

Unique expression pattern of protein kinase C- θ : high mRNA levels in normal mouse testes and in T-lymphocytic cells and neoplasms

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A 2.2-kb cDNA that contains the entire coding region of mouse protein kinase C- θ (PKC- θ) was cloned from skeletal muscle mRNA using reverse transcription and the polymerase chain reaction (PCR). This clone was used as a probe to study the expression of this PKC isoform in normal and transformed hemopoietic cells and other normal tissues. By far the highest steady-state level of PKC- θ mRNA was found as a 2.8-kb transcript on a Northern blot of poly(A)⁺ RNA from testes. High levels were also found in skeletal muscle, spleen, T lymphomas and purified normal T lymphocytes, but these tissues and cells expressed two transcripts, 3.3 kb and 3.8 kb. Lower levels of similar size transcripts were found in normal brain, B lymphocytes and B-lymphocytic tumors and cell lines.

Protein kinase C; Isoenzyme; Expression; Lymphocyte; Testis; Lymphoma; Skeletal muscle

1. INTRODUCTION

Protein kinase C (PKC) is a multi-gene family of at least nine serine/threonine kinases that are central to many signal transduction pathways [1,2]. cDNA cloning and sequencing studies have shown that eight of the PKCs are encoded by different genes, and one, PKC- β II, represents the product of alternative splicing of the same gene that encodes PKC- β I [3–8]. PKC is the major intracellular receptor for phorbol esters, which are potent activators for most of the isozymes and well known tumor promoters [9,10]. The physiological activator of PKC is thought to be SN-1,2-diacylglycerol, produced as a lipophilic second messenger either from turnover of inositol phospholipids or phosphatidylcholine as a result of external stimulation by hormones, growth factors, etc. [1,2]. A required cofactor is anionic phospholipid, preferably phosphatidylserine. Additionally, PKC- α , - β and - γ are dependent on the presence of Ca²⁺ for activation of their kinase activity [1–3], whereas PKC- δ , - ϵ , - η and - θ are Ca²⁺-independent [7,8,11,12]. The Ca²⁺-independent isoforms, also referred to as novel or nPKCs, are activated by micelles composed of phosphatidylserine and diacylglycerol. Another member of the PKC gene family, PKC- ζ , does not bind

phorbol esters and is not activated by Ca²⁺ [6,13,14]. Its mechanism for activation is unknown.

Most cells express several of the PKC isozymes, and most PKCs are found in a wide variety of cell types. The combination of isozymes that are expressed seem to be unique to each tissue. We have recently reported that the expression of PKC- α through - η in hemopoietic cells is cell type- and, at least for B lymphocytes, differentiation stage-specific [15]. Cell type- and differentiation stage-specific patterns of PKC isozyme expression have been reported for other cell lines and tissues [16–18]. The cloning of an additional member of the PKC gene family, PKC- θ [8], which appeared to be the principal isoform expressed in mouse skeletal muscle, prompted us to investigate its expression more thoroughly in different mouse hemopoietic cell lines and tumors, as well as in normal tissues. During the preparation of this manuscript, a report appeared in which PKC- θ was shown to be abundantly expressed in hemopoietic cells [19].

2. MATERIALS AND METHODS

2.1. Cloning of mouse PKC- θ

1 μ g of mRNA from BALB/c skeletal muscle was reverse transcribed using random hexamers and the Superscript kit (BRL) following the manufacturer's protocol. 10 ng of cDNA were used in each 100 μ l polymerase chain reaction (PCR) using *Taq* Polymerase (BRL or Perkin Elmer) or Vent DNA polymerase (New England Biolabs, Beverly, MA). The following primers used for PCR were taken from the sequence published for PKC- θ from mouse muscle [8]. For location of the primers in the published sequence the position of the final base of each primer is given in parentheses. GAATTCG-

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CAAATCCCTTCCAGTCTGGA (2,161); GAATCCGACAC-CAGGGAACAACC (-18). Each of the 30 cycles of PCR included 1 min annealing at 58°C, 2 min extension at 72°C and 30 s denaturation at 94°C. The initial denaturing step lasted 3 min, and the final extension lasted 5 min. The reaction products were resolved on a 1% low-melting point agarose gel (BRL) and the expected bands were isolated as described [20]. 20 ng of the DNA fragment were phosphorylated using T4-kinase, ligated into 10 ng of *Sma*I-digested pBS (Stratagene), and the resulting DNA was transfected into electrocompetent DH10B cells (BRL) using electroporation. Positive colonies were identified by colony hybridization using the PCR fragment and confirmed by restriction digestion of DNA isolated from positive clones.

2.2. Northern blot analysis

Tissue, tumors, cell lines or purified normal T and B cells were obtained as described [15]. Poly(A)⁺ RNA was directly isolated from frozen cell pellets or pulverized tissue [21]. 5 µg of mRNA was loaded onto each lane of 1% agarose gels containing formaldehyde, and electrophoretically resolved. The RNA was transferred to Hybond^N membranes (Amersham) by capillary transfer, and the blots were probed with 3 × 10⁶ cpm per ml of a ³²P-radiolabeled 1.1-kb *Pst*I fragment of glyceraldehyde-phosphate dehydrogenase (GAPDH, [22]) to normalize for mRNA content in each lane. Subsequently, the blot was stripped with boiling water and re-probed with a 2-kb *Eco*RI fragment of mouse PKC-θ cDNA. For the detection of additional PKC transcripts, full-length cDNAs from mouse PKC-α [23], rat PKC-β and -γ [3], mouse PKC-δ [11], -ε [24], -ζ [14] and -η [15] were used.

3. RESULTS

We decided to use PCR for cloning PKC-θ cDNA since this method, if successful, should be much faster than conventional cloning approaches. We obtained a 2.2-kb DNA fragment that contained the entire protein-coding region using the two primers described. The 2.2-kb DNA fragment was cloned into pBS and identified as PKC-θ by restriction mapping and partial sequencing (data not shown), both of which revealed 100% identity with the previously published mouse PKC-θ [8].

We first used the PKC-θ cDNA as a probe to investigate the expression of PKC-θ in different normal mouse tissues (Fig. 1). Two transcripts for PKC-θ of 3.3- and 3.8-kb were found in easily detectable levels in skeletal muscle tissue and spleen, while very low levels of similar size transcripts were found in almost every other tissue examined. The 1.3-kb bands that are visible in several lanes of all figures represent remnants of GAPDH bands from a previous hybridization. The highest level of PKC-θ expression, however, was in testes, which revealed an mRNA of different size, ca. 2.8 kb.

To investigate more thoroughly the expression of PKC-θ in hemopoietic cells, we probed Northern blots of an extensive set of mRNAs from 67 B-lymphocytic, 9 T-lymphocytic and 9 myeloid tumors and cell lines (for a more detailed description of the cell lines see [15] and [25]), as well as normal B and T lymphocytes.

Fig. 2 is a Northern blot of RNAs from a series of B-lymphocytic cell lines that vary from early progenitor B cells on the left through pre-B and B cells to plasma-

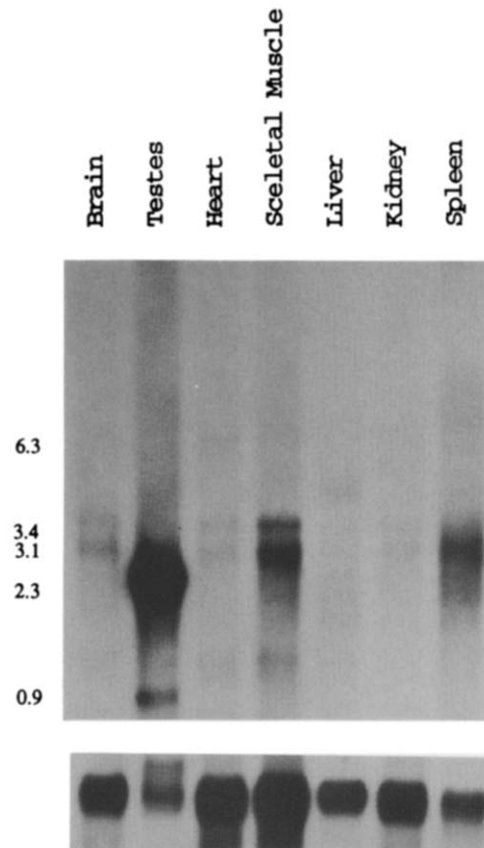


Fig. 1. Northern blot of 5 µg poly(A)⁺ RNA from normal mouse tissues probed with the 2.2-kb PKC-θ cDNA. The sizes (in kb) and positions of RNA markers are indicated on the left. Hybridization of the same blot with GAPDH is shown in the lower panel.

cytomas on the right. PKC-θ is expressed as 3.3- and 3.8-kb mRNAs in most of the B lymphocytic cell lines examined, but at variable, generally low levels that do not change significantly during B cell development. The presence of the occasional 1.8 and 4.8 kb bands is due to cross-reactivity with ribosomal RNA. Only two of the tumors, BAL 17, a B cell lymphoma, and ABPC 103, a plasmacytoma, expressed PKC-θ in easily detectable amounts. Generally the 3.3-kb mRNA predominated over the larger form, and sometimes the 3.8-kb mRNA was not seen at all, e.g. SJL-4.

RNAs from 9 myeloid cell lines were examined (7 examples are shown in Fig. 3) and no 3.3- or 3.8-bands of PKC-θ mRNA were detectable.

In contrast, normal T lymphocytes and most T-cell lines express high levels of both 3.3- and 3.8-kb PKC-θ mRNA (Fig. 4), as can be seen from our Northern blot analysis. Only BW 5147 and two clones obtained from cell fusions with BW 5147, T5 and 2B4, failed to express detectable amounts of PKC-θ mRNA.

To study whether other members of the PKC-gene family are expressed as different size messages in testes, we probed Northern blots of poly(A)⁺ RNA from brain and testes with cDNA probes of all known PKC genes.

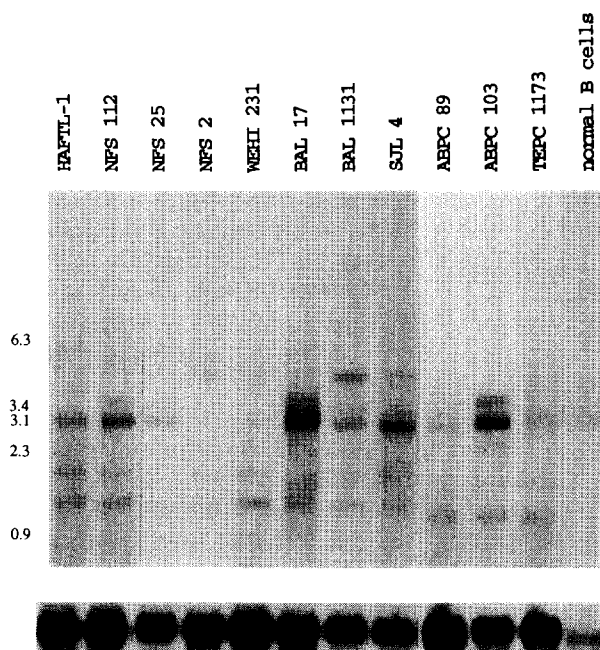


Fig. 2. Northern blot of 5 µg poly(A)⁺ RNA from different mouse B-lymphocytic cell lines in increasing degrees of maturation from left to right, and normal splenic B cells. The order of individual RNAs corresponds to the differentiation stage of the cells. HAFTL-1 (pro-B), NFS 112 and NFS 25 (pre-B), WEHI 231, BAL 17, and BAL 1131 (mature B), SJL 4 (plasmablast) and ABPC 89, ABPC 103 and TEPC 1173 (plasmacytomas). The sizes (in kb) and positions of RNA markers are indicated on the left; an exposure of the same blot probed with GAPDH is shown below.

As shown in Fig. 5, none of the Ca²⁺-dependent PKCs is expressed in detectable amounts in testes. Only PKC- δ , - ζ and - θ mRNAs can be easily detected in this tissue, and all three transcripts differ in size from the respective PKC mRNAs expressed in brain.

4. DISCUSSION

Our experiments demonstrate that under proper conditions, a > 2-kb cDNA can easily be cloned by using RT-PCR. In our hands, however, these results could only be obtained with Vent DNA polymerase. We were unable to amplify DNA fragments > 1 kb in our RT-PCR when we used *Taq* DNA polymerase.

Our results confirm the previous report [8] that PKC- θ is expressed in quite high amounts in skeletal muscle tissue and in low, but easily detectable amounts in spleen, brain and heart. Unexpectedly, expression is highest not in skeletal muscle, but in testes, although we detected only one transcript of ca. 2.8 kb. Our probe is specific for the θ isoform, because it does not detect any other PKC transcript under the high stringency conditions utilized, even in cells that over-express other PKC isozymes (data not shown). We also probed this blot with the mouse brain PKC- θ cDNA that was previously published [8], and found identical results (data not

shown). The results reported here differ from those of Baier et al. [19], who used a 'semi-quantitative PCR' approach and could detect only very low levels of PKC- θ mRNA in human testes. We suspect that the discrepancy in results stems from the use of PCR with only one pair of primers on testes transcripts which, as indicated by our Northern blot data, are 500–1,000 bp shorter than those in any other tissue. Alternatively, man and mouse might differ in their PKC expression patterns.

Testes express mRNAs of unusual size for a variety of genes [26], including other PKC genes. As shown in Fig. 5, only three of the eight known PKC genes are expressed at easily detectable levels in testes, and all three isoforms appear as transcripts that are different in size from those in brain. Transcripts of PKC- θ and - δ (the isotype most similar in structure to PKC- θ) in testes are ca. 500 bp shorter than those in brain or any other tissue and are unique to testes ([11,15], and this manuscript). The 2.5-kb PKC- ζ transcript in testes is the same size as the lower molecular weight transcript seen in other tissues or cell lines [14,17], although most of them contain an additional 4.5-kb PKC- ζ mRNA, which is completely absent in testes. Brain is the unique

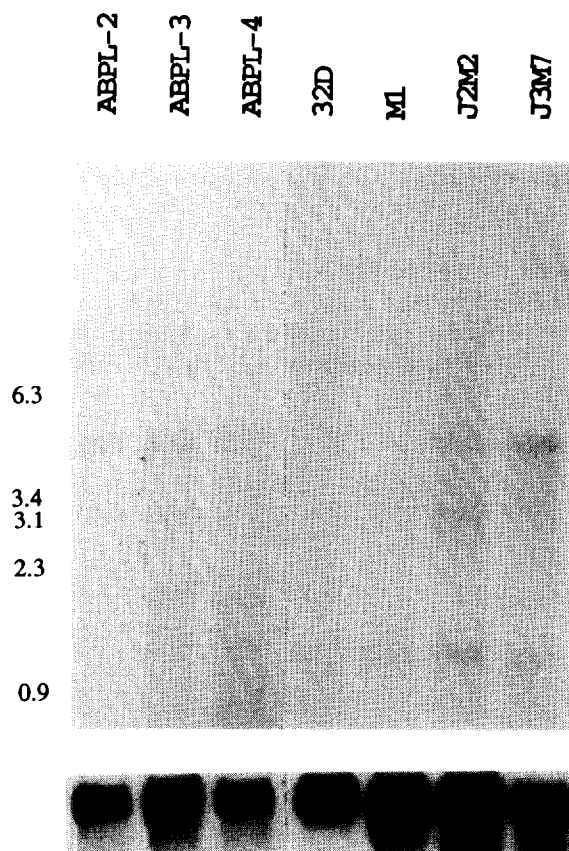


Fig. 3. Northern blot of 5 µg poly(A)⁺ RNA from different myeloid tumors (ABPL-2, ABPL-3, ABPL-4, J2M2 and J3M7) and cell lines (32D and M1). The sizes (in kb) and positions of RNA markers are indicated on the left. Hybridization of the same blot probed with GAPDH is shown below.

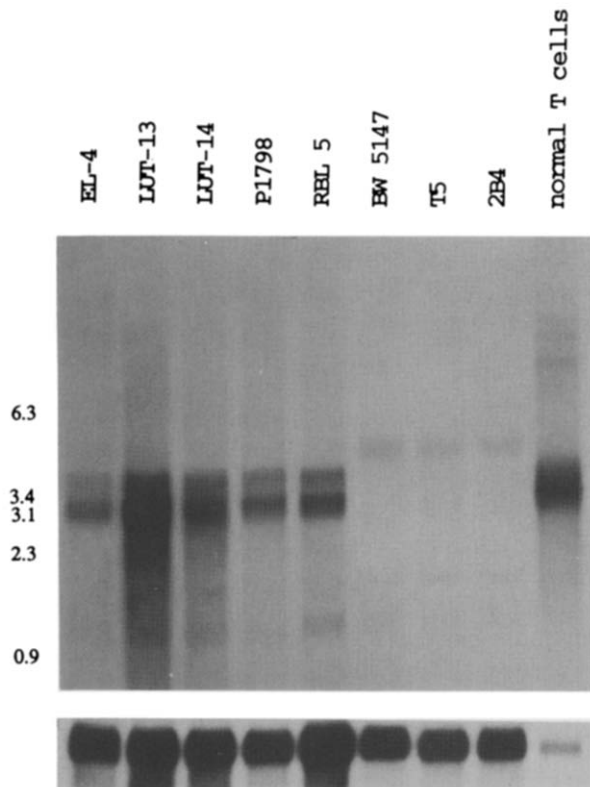


Fig. 4. Northern blot of 5 µg poly(A)⁺ RNA from different T lymphomas (LUT-13, LUT-14, P1798 and RBL 5), cell lines (EL-4, BW 5147 and two hybridomas, T5 and 2B4). The last lane contains 5 µg poly(A)⁺ from normal CD4⁺ and CD8⁺ T cells that were purified from lymph nodes as described [15]. The sizes (in kb) and positions of RNA markers are indicated on the left; an exposure of the same blot probed with GAPDH is shown below.

tissue in the case of PKC-ζ in that its transcripts are ca. 200 bp smaller than those of testes or any other tissue.

We previously examined the expression of PKC-α through -η in hemopoietic cells [15] and concluded that

the patterns of expression of PKC isozymes are different for each cell type, and, in the case of B lymphocytes, also differentiation stage-specific. Expression of PKC-θ also differs among hemopoietic cells. In all myeloid cell lines examined PKC-θ mRNA is absent, or at least below our detection limit, whereas most of the cell lines of the B-lymphocyte lineage show low-to-moderate levels of expression, as do normal splenic B lymphocytes.

The steady-state levels of PKC-θ mRNA in T-lymphocytic cell lines and normal lymph node T lymphocytes was very high, approximately twice that seen in skeletal muscle. Curiously we did not find any PKC-θ expression in the T-cell fusing line, BW 5147, or in the two hybridomas derived from it. BW 5147 has lost several chromosomes or portions thereof during mutation and selection, and it lacks expression of most PKC isozymes and several other unrelated genes ([15], J.F.M. unpublished). In fact, the only PKC isoform expressed in these lines is PKC-δ [15].

Our Northern blot results are generally similar to those obtained by RNase protection and RT-PCR methods on human tissues and tumors [19], however, the interpretation of Baier et al. [19] was that PKC-θ was predominantly expressed in hemopoietic cells based on their results with mostly T-cell lines. Our results from a survey of 85 hemopoietic tumors and cell lines, as well as purified lymphocytes, suggests that PKC-θ mRNA is rarely found in B lymphocytes or myeloid cells, but it is commonly expressed in T lymphomas and purified T lymphocytes. Still, the highest level of expression was not in T lymphocytes nor in skeletal muscle but in testes, as determined by Northern blot analysis.

Although conclusive data indicating that the different PKC isozymes play unique roles in mediating certain biological responses are still scarce, the tissue-specific pattern of PKC isozyme expression, such as that reported here for PKC-θ, point in this direction. Over-

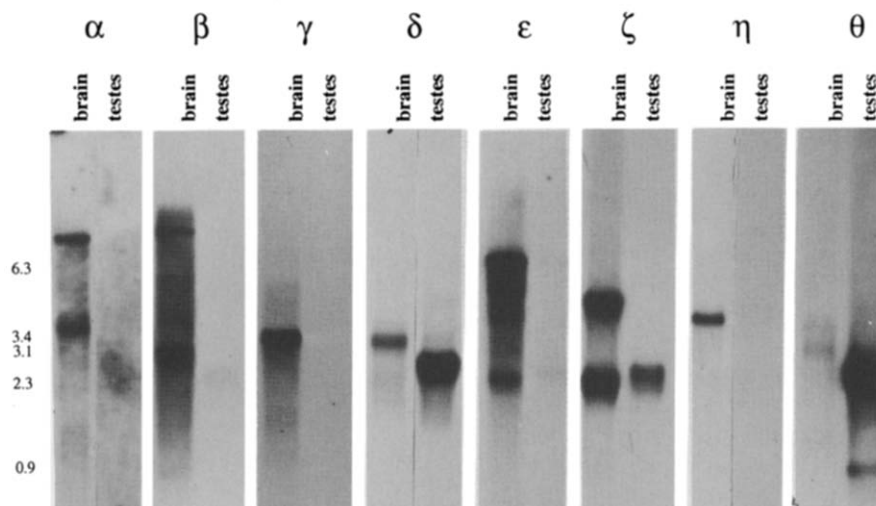


Fig. 5. Northern blots of 5 µg poly(A)⁺ RNA from normal mouse brain and testes probed for the expression of eight different PKC genes as indicated on the top. The sizes (in kb) and positions of RNA markers are indicated on the left.

expression of PKC isozymes in cell lines that do not express particular isozymes (e.g. PKC- α , - β , - η and - θ in BW 5147) might be a promising approach to study the effects of the different PKC isozymes in certain cells.

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