Abbreviations used:* TPA, 12-*O*-tetradecanoylphorbol-13-acetate ALAS, 5-aminolevulinate synthase; AP-1, activator protein-1; CAT, chloramphenicol acetyltransferase; CREB, cAMP-responsive element protein; CBP, CREB-binding protein; CRE, cyclic AMP-responsive element; ERK, extracellular-signal regulated kinase; JIP-1, JNK-interacting protein-1; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MEK, MAPK/ERK kinase; PI3K, phosphatidyl inositol 3-kinase; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PDK-1, 3-phosphoinositide-dependent kinase-1; PKA, PKB, and PKC, protein kinase A, B, and C, respectively; SEK, SAPK/ERK kinase-1; TRE, TPA-responsive element.

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role for this drug in the modulation of a variety of cellular processes, including those that affect the development, progression, and therapy of human malignancies [1].

Since TPA shares some structural similarities with diacylglycerol, the protein kinase C (PKC) physiological activator, it is commonly used to activate classical and novel PKC isoenzymes. Most of TPA-induced effects are mediated by the temporal activation, translocation, and suppression of selected PKC isoforms. These kinases play central roles in signaling pathways, participating in a variety of protein phosphorylation cascades that regulate gene expression.

It has been reported that the biological effects of the activation of a given subset of PKC isoforms in any cell will be variable, dependent upon the status of several interwoven signaling and regulatory pathways controlled by a variety of extracellular and intracellular stimuli [2]. Although cellular targets of phorbol esters are the aforementioned PKC isoforms, TPA signaling also triggers activation of other kinases, including those of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) families [3]. There are several PKC effects mediated by activation of MAPKs, ERK, and c-Jun aminoterminal kinase (JNK), and subsequent activation of activator protein-1 (AP-1) [4].

Diacylglycerol and phorbol ester-dependent regulation of gene expression is often mediated by AP-1 transcription factors. AP-1, dimeric complexes comprising members of Fos and Jun family proteins, are early response genes involved in a diverse set of transcriptional regulatory processes [5]. They bind to and direct transcription from TPA-response elements (TREs) that are therefore known as AP-1-binding sites. Mitogens, oncoproteins, cytokines, and stress agents such as ultraviolet light activate AP-1. Frequently, AP-1 plays a role as a positive regulator of gene expression [6]. Recently, we have described a distinct mechanism of negative regulation by AP-1 on 5-aminolevulinate synthase (ALAS) gene promoter. We found that an AP-1-binding site, located at −261 bp, was crucial for the repression of ALAS promoter activity. This event involves competition between cAMP-response element (CRE) protein (CREB) and AP-1 for the coactivator CREB-binding protein (CBP) [7].

The ALAS gene has provided a useful model system for studying the integration of multiple hormonal signals at the level of a single gene [8–10]. ALAS is a mitochondrial matrix enzyme that catalyzes the first and rate-limiting step of heme biosynthesis [11]. In eukaryotes, there are two related ALAS isozymes that are encoded by two separate genes. The erythroid form of ALAS is expressed in hematopoietic tissue and is essential for the generation of functional hemoglobin in erythrocytes. The second enzyme, ubiquitous or liver type ALAS, is probably expressed in all tissues, providing heme for cytochromes and other hemeproteins [11]. Defects in genes encoding enzymes in the heme biosynthesis pathway are associated with a family of serious disorders known as porphyrias. Additionally, heme synthesis decline could explain the loss of iron homeostasis, because insufficient levels of heme would compromise iron regulation [12].

Acute hormonal regulation of ALAS is often exerted by regulating transcription. We have already demonstrated in rat hepatocytes and in human hepatoma cells that cAMP induces ALAS gene through protein kinase A (PKA) [13], and insulin represses it through extracellular-signal regulated kinase (ERK) and protein kinase B (PKB) activation [9]. These regulatory events are mediated through a region comprising 450 bp of ALAS promoter. The cAMP response is elicited through the binding of CREB to the two CRE sites located at −149 and −45 bp, respectively [13]. Moreover, insulin exerts its end point response by modulating hepatic nuclear factor 3 (HNF3) transactivation ability and through a *cis*-acting insulin-response element (IRE), present at −389 bp [10].

Here, we explored and characterized the pathways and effector targets involved in the transduction of TPA signal on ALAS gene regulation. We analyze the participation of TPA-responsive PKC isoforms, and the involvement of PI3K, ERK, and JNK pathways. To achieve this, we combined the transient overexpression of mutant versions of effector proteins involved in these pathways, and the use of small cell permeant protein inhibitors in HepG2 cells. Our findings indicate that among the TPA-responsive PKC isoforms, activation of PKCα is required for the transduction of the signal. Moreover, PI3K is involved, needless the participation of its downstream effector, PKB. The ERK, activated in a MAPK/ERK kinase (MEK)-independent manner, also contributes to the transduction of this signal. Finally, the Jun component of AP-1 complex needs to be induced and activated by JNK. This complexity indicates that activation of multiple pathways is jointly required for TPA-mediated repression of ALAS gene.

Materials and methods

Expression vectors

The following expression vectors were used as indicated in each experiment. Deletion mutant plasmid p-354ALAS/CAT, derived from parental pALAS/CAT containing the 5′-flanking region (−833 to +42 bp) of rat ubiquitous ALAS gene cloned upstream the CAT reporter gene in vector pBLCAT6, was described previously [13]. Vectors encoding wild type, constitutively active or kinase dead mutants of α, δ or ε PKC isoforms [14] were kindly provided by Dr. Shigeo Ohno (Yokohama City University, Kanagawa, Japan).