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Cloning and Characterization of the 5′-Flanking Region for the Mouse Phospholipase C-δ1 Gene

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To date, little is known about the molecular mechanisms controlling the regulation of phospholipase C-δ1 (PLC-δ1) gene expression. To understand the mechanisms responsible for the regulation of PLC-δ1 gene expression, the 5′-flanking region of the mouse PLC-δ1 gene was isolated from a mouse genomic DNA library. Primer extension analysis revealed that there is a single transcriptional start site located at 127 bases upstream from the translation start codon in the mouse PLC-δ1 gene. DNA sequence analysis showed that the sequence around the transcriptional start site is very GC-rich and has no TATA or CAAT boxes. Transient expression of a luciferase reporter gene under the control of serially deleted 5′-flanking sequences revealed that the 160-base-pair region from −622 to −462 upstream of the transcriptional start site includes a positive *cis*-acting element(s) for the efficient expression of the PLC-δ1 gene. Gel retardation analysis suggests that multiple transcription factors bind to separate sites on the promoter region. Based on these results, our study suggests that the minimal essential region located at −622 to +70 is fully sufficient to confer high-level transcriptional activity and contains high-affinity binding elements for multiple transcription factors.

Phosphatidylinositol-specific phospholipase C (PLC) is one of the key enzymes in the intracellular signal transduction pathway (1–3). PLC catalyzed mainly the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3), which serve as intracellular messengers for protein kinase C activation and intracellular Ca^{2+} mobilization, respectively (4, 5). All PLC isozymes contain a conserved catalytic domain, a pleckstrin homology (PH) domain in the amino terminus of the protein, and additional regulatory sequences (1, 3, 6). PLC-β is activated by α- or βγ-subunits of the heterotrimeric Gq protein (7, 8). PLC-γ, unlike the other PLC isozymes, contains two Src homology 2 domain (SH2) and one Src homology 3 domain (SH3), is activated by both receptor and nonreceptor tyrosine kinases (9, 10). The regulatory mechanisms of the PLC-δ isozyme, however, are not well understood.

Recently, the three-dimensional structure of a PLC-δ1 molecule has been determined (11, 12). PLC-δ1 consists of four domains: a PH domain, an EF-hand domain, a catalytic X and Y domain, and a C2 domain. On the basis of both structural and biochemical studies it was concluded that the PH domain of PLC-δ1 would tether the enzyme to the membrane and the C2 domain would fix the catalytic domain (13, 14). Several lines of experiment reported a modulator of PLC-δ1 activity in cells (15–17). However, the regulatory mechanism of protein level needless to say the gene of PLC-δ1 still remains to be determined.

We have previously demonstrated that the mouse PLC-δ1 was expressed significantly depending on tissue-specific manner and may carry out fundamental roles in almost all of mouse tissues (18). Although endogenous expression of mouse PLC-δ1 gene was found in multiple somatic tissues, the mechanisms of transcriptional regulation of the PLC-δ1 gene at the promoter level are not known. In view of these considerations, we investigated the structural organization and functional characterization of the mouse PLC-δ1 gene promoter.

MATERIALS AND METHODS

Isolation of the mouse PLC-δ1 gene 5′-flanking region. A mouse strain 129/SVJ genomic library constructed in the phage vector λ-FIX II (Stratagene) was screened by plaque hybridization using a

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500-bp cDNA probe encoding the 5′ part of the mouse PLC-δ1 cDNA (18). The library screening method have been previously described (18). Four positive plaques were identified, and its DNA inserts subjected to characterization by restriction endonuclease mapping and Southern blot analysis. Digestion with *Hin*dIII restriction endonuclease and Southern blot hybridization with a synthetic oligonucleotide specific to the 5′-untranslated region of the PLC-δ1 cDNA (5′-GCAAGGACCCCAGGCCGCTTGGTG-3′) revealed that 5-kb fragment contained the 5′-flanking region of the PLC-δ1 gene. This 5-kb fragment was subcloned into the *Hin*dIII site of pBluescript SK(+) (Stratagene) and sequenced with an ALFexpress DNA Sequencer (Amersham Pharmacia Biotech).

Northern blot analysis. Total cellular RNA was isolated from exponentially growing cells using Trizol reagent (Sigma). RNA samples (30 μg/lane) were denatured and electrophoresed in 1% agarose gels containing 3% formaldehyde and transferred to Hybond N^+ nylon membrane (Amersham Pharmacia Biotech) by the capillary blot method in 10× SSC (1× SSC: 0.15 M NaCl and 0.015 M sodium citrate). A probe consisting of a 593-bp *Eco*RI fragment of the mouse PLC-δ1 cDNA was labeled, hybridized and autoradiographed as described previously (18). The quality and quantity of the electrophoresed RNA were determined by rehybridization of the same blot with a 1.0-kb glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

Primer extension analysis. A 24-nucleotide antisense primer with sequence 5′-ATCCTGCAGCCCGTGCAA GGTCAG-3′ was synthesized corresponding to the cDNA region 22–45 bp downstream of the translation initiation codon. An oligonucleotide was radiolabeled at the 5′-end with T4 polynucleotide kinase and [γ-^{32}P]ATP (3,000 Ci/mmol, Amersham Pharmacia Biotech). The radiolabeled primer (1 ng) was allowed to anneal to 10 μg of total cellular RNA isolated from NIH3T3 cells by incubation for 1 h at 62°C in Superscript II reverse transcriptase buffer (GIBCO BRL) containing RNasin. The reactions were cooled to 45°C, and then extended for 30 min by addition of dNTPs and Superscript II reverse transcriptase. The mixtures were subjected to RNase A digestion, Phenol/chloroform extraction, and ethanol/sodium acetate precipitation. Samples were analyzed by electrophoresis on denaturing 6% polyacrylamide gels alongside DNA sequencing reactions of the 5-kb *Hin*dIII genomic subclone using the same oligonucleotide as used for the primer extension.

Construction of promoter-luciferase chimeric plasmids. A series of plasmids containing various sizes of the 5′-flanking region of the mouse PLC-δ1 gene were constructed by inserting DNA fragments between the *Kpn*I and *Hin*dIII sites of the vector pGL3 Basic (Promega). Briefly, 5′ deletion DNA fragments were obtained by PCR using the following synthetic oligonucleotides incorporating 5′-*Kpn*I and 3′-*Hin*dIII: for the 5′-ends of the inserts, 5′-CACGGTACCATACTAGACCTCTCTGACCTG-3′ (−1787 to −1767 from the transcription start), 5′-CACGGTACCACCAGATTCCACAACCATCC-3′ (−1506 to −1487), 5′-CACGGTACCCAGAACCTGGCTGCTTTGGAC-3′ (−1357 to −1337), 5′-CACGGTACCAACAGAGGCTACATATGTGTC-3′ (−1256 to −1236), 5′-CACG GTACCGGCCCTAGTGGTGGGTAATGC-3′ (−1165 to −1145), 5′-CACGGTACCA CTCATGGATGCGAATACCTG-3′ (−1023 to −1003), 5′-CACGGTACCACCCAGCCT TAACACTCAGAATC-3′ (−717 to −695), 5′-CACGGTACCCTAGACTCTTCCAGGC CAACTG-3′ (−622 to −601), 5′-CACGGTACCTGAGACGTCTCTGAGAGCTAAAG-3′ (−462 to −440), 5′-CACGGTACCTTAGCAGTGATTCCTGTCCCC-3′ (−318 to −298), 5′-CACGGTACCAGGAGGGATCTGACTATTTGTGG-3′ (−167 to −145), and for the 3′-end of the inserts, 5′-CACAAGCTTGCGTGATTCACTTGAGCAAAA TACC-3′ (+70 to +95 from the transcription start). Amplified DNA fragments were digested with *Kpn*I and *Hin*dIII and inserted into the immediate upstream of the luciferase reporter gene. All constructs were subjected to restriction enzyme digestion analysis and nucleotide sequencing to verify correct sequence and orientation.

Cell culture and transient transfection assay. TM3 (Leydig cell), Nor10 (muscle fibroblast cell), MLg (lung fibroblast cell), TCMK-1 (kidney epithelial cell), and NIH3T3 (embryonal fibroblast cell) were obtained from the Korean Cell Line Bank (KCLB) and maintained at 37°C in a humidified environment with 5% CO_2. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO BRL). All culture media were supplemented with penicillin G (100 units/ml) and streptomycin (100 units/ml). Cells were plated on six-well plates, each well containing 3×10^5 cells. Following 24 h incubation, cells in each well were transfected with 2 μg of promoter-reporter construct and 0.002 μg of pRL-SV40 plasmid (Promega) using the calcium phosphate method. The pRL-SV40 plasmid was co-transfected to normalize the variation in transfection efficiency. pRL-SV40 encodes the *Renilla* luciferase, and its activity can be distinguished from that of the *firefly* luciferase encoded in pGL3 Basic in the dual-luciferase assay system (Promega).

Dual-luciferase activity assay. Activities of the *firefly* luciferase and *Renilla* luciferase in a single sample were measured sequentially using the Dual-Luciferase Report Assay System (Promega) according to the manufacturer's instructions. Briefly, cells were rinsed twice with phosphate-buffered saline and then lysed in 500 μl of passive lysis buffer at room temperature for 15 min. Twenty microliters of the cell lysate was quickly mixed with 100 μl of Luciferase assay reagent in a luminometer tube. The light emission for the firefly luciferase was recorded immediately for 10s after a 3-s premeasurement delay using a MiniLumat LB9506 (EG & G Berthold). Subsequently, 100 μl of Stop&Glo reagent was added to the same tube to inactivate the *firefly* luciferase while activating the *Renilla* luciferase. The light output from the *Renilla* luciferase was integrated under same conditions. Variation in transfection efficiency was normalized by dividing the measurement for the *firefly* luciferase activity with that for the *Renilla* luciferase activity.

Gel retardation assay. NIH3T3 cell nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagent (PIERCE) according to the manufacturer's instructions. For gel retardation assay, duplex probes were end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Approximately 1 ng of the labeled probe was mixed with 2 μg of nuclear protein in a total 20 μl of the binding buffer (10 mM Tris–HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, 0.5 mM PMSF) containing 1 μg of poly(dI · dC). After incubation in ice for 20 min, the reaction mixture was separated on a 6% nondenaturing polyacrylamide gel with 0.5× TBE. The gel was dried and subjected to autoradiography. For competition experiments, the molar excess of unlabeled competitor DNA was added prior to the addition of labeled probe as specified.

RESULTS

Cloning and Sequencing of the Mouse PLC-δ1 Promoter Region

A mouse genomic DNA library was screened with a probe consisting of the extreme 5′-end of the cloned mouse PLC-δ1 cDNA (18). DNA was purified from positive plaques and mapped by restriction enzyme digestion combined with Southern blot analysis. A 5-kb *Hin*dIII fragment, which contained sequences upstream of the previously reported 5′-end of the mouse PLC-δ1 cDNA, was subcloned into pBluescript SK(+) and sequenced (Fig. 1). A particular striking feature of the PLC-δ1 gene promoter was its high G + C content. The 580-bp region upstream of the ATG initiation codon in our study had a G + C content of 76%. Consensus binding sites in the PLC-δ1 promoter sequence were identified for transcription factors by the Dnasis, such as AP1 (19),

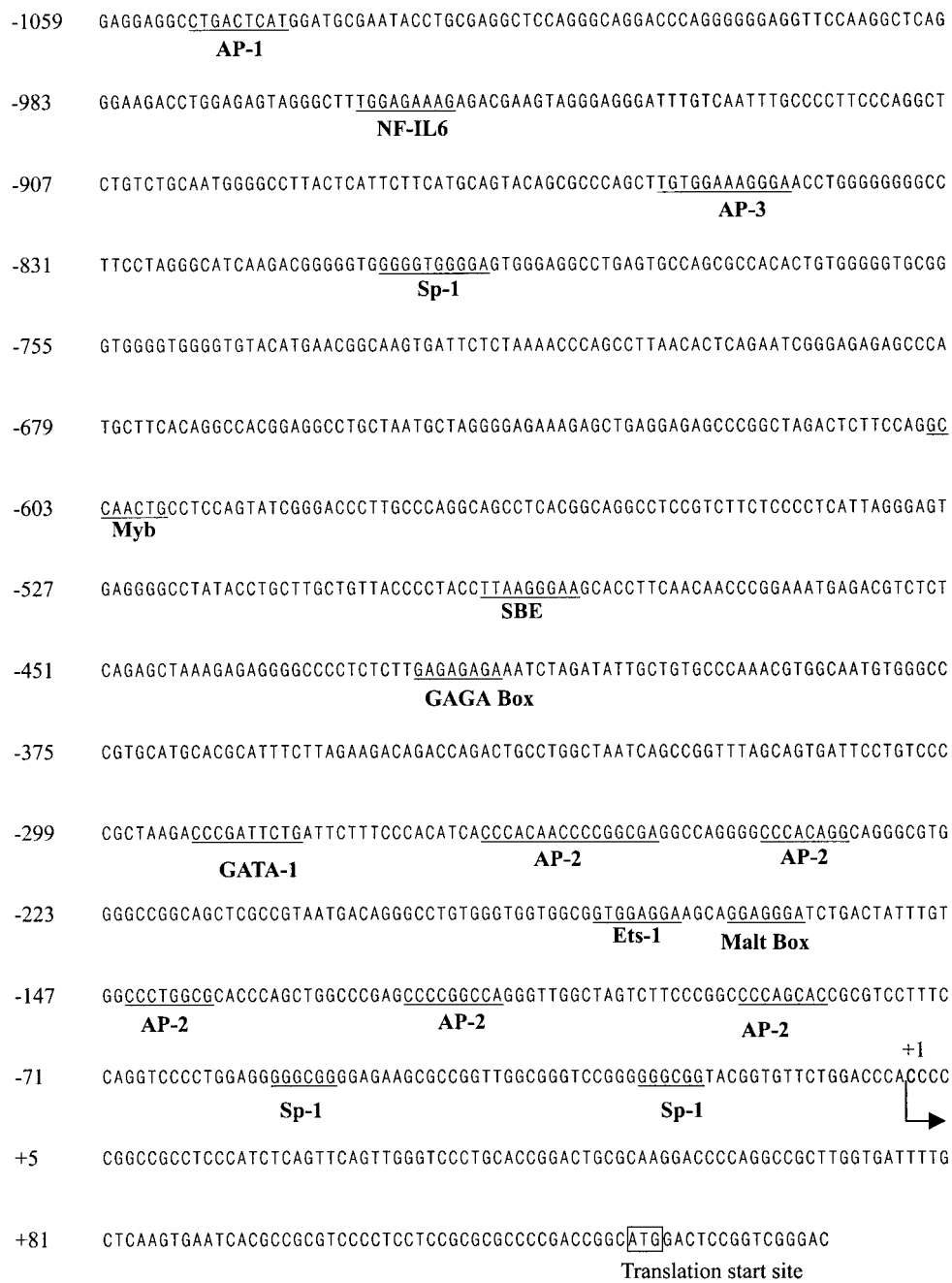

FIG. 1. Nucleotide sequence of the 5′-flanking region of the mouse PLC-δ1 gene. Nucleotide numbering is referenced to the +1 nucleotide (C) identified major transcription site (bent arrow) by primer extension. Potential *cis*-acting elements are underlined with their names indicated below. The ATG translation initiation codon is boxed. The sequence was submitted to GenBank with Accession No. AF238293.

AP2 (19), NF-IL6 (19), AP3 (19), Sp1 (20), Myb (21), Ets-1 (19), Malt box (22), and SBE (23) (Fig. 1). No apparent TATA box or CAAT box was found in the 5′-flanking region. Lack of the TATA- and CAAT-box elements are a common feature for many GC-rich promoters and usually contain multiple sequences with the CCGCCC core for binding the Sp1 transcription factor.

Identification of PLC-δ1 Transcription Start Site

To determine the transcription initiation sites of the PLC-δ1 gene, primer extension was performed as described under Materials and Methods. A radiolabeled antisense primer was hybridized to total RNA isolated from NIH3T3 cells, and the extension products were analyzed on a sequencing gel (Fig. 2). As deduced from

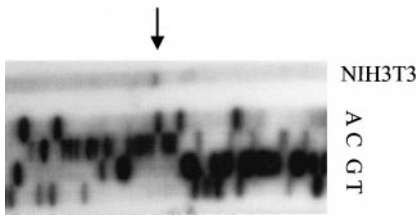

FIG. 2. Determination of the mouse PLC-δ1 gene transcription initiation site by primer extension analysis. A radiolabeled oligonucleotide corresponding to position 22–45 bp downstream of the translation initiation codon was used as a primer in a reverse transcription reaction using NIH3T3 cell total RNA. Extension products were analyzed by electrophoresis in denaturing polyacrylamide gel with a sequencing ladder of PLC-δ1 genomic DNA prepared using the same primer. The arrow indicates anticipated transcription initiation site.

the extension products, the major transcript is being initiated from cytosine residue located 127-bp upstream of the ATG codon. Accordingly, this base was designated hereafter as +1 bp unless otherwise stated and extended the 5′-end of mouse PLC-δ1 cDNA previously reported by 34 base (18). The same primer extension product was obtained using total RNA isolated from other mouse cell lines (data not shown).

Expression of the PLC-δ1 mRNA in Various Cell Types

PLC-δ1 mRNA expression was analyzed in various mouse cell lines by Northern blotting analysis (Fig. 3). Blot containing total RNA from five mouse cell lines was hybridized with a probe consisting of a 600-bp fragment of the mouse PLC-δ1 cDNA. One distinct PLC-δ1 transcript was detected, and the level of PLC-δ1 mRNA was varied significantly depending on cell types examined. Control hybridizations with a glyceraldehyde-3-phosphate dehydrogenase probe confirmed nearly equal mRNA amounts in each lane. These findings suggesting that PLC-δ1 expression in various cells are regulated at the transcription level.

Transcription from PLC-δ1 Promoter in Various Cells

The promoter activity of PLC-δ1 gene was examined by a transient transfection method. A reporter plasmid containing the luciferase gene under the control of the 5′-region of PLC-δ1 gene (from −1787 to +70) was transfected into TM3, TCMK-1, Nor10, and NIH3T3. The control SV40 promoter showed 40- to 50-fold activation in these cells when compared with the promoterless construct (Fig. 4). The PLC-δ1 promoter directed a higher level of expression than the SV40 promoter in kidney epithelial cells, TCMK-1. In contrast, the PLC-δ1 promoter exhibited much lower activity than SV40 promoter in Leydig cells, TM3. These results indicated that the 5′-region of PLC-δ1 gene was sufficient to direct cell type-specific expression.

To determine the regulatory elements responsible for PLC-δ1 gene expression, a series of deletion constructs of the 5′-region were generated (Fig. 5). These deletion constructs were transiently transfected into NIH3T3 cells, and luciferase activity was determined. Figure 5 shows that deletions up to position −622 did not result in significant changes in the promoter activity, with an activity of the longest PLC-δ1 promoter-reporter construct (−1787/+70) being only two times higher when compared with the PLC-δ1 promoter-reporter construct (−622/+70). The promoter activity was significantly lost using the construct containing sequences from −462 to +70. These results indicated that the minimal promoter comprises a region spanning from −622 to +70, and a potential positive regulatory element(s) appear to be located in the sequences from −622 to −462 and that this region is essential for a high level expression of the PLC-δ1 gene. Transfection of the −1787/+70 fusion construct resulted in a lower level of luciferase activity (10–20%) compared with that of the −1506/+70 construct. This suggested the presence of another positive regulatory element(s) between −1787 and −1506.

In an attempt to identify transcription factors with the potential to interact with promoter region, four synthetic double-stranded DNA probes, designated P1 (−126/−112), P2 (−189/−160), P3 (−498/−478), and P4 (−65/−41), were subjected to gel retardation assays. Each probe used contained one or two known consensus sequences, i.e., AP2 in P1, Ets and Malt box in P2, SBE in P3, and Sp1 in P4. As shown in Fig. 6, multiple DNA-protein complexes were formed with nuclear extracts from NIH3T3 cells. The specificity of these complexes for the sequence was shown by a competition experiment, in which the complexes were completely abolished by competition with a 50- or a 100-fold excess of an unlabeled probe. The above reporter and gel retardation analyses suggest that multiple pro-

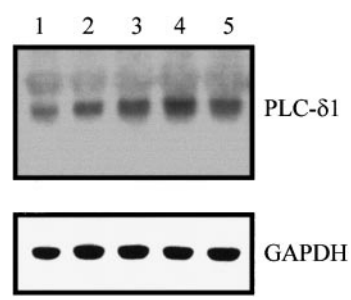

FIG. 3. Mouse PLC-δ1 mRNA expression by mouse cell lines. PLC-δ1 mRNA expression was examined by Northern blot analysis. RNA samples from various mouse cell lines were separated on 1% formaldehyde-agarose gel, transferred, and probed with PLC-δ1 cDNA. The quality and quantity of the total RNA were determined by rehybridization of the same blot with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe. Lane 1, TM3 (Leydig cell); lane 2, Nor10 (muscle fibroblast cell); lane 3, MLg (lung fibroblast cell); lane 4, TCMK-1 (kidney epithelial cell); and lane 5, NIH3T3 (embryonal fibroblast cell).

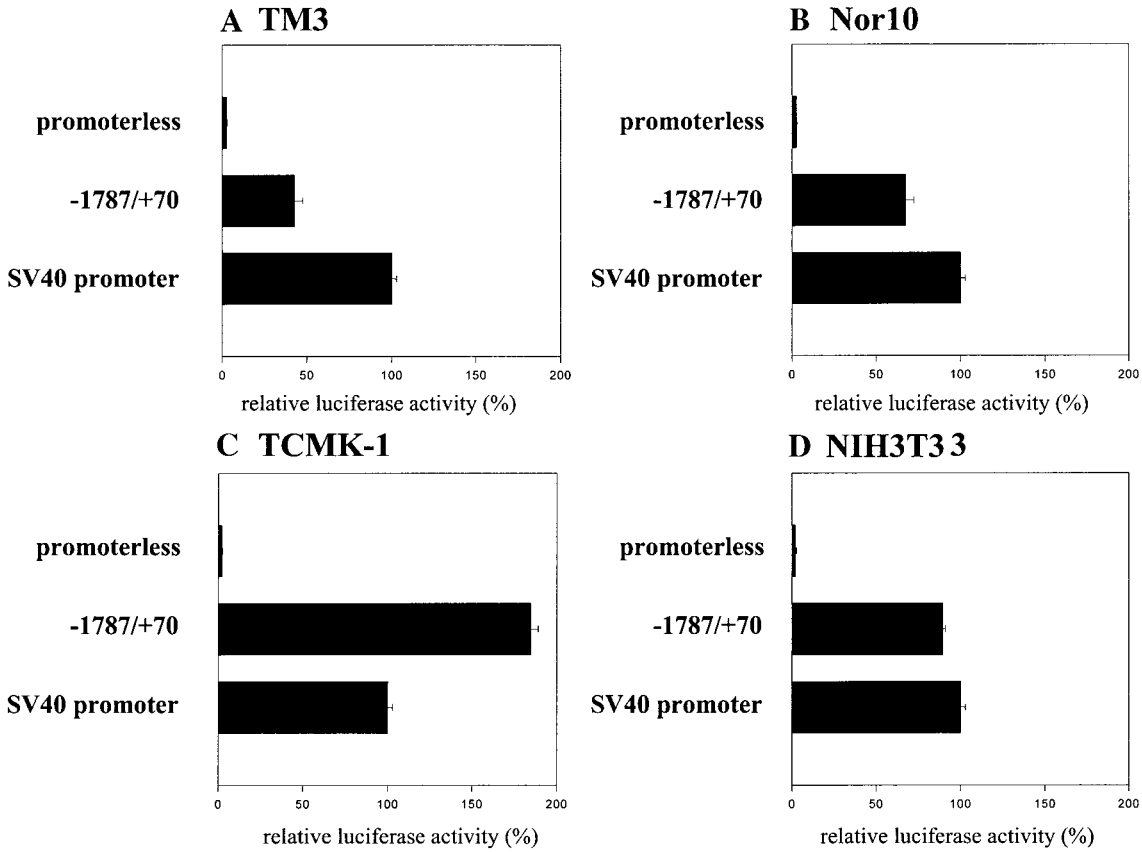

FIG. 4. Cell type specific expression by PLC-δ1 gene promoter. The luciferase reporter plasmid was transfected into TM3 (A), Nor10 (B), TCMK-1 (C), and NIH3T3 cells (D). The SV40 promoter exhibited 40- to 50-fold activation of the luciferase activity when compared with the promoterless construct. The activity of the SV40 promoter was designated as 100, and the relative activity of PLC-δ1 promoter (from −1787 to +70) was determined. Each activity was the average of at least three independent experiments and was also normalized by the activity of cotransfected pRL-SV40.

teins bind to separate sites on the promoter and some of these complexes were needed simultaneously for transcriptional activation on the PLC-δ1 gene.

DISCUSSION

In previously our study, a mouse cDNA encoding the PLC-δ1 has been identified (18). This enzyme probably present in many different cell types as manifested by Northern analysis of PLC-δ1 mRNA levels in multiple somatic tissues. Mouse PLC-δ1 was highly expressed in the testis in comparison with other tissues, suggesting that it might be transcriptionally regulated in a tissue- and cell type-specific manner.

In this study, we performed molecular cloning and functional characterization of the upstream regulatory region of the mouse PLC-δ1 gene. A 5-kb fragment of genomic DNA was isolated, encompassing the 5′-flanking region and the first exon. The PLC-δ1 promoter region is high in GC content and lacks a canonical TATA box, suggesting that PLC-δ1 promoter has overall similarity to promoters of a number of housekeeping gene. Previously, we found that transcripts of mouse PLC-δ1 are present in almost all regions of brain examined, implying that the enzyme may play a role in some “housekeeping” cellular process in brain. The transcription initiation site determined by primer extension analysis showed a single major site at the cytosine residue located 127-bp upstream from the ATG codon. Like many housekeeping genes, the promoter of PLC-δ1 gene contains GC boxes around the transcription initiation site with a potential for binding of the transcription factor Sp1. Some TATA-less promoters retain the ability to direct transcription initiation from a specific nucleotide, whereas others direct transcription initiation at multiple start sites (24).

A DNA sequence containing this promoter region (−1787 to +70) was inserted upstream of the promoterless luciferase gene in the pGL3 basic vector. When transfected into confluent NIH3T3 cells, this construct produced a 20- to 30-fold increased luciferase expression over that observed with the pGL3 basic vector. The PLC-δ1 promoter had high basal activity in TCMK-1 and NIH3T3 cells. Activity was slightly less in Nor10 cells,

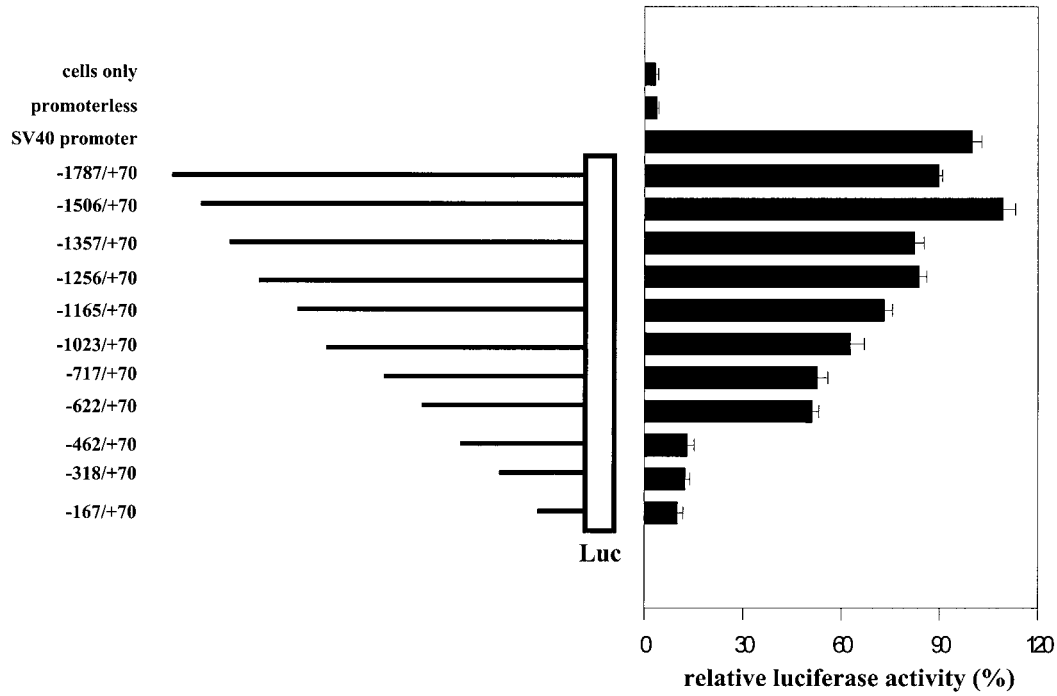

FIG. 5. Deletion analysis of PLC-δ1 gene promoter. The reporter plasmid containing the luciferase gene under the control of various sizes of PLC-δ1 promoter was transfected into NIH3T3 cells. The promoter regions used for the assay are shown on the left. The activity of the SV40 promoter was designated as 100, and the relative activity of PLC-δ1 promoter was determined. Each activity was the average of at least three independent experiments and was also normalized by the activity of cotransfected pRL-SV40.

and lower again in TM3; however, the profile of PLC-δ1 promoter activity within a deletion series of promoter constructs was similar between all cell lines (data not shown). Our results suggest that the major tissue- or cell-specific transcriptional activators of PLC-δ1 gene expression were either not present in the cell lines used, or could not transactivate the promoter fragments used in this analysis. It is not unusual for tissue-specific regulatory sequences to reside at a long distance from transcription start sites. Using different deletion mutants, we mapped the minimal essential region located between −622 and +70, and a potential regulatory element appears to be located in the sequences from −1787 to −622. This appears to indicate that this region contains *cis*-acting regulatory elements responsible for the PLC-δ1 gene expression and that a number of different DNA binding factors are involved in the basal PLC-δ1 expression. Several putative binding sites were identified up-

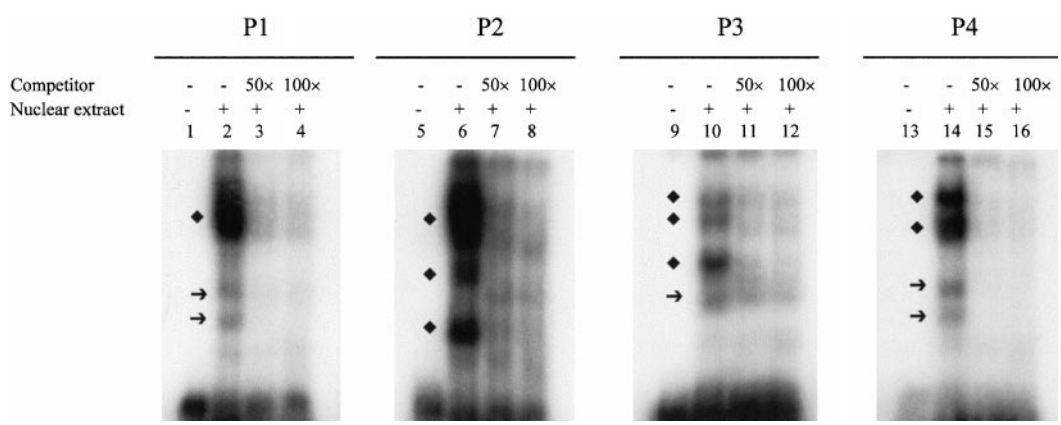

FIG. 6. Gel retardation assay of nuclear protein factors for the *cis* element of the PLC-δ1 promoter. Gel retardation assays were performed using P1 (−126/−112), P2 (−189/−160), P3 (−498/−478), and P4 (−65/−41) probes and nuclear extracts from NIH3T3. Major DNA-protein complexes are indicated by ◆, and minor DNA-protein complexes are indicated by →. Lanes 1, 5, 9, and 13 contained control samples incubated without nuclear extracts.

stream of the transcription start sites for transcription factors AP2, AP3, SP1, Ets-1, Malt box, NF-IL6, Myb, and SBE. Gel retardation assays detected several DNA-protein complexes in promoter region, suggesting that other, undetermined factors are also present. The functional importance of each of these transcription factors with respect to modulation of PLC-δ1 gene expression will need to be addressed. Although the essential promoter region is necessary for basal transcription, other regulatory elements may also be used to induce transcription under different physiological conditions. The studies described here provide a starting point for the examination of the regulation of the mouse PLC-δ1 gene in more detail. Further work is required to define factors and elements necessary for PLC-δ1 gene transcription regulation.

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REFERENCES

1. Rhee, S. G., and Bae, Y. S. (1997) *J. Biol. Chem.* **272,** 15045–15048.
2. Singer, W. D., Brown, H. A., and Sternweis, P. C. (1997) *Annu. Rev. Biochem.* **66,** 475–509.
3. Katan, M. (1998) *Biochim. Biophys. Acta* **1436,** 5–17.
4. Cockcroft, S., and Thomas, G. M. H. (1992) *Biochem. J.* **288,** 1–14.
5. Berridge, M. J. (1993) *Nature* **361,** 315–325.
6. Noh, D. Y., Shin, S. h., and Rhee, S. G. (1995) *Biochim. Biophys. Acta* **1242,** 99–114.
7. Park, D., John, D. Y., Lee, C. W., Ryu, S., and Rhee, S. G. (1993) *J. Biol. Chem.* **268,** 3710–3714.
8. Wu, D., Jiang, H., Katz, A., and Simon, M. I. (1993) *J. Biol. Chem.* **268,** 3704–3709.
9. Wahl, M. I., Jones, G. A., Nishibe, S., Rhee, S. G., and Carpenter, G. (1992) *J. Biol. Chem.* **267,** 10447–10456.
10. Goldschumidt-Clermont, P. J., Kim, J. W., Machesky, S. G., Rhee, S. G., and Pollard, T. D. (1991) *Science* **251,** 1231–1233.
11. Lemmon, M. A., Ferguson, K. A., Sigler, P. B., and Schlessinger, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10472–10476.
12. Ferguson, K. A., Lemmon, M. A., Schlessinger, J., and Sigler, P. B. (1995) *Cell* **83,** 1037–1046.
13. Cifuentes, M. E., Honkanen, L., and Rebecchi, M. J. (1993) *J. Biol. Chem.* **268,** 11586–11593.
14. Lemmon, M. A., Ferguson, K. A., and Schlessinger, J. (1996) *Cell* **85,** 621–624.
15. Im, M.-J., Russell, M. A., and Feng, J.-F. (1997) *Cell. Signal.* **9,** 477–482.
16. Hwang, G.-C., Gray, C. D., Sivasubramanian, N., and Im, M.-J. (1995) *J. Biol. Chem.* **270,** 27058–27062.
17. Park, E. S., Won, J. H., Han, K. J., Suh, P. G., Ryu, S. H., Lee, H. S., Yun, H. Y., Kwon, N. S., and Baek, K. J. (1998) *Biochem. J.* **331,** 283–289.
18. Lee, W. K., Kim, J. K., Seo, M.-S., Cha, J.-H., Lee, K. J., Rha, H. K., Min, D. S., Jo, Y.-H., and Lee, K.-H. (1999) *Biochem. Biophys. Res. Commun.* **261,** 393–399.
19. Faisst, S., and Mayer, S. (1992) *Nucleic Acids Res.* **20,** 3–26.
20. Kadonaga, J. T., Jones, K. A., and Tji, R. (1986) *Trends Biochem.* **11,** 20–23.
21. Biedenkapp, H., Borgmeyer, U., Sippel, A. E., and Klempnauer, K.-H. (1988) *Nature* **335,** 835–837.
22. Richet, E., and Raibaud, O. (1989) *EMBO J.* **8,** 981–987.
23. Zhang, Z., Fuentes, N. L., and Fuller, G. M. (1995) *J. Biol. Chem.* **270,** 24287–24291.
24. Smale, S. T. (1998) *Biochim. Biophys. Acta* **1351,** 73–88.