Vol. 212, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 6, 1995 Pages 190-195

STRUCTURAL ORGANIZATION AND EXPRESSION OF THE HUMAN PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C β-3 GENE [1]

K. Mazuruk, T.J. Schoen, G.J. Chader, and I.R. Rodriguez*

Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892

Received May 9, 1995

SUMMARY: We have cloned and fully sequenced the phospholipase C β-3 (PLC β-3) gene. The gene spans approx. 17 kb and consists of 31 exons and 30 introns. All intron-exon junctions obey the GT/AG rule. The gene is highly expressed in several human tissues including retina, brain and kidney; PLC β-3 mRNA is detected at a much lower level in liver. Because of its importance in signal transduction, its chromosomal localization and its high expression in CNS and other tissues, the PLC β-3 gene is a candidate in several human genetic diseases which, with the present genomic sequence, can now be fully examined.

Phosphatidylinositol-specific phospholipase Cs (PI-PLCs) are a family of phosphodiesterases that cleave phosphatidylinositol-4,5 *bis*phosphate into two potent second messengers, 1,2- diacylglycerol and inositol 1,4,5-*tris*phosphate (1). In particular, PLCs of the β-class seem to play important roles in signal transduction in many cell types. In the neural retina, for example, several different form of the enzyme have been identified including a PLC specific to cone photoreceptor neurons (2,3). Importantly, in *Drosophila*, mutant alleles of the norpA gene, a PLC of the β-class, result in degeneration of retinal photoreceptor cells (4).

Recently, the complete cDNA sequence for the human PI-PLC β-3 has become available as well as the chromosomal sublocalization (5, 6, 7). Specifically, the gene

[1] The sequence data have been deposited in GenBank under Accession # U26425.

*To whom correspondence should be addressed at National Eye Institute , National Institutes of Health, Bldg.6, Rm. 304, Bethesda, MD 20892. Fax: 301-402-0750. E-mail: ignacio@helix.nih.gov.

0006-291X/95 $12.00

localizes to a region within 500 Kb telomeric of the PYGM marker on 11q13, an area containing genes thought to be involved in both a hereditary cancer and neurodegenerative diseases. PLC β-3 thus is an important candidate gene for these conditions. In order to adequately perform analyses for other than gross mutations of this gene, however, substantial knowledge of the genomic sequence is necessary. For this purpose, we now report the complete PLC β-3 gene sequence, the intron/exon boundaries and the genomic organization as well as its general tissue expression in the human.

METHODS

Northern blotting. Total RNA (20 μg) from various human tissues was run on a 1% agarose-formaldehyde gel at 60 volts for 3 hours. The gels were blotted, transferred, hybridized and washed using standard techniques (8) . Molecular weight estimation of the hybridizing bands was obtained by comparison with the migration of RNA molecular weight standards (Life Technologies, Gaithersburg, MD).

Polymerase chain reaction (PCR). A 2X PCR mix was prepared containing 1.6 μmoles/ml of GeneAmp dNTPs (400 μM each), 2X GeneAmp PCR buffer and 50 U/ml Taq polymerase. These reagents were purchased from Perkin-Elmer (Norwalk, CT). In general, the template and oligonucleotides (200 ng of each oligo) were mixed in 25 μl volume and 25 μl of the 2X mix were then added followed by 50 μl of mineral oil. The template was initially denatured for 2 min at 95°C followed by a 30 cycle program with 1 min at 95°C, 30 sec annealing (temperature between 55 and 65°C depending on the primers) and an extension at 72°C for 1-5 min depending on the length of the product amplified.

cDNA synthesis on Dynabeads oligo (dT)25. cDNA was synthesized on Dynabeads as previously described (9).

5' Rapid Amplification of cDNA Ends (RACE). The 5'-RACE was performed using a modified method based on the 5'-AmpliFINDER RACE kit purchased from Clontech (Palo Alto, CA) as previously described (9).

Oligonucleotides. Oligonucleotide primers were synthesized in an Applied Biosystems Inc. (Foster City, CA) DNA synthesizer model 392. The oligonucleotides were deprotected and used without further purification.

Plasmid purification. The plasmid, cosmids and P-1 DNA were purified using the Plasmid Midi Kit purchased from Qiagen Inc. (Chatsworth, CA) following the manufacturer's protocol.

Purification of PCR products. The PCR products were purified before sequencing using the Wizard PCR Preps DNA purification system (Promega, Madison, WI) following the manufacturer's protocol.

Cloning of PCR products. PCR products were cloned using the Promega pGEM -T vector kit following the manufacturer's protocol. The transformation and plating was performed as described by the manufacturer. Selection of insert-containing clones was performed by PCR using vector-specific primers (T7 and SP6).

Automated fluorescent DNA sequencing. Fluorescent sequencing was performed using PRISM ready reaction DyeDeoxy terminator cycle sequencing kits and a Perkin-Elmer/Applied Biosystems model 370A instrument and software. In general, 0.5 pmoles of template and 3 pmoles of primer were used per sequencing reaction. The reactions were purified using Select-D G-50 columns purchased from 5Prime-3Prime (Boulder, CO).

RESULTS

Expression of PLC β-3. we performed a northern blot using a PCR probe generated from the PLC β-3 sequence previously reported (5) on RNA samples from several human tissues (Fig.1). A single transcript of approximately 4.4 Kb in length was observed in all tissues examined. The PLC β-3 is highly expressed in kidney, brain and retina as well as in most other tissues with the interesting exception of liver. Although no attempts to quantitate the messege levels were made, it should be noted that the retina with lowest RNA load in this particular experiment, shows a significant level of PLC β-3 expression.

Cloning of the human phospholipase C beta-3 gene. To clone the PLC β-3 gene we first identified a primer pair that would unequivocally amplify the PLC β-3 gene from human genomic DNA. We performed several PCR amplifications using oligonucleotides to different portions of the reported cDNA (5). A single specific product of 285 bp in length crossing a small intron was obtained using oligonucleotides 3565 (GGGCAAGCCATACCTGACGC) and 3566 (CCATTCTCCTCGCCTCCCAG). We used these oligos to isolate two overlapping cosmids (pPLC2 and pPLC6) and three P-1 clones (2098, 2099, 2100). The P-1 clones were isolated by Genome Systems Inc.,(St. Louis, MO) using primers 3565/3566.

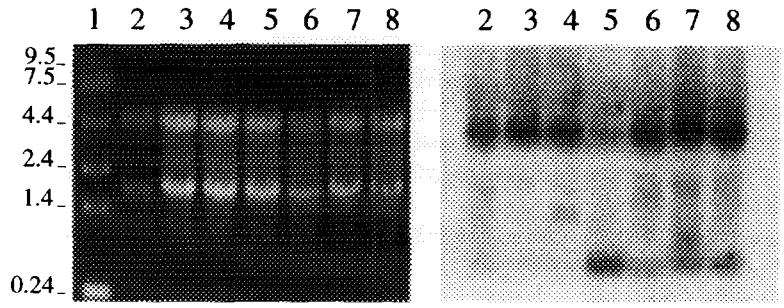

Figure 1. Multitissue northern blot showing the expression of the phospholipase C β-3 message in different human tissues. The left picture shows the ethidium bromide stained samples and the right picture shows the autoradiograph after probing with a PLC β-3 PCR product. Similar results were obtained using a different PCR probe. Lane 1 contains the RNA size standards and the sizes are listed in kb. Lanes 2-8 contain human RNA samples as follows: lane 2 adult retina; lane 3 adult heart; lane 4 adult skeletal muscle; lane 5 adult liver; lane 6 placenta; lane 7 adult kidney and lane 8 adult brain.

5' RACE. To complete the sequence of the gene, we performed solid-phase 5'RACE as described () to obtain the 5' portion of the cDNA (data not shown). Products obtained from the 5'RACE amplification were cloned and sequenced.

Sequence of PLC β-3 gene. Using primers initially obtained from the cDNA sequence (5) and later from the 5'RACE products, we amplified portions of the PLC β-3 gene using the P-1 clone 2098 as template. The PCR products were purified and sequenced directly or after subcloning. All exons and intron/exon junctions were sequenced multiple time in both directions. Portions of intron 1, exon 1 and the 5' end of the gene were subcloned directly from p2098 into the BamHI (pBB 3.5 kb), HindIII (pHH17 311 bp) and BamHI/HindIII (pBH3 2.0 Kb, pBH2 1.1 Kb) sites of pBluescript SK (Fig. 2). The gene contains 31 exons and 30 introns (Table 1) and spans approximately 17 Kb in length. All of the intron exon junctions obey the GT/AG rule. A scale map of the genomic organization is shown in Fig. 2.

DISCUSSION

The PLC β-3 gene, although containing 31 exons, is a relatively compact 17 kb with the largest intron less than 3 kb in size. Studies analyzing the 5' flanking region and examination of promoter elements important in expression are currently underway in our laboratory. The chromosomal location of the PLC β-3 gene and the general biological function of the enzyme in signal transduction mechanisms make it a promising candidate for several human genetic disorders (6, 7) including at least two with severe ocular

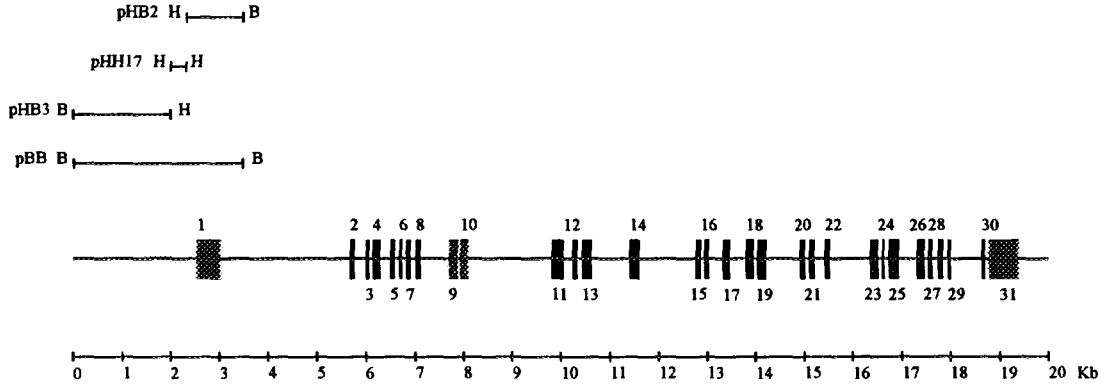

Figure 2. Structural organization of the human phospholipase C β-3 gene. The exons are shown in solid boxes and numbered. The clones containing exon 1 and the 5' flanking region are shown in bars at their relative positions.

Table 1. Intron-exon juctions of human phospholipase C β-3 gene

Exon No.	Size (bp)	Exon	Intron		Intron	Exon	Exon No.
					5' flanking	AGGGGGAGC	1
1	316	TGGGACGAG	gtaagcgcg...	2679 bp	...tgtcctcag	GAGACCTCC	2
2	78	CCCAACATG	gtgagggtg...	255 bp	...tccccccag	GAGGTGGAC	3
3	69	CTGCCCAAG	gtgagtgat...	81 bp	...cctactcag	GACCCCAAG	4
4	141	ACAGCCAAG	gtgggctgg...	195 bp	...gccccccag	GTCTGGTCT	5
5	80	GCGCAAAGC	gtgagcccc...	82 bp	...ccttcccag	ATACACGGA	6
6	54	CGTCAAGAA	gtgagcacc...	91 bp	...ccgcatcag	CATCCTGAA	7
7	76	TTCAACCGG	gtgtgtggg...	106 bp	...gacctccag	AGTGAGTCC	8
8	101	GCTGGAGAT	gtgagtggg...	555 bp	...cccctccag	AGGCGCCAA	9
9	167	TGGAGCGAG	gtgagctgg...	75 bp	...ccaccccag	ACCAGATGT	10
10	147	ATCTCACTG	gtgagtggg...	1730 bp	...ccgccccag	CGGGGCAGC	11
11	241	TGTGGACTC	gtgagtgag...	171 bp	...gttccgcag	GGCAAAGCA	12
12	85	AAGTACCCG	gtacgggag...	88 bp	...cctgcccag	CTGGCCCCA	13
13	187	CGCAGCTGG	gtaggcccc...	787 bp	...cctctgcag	GGTCTCCCA	14
14	206	ACAGATGAG	gtcaggccc...	1163 bp	...ggcccacag	GGCACAGCC	15
15	97	CTGCTCGAA	gtgagtggg...	84 bp	...accctaag	AGAGGAACA	16
16	85	GTTTGTGGA	gtatccttt...	286 bp	...caatctcag	ATACAACAA	17
17	125	AGACCCTCG	gtgagccct...	330 bp	...gcaccccag	ATGTGGCGA	18
18	155	CGGGTCAAG	gtggggctt...	85 bp	...caccccag	GTGATCTCA	19
19	162	TTCCCCAAG	gtgagcctg...	689 bp	...ggcccacag	GTGGTGCTG	20
20	100	TCCGCTCCG	gtgaggcct...	93 bp	...accacccag	GATACCACT	21
21	105	ACCACCAGG	gtgagctgg...	225 bp	...tccccccag	ACTATGCGG	22
22	92	GAGAGTGAG	gtgagccgg...	842 bp	...tccctatag	GCTCAGGCT	23
23	154	GCAGCCCAG	gtaaggagt...	87 bp	...gctccccag	GGCAGCGTG	24
24	36	TCCTCTCAG	gtagggggc...	82 bp	...cgctcccag	AGGTGGCCC	25
25	193	AGGTGCCCT	gtgagtgtc...	384 bp	...tcctcgcag	AGGTGGGGC	26
26	154	CTGAGACAG	gtaggggggc...	86 bp	...tgtccacag	GCTCTGCAG	27
27	77	GAACGAGAG	gtgaaagcc...	112 bp	...cttctccag	GGAGAAGAA	28
28	90	GAAGGAGGC	gtaagggca...	90 bp	...cccgtgcag	GGAACTGAC	29
29	58	ATCCGTCGG	gtgagtcag...	632 bp	...cgcctgtag	CTGGAGGAG	30
30	87	GAGCCCAAG	gtgaggcca...	81 bp	...ctcccacag	CTGCTGGCC	31
31	569	GATTTCAGG	gcaccctgt...	3' flanking			

complications (10, 11). A recent report has also demonstrated the loss of PLC expression in MEN1 (multiple endocrine neoplasia type 1) tumors (6); however, no mutations have yet been reported.

The high expression of this gene in the retina, its possible role in normal visual transduction, its relation to the norpA gene in *Drosophila* retinal degeneration (4) and its proximity to the loci for the human Bardet-Biedl syndrome type I (10) and Best's vitelliform dystrophy (11) make PLC β-3 of particular interest in these latter cases. Along with pigmentary retinopathy, mental retardation and obesity are major sequelae associated with the Bardet-Biedl syndrome type I, findings that correlate well with the high PLC β-3 expression we find in brain and retina. Our results should thus facilitate studies on all these hereditary conditions as well as increase our knowledge as to the gene characteristics of this important family of enzymes.

REFERENCES

1. Berridge, M.J. and Irvine R.F. (1984) Nature 312, 315-321.
2. Ferreire, P.A. and Pak, W.L. (1994) J. Biol. Chem. 269, 3129-3131.

3. Ferreira, P.A. Shortridge, R.D. and Pak, W.L. (1993) Proc. Natl. Acad. Sci USA 90, 6042-6046.
4. Meyerholen, E.P., Stein, P.J., Williams, M.A. and Ostroy, S.E. (1987) J. Comp. Physiol. 161, 793-798 .
5. Carozzi, A., Kriz, R.W., Webster, C. and Parker, P.J. (1992) Eur. J. Biochem. 210, 521-529.
6. Weber, G., Friedman,E., Grimmond, S., Hayward, N.K., Phelan,C., Skogseid, B., Gobl, A., Zedenius, J., Sandelin, K., The, B.T., Carson, E., White, I., Oberg, K., Shepherd, J., Nordenskold, M. and Larsson, C. (1994) Hum. Mol. Gen. 3, 1775-1781.
7. Sinke, R.J. and Vankessel, A.G. (1995) Genomics 25, 568-568.
8. Sambrook J., Fritsch E.F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. *Cold Spring Harbor Laboratory Press.* Cold Spring Harbor, N.Y.
9. Rodriguez, I.R., Mazuruk, K., Schoen, T.J. and Chader, G.J.(1994) J. Biol. Chem. 269, 31969-31977.
10. Leppert, M, Baird, L., Anderson, K.L., Otterud, B., Lupski, J.R. and Lewis, R.A. (1994) Nature Genetics 7, 108-112.
11. Weber, B.H.F., Walker, D., Muller, B. and Mar, L. (1994) Genomics 20, 267-274.