Transcriptional activation of the gene for the large subunit of human m-calpain by 12-o-tetradecanoyl-phorbol-13-acetate

Akiko Ham, Shigeo Ohno* and Koichi Suzuki**

Department of Molecular Biology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Hon-komagome, Bunkyo-ku, Tokyo 113, Japan

Received 23 April 1992

The effect of the treatment of HeLa cells with a tumor-promoting phorbol ester, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) on the expression of the genes for the calpain family has been examined. Among the mRNAs for the calpain family, only the mRNA for the large subunit of human m-calpain (calpain mL) was specifically induced by treatment of cells with TPA, suggesting its specific function in response to cellular stimuli. The effect of TPA on the expression of the calpain mL gene was further examined using fusion genes containing the promoter/enhancer region of the calpain mL gene fused upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene, showing that the promoter/enhancer sequence of the calpain mL gene contains a cis-acting element which responds to TPA and activates transcription of the downstream sequence.

Culpain; Protein kinase C; Transcription; TPA; HeLa cell

1. INTRODUCTION

Three types of calpain (calcium-dependent protease, calcium-activated neutral protease, CANP) isozymes, m-, μ-, and n-calpain, have been found. Although n-calpain is expressed only in muscle, other two types of calpain, m- and μ-calpain, locate in various animal tissues and cells [1,2]. Ubiquitous distribution of m- and μ-calpains suggests their physiological importance especially in the cellular functions regulated by Ca~++. Although there is a clear difference between the two calpain isozymes, m- and μ-calpain, in the Ca~++ concentrations required for activation, no clear differences are found in other biochemical properties such as substrate specificity [1,2]. This suggests that these two isozymes act in distinct physiological situations.

Previous experiments have established that both m- and μ-calpains are composed of heterogeneous subunits, large (L) and small (S), where the small subunit is common to both isozymes [3]. Thus the difference in the Ca~++-requirements resides in the large subunits, mL and μL. The structural similarity of these calpain proteins, mL, μL, S and the third large subunit, nL, suggests their evolutionary relationship, whereas their genes locate on four distinct human chromosomes, respectively [4].

Isolation and characterization of the promoter/enhancer region of the human calpain mL gene revealed the presence of tandemly repeated negative enhancer-like cis-acting elements which negatively regulate the transcription of the calpain mL gene as well as a promoter sequence [5]. The promoter/enhancer sequence of the calpain mL gene contains a sequence motif called TRE (TPA-response element) shown to be involved in transcriptional activation mediated by protein kinase C, a major target of TPA [6–8].

In the present study, we examined the expression of the genes for the calpain family as well as that for the specific proteinous inhibitor, calpastatin, and found that TPA specifically induces the expression of the calpain mL mRNA and the induction is very likely regulated at the level of transcription through the promoter/enhancer region of the calpain mL gene.

2. EXPERIMENTAL

2.1. Northern blot analysis

HeLa cells were cultured in Eagle's minimum essential medium (E-MEM) supplemented with 5% fetal calf serum. After incubation with TPA (100 ng/ml) for the indicated time, total RNA was prepared as described [5] and subjected to Northern hybridization following a standard procedure [9]. DNA probes used for hybridization were cDNA inserts of plasmids p21 (human calpain mL) [10], p31 (human calpain μL) [11], p276 (human calpain S) [12], and pC313 (rat calpastatin) [13]. A human β-actin genomic DNA segment was purchased from Wako (Osaka, Japan).
2.2. DNA transfection and CAT assay

A rat fibroblast 3Y1 cell stable transfectants, W3, was cultured as described [14] and used in the transient expression experiments. The constructs of the CAT-calpain mL fusion genes, p-460/-20CAT, p-202/-80TKCAT, and pTKCAT was described previously [5].Cell extracts equivalent to 15 mg of protein were used for the CAT assay. The CAT activity of each construct was determined by at least two independent transfection experiments and the average value is shown.

3. RESULTS AND DISCUSSION

3.1. Specific induction of the mRNA for the large subunit of human m-calpain in HeLa cells treated with a tumor-promoting phorbol ester, TPA

In order to further obtain an evidence supporting the difference of these two calpain isoforms in various physiological situations, we examined the expression of their genes in HeLa cells. We have previously isolated cDNA clones encoding mL, µL, S, and calpastatin. These mRNA species were shown to be expressed ubiquitously in a variety of tissues and cells [1]. In order to examine whether the amount of these calpain proteins change in response to a certain extracellular stimuli, we examined their mRNA expression by Northern blotting using corresponding cDNA as hybridization probes after treatment of HeLa cells with TPA which is shown to activate protein kinase C (PKC) in cells and activates PKC-mediated signaling pathway [6–8].

Exponentially growing HeLa cells were treated with TPA, total RNA was extracted, and were analyzed in terms of Northern blot analysis. As shown in Fig. 1, all the calpain mRNAs (mL, µL, and S) and calpastatin mRNA were expressed in HeLa cells and the amount of mRNA for µL did not change. However, mL mRNA greatly increased upon treatment of cells with TPA for several hours. The mRNAs for S and calpastatin seem to increase to some extent. This is the first demonstration that the amount of calpain mRNA change in response to an extracellular stimuli. Moreover, it is quite interesting that only mL mRNA was induced and the other mRNAs did not change upon TPA treatment, suggesting that the amount of mL and thus that of m-calpain is regulated under intra-cellular signaling system involving PKC.

3.2. The induction of calpain mL mRNA is regulated through a cis-acting transcriptional elements

In order to ascertain whether the induction of calpain mL mRNA is regulated at the transcriptional level, we next examined the transcriptional activity of the cloned promoter/enhancer region of the human calpain mL gene. A set of DNA construct which contains the promoter/enhancer region of the calpain mL gene fused upstream of the bacterial CAT structural sequence was transfected to a rat fibroblast cell clone W3, which is a stable transfectant of 3Y1 cell. W3 contains an exogenous sequence encoding a rabbit PKCa cDNA under the control of mouse mammary tumor virus (MMTV) long terminal repeat (LTR) promoter which responds to dexamethasone (Dex) and results in the increase of the transcription of the downstream sequence [14]. W3 cells treated with Dex express an approximately 20-fold amount of PKCa compared with the endogenous protein and is highly responsive to TPA when examined in terms of transcriptional activation of

![Fig. 1. Expression of calpain and calpastatin mRNAs in HeLa cells treated with TPA. Total cellular RNA (2 µg/lane) isolated from HeLa cells cultured in the presence of TPA (100 ng/ml) for various lengths was analyzed by Northern blot analysis. Length of TPA treatment is shown as follows: (lane 1) 0 h; (lane 2) 0.5 h; (lane 3) 1 h; (lane 4) 2 h; (lane 5) 4 h; (lane 6) 8 h. Probes used were calpain mL (mL), calpain µL (µL), calpain S (S), calpastatin (CS), and β-actin. Arrow-heads indicate 28S and 18S ribosomal RNAs. Arrows indicate corresponding mRNA species. Small arrow in S indicates the cross-hybridization with rRNA.](image)
Fig. 2. TPA-responsive expression of the CAT-calpain mL fusion genes. The fragments of calpain mL gene upstream sequences inserted into the CAT expression plasmid pKSCAT or pTKCAT [5] are shown as arrows. The CAT activity of each construct was determined and indicated as percent acetylation as described in section 2. Open box shows the promoter of HSV TK gene which includes both CAAT box (*) and TATA box (o). Closed box shows bacterial CAT gene.

the reporter CAT fusion gene containing TPA-response elements such as TRE and serum-response element (SRE) (data not shown). 

pTKCAT contains the promoter sequence of the herpes simplex virus (HSV) thymidine kinase (TK) gene and is constitutively active with or without TPA (Fig. 2). Addition of the promoter sequence (P) region of the calpain mL gene upstream of the TK gene promoter (p-202/80TKCAT) does not alter the CAT activity without TPA. However, the CAT activity of p-202/80TKCAT is enhanced 4-fold by treatment with TPA. This indicates that the -202/-80 (P) region contains cis-acting element(s) which responds to TPA and enhances the transcription. As previously reported, this region of the calpain mL gene promoter contains a sequence motif TGAATCA at -132 closely related to the collagenase TPA-response element (TRE) which leads to irreversible molecular transformation of these substrate proteins [1]. The cellular proteins which are digested by calpain involves receptors for insulin and epidermal growth factors, protein kinases such as PKC, and cytoskeletal proteins. It is also reported that activation of calpain involves irreversible transformation of itself; auto-digestion [2] and the activated calpain species is very unstable [16]. This implies that calpains are used only once in cells, and that activation of calpain requires a mechanism which compensates the used-up molecules and prepare for the next signal. The induction of calpain mL gene by TPA suggests that various physiological stimuli may induce the synthesis of mL-calpain to maintain the level of this protein.

Acknowledgement: This work was supported in part by research grant from the Ministry of Education, Science, and Culture of Japan.

REFERENCES


