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Molecular cloning and characterization of PLC-η2

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PLC (phospholipase C) isoenzymes catalyse the conversion of PtdIns(4,5)P_2 into the Ca^{2+}-mobilizing second messenger, Ins(1,4,5)P_3, and the protein kinase C-activating second messenger, diacylglycerol. With the goal of identifying additional mammalian PLC isoenzymes, we screened the NCBI non-redundant database using a BLAST algorithm for novel sequences with homology with the conserved PLC catalytic core. Two unique sequences corresponding to two unknown PLC isoenzymes were identified, and one of these, designated PLC-η2, was cloned and characterized. Most of the coding sequence of PLC-η2 was constructed from two ESTs (expressed sequence tags), which included an overlapping sequence that was confirmed by multiple ESTs and mRNAs. 5′-RACE (rapid amplification of cDNA ends) also identified an upstream exon not deduced from available EST or mRNA sequences. Sequence analysis of PLC-η2 revealed the canonical domains of a PLC isoenzyme with an additional long C-terminus that contains a class II PDZ-binding motif. Genomic analyses indicated that PLC-η2 is encoded by 23 exons. RT-PCR (reverse transcriptase-PCR) analyses illustrated expression of PLC-η2 in human retina and kidney, as well as in mouse brain, eye and lung. RT-PCR with exon-specific primers also revealed tissue-specific expression of four splice variants in mouse that represent alternative use of sequences in exons 21, 22 and 23. PLC-η2-specific antisera recognized one of these splice variants as an approx. 155 kDa species when expressed in COS-7 cells; PLC-η2 natively expressed in 1321N1 human astrocytoma cells also migrated as an approx. 155 kDa species. PLC activity was observed *in vitro* and *in vivo* for three different constructs of PLC-η2, each containing possible alternatively spliced first exons. Co-expression of PLC-η2 with G$\beta_1\gamma_2$ dimers of heterotrimeric G-proteins resulted in marked stimulation of inositol lipid hydrolysis. Thus PLC-η2 may in part function downstream of G-protein-coupled receptors.

Key words: G-protein, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], phospholipase C-η2, phospholipase C-eta2, G$\beta\gamma$.

INTRODUCTION

PLC (phospholipase C) catalyses the conversion of PtdIns(4,5)P_2 into the Ca^{2+}-mobilizing second messenger, Ins(1,4,5)P_3, and the protein kinase C-activating second messenger, diacylglycerol [1,2]. Inositol lipids also specifically bind to conserved PH (pleckstrin homology), FYVE, PX (Phox homology) and other domains [3,4], and therein play additionally important roles, directly regulating membrane association and/or activity of a broad range of proteins. By altering the ratio of membrane phosphoinositides, PLC isoenzymes potentially affect membrane association and activity of many signalling proteins. Whereas the original conception of inositol lipid signalling focused on receptor-promoted regulation of PLC at the plasma membrane, inositol lipids regulate protein function at many subcellular locations [5–7], and the presence of PLC isoenzymes in most cellular compartments suggests diverse activities of these signalling proteins.

Given the broad physiological roles played by phosphoinositides, it is not surprising that a large family of PLC isoenzymes exist [8]. All five classes of PLC (PLC-β, -γ, -δ, -ζ and -ε) contain canonical PH, EF (elongation factor)-hand, catalytic TIM (triose phosphate isomerase) barrel and C2 domains, with the exception of sperm-specific PLC-ζ [9], which lacks a PH domain. PLC-δ isoenzymes are broadly distributed, but their mechanism of regulation remains elusive [8]. PLC-γ isoenzymes contain SH2 and SH3 (Src homology 2 and 3) domains, and are regulated by tyrosine phosphorylation by tyrosine kinase receptors and non-receptor tyrosine kinases [8,10–13]. PLC-β isoenzymes are elaborated with a helical C-terminus [14], and are activated directly by Gα subunits of the G_q family of heterotrimeric G-proteins [15–17]. G$\beta\gamma$ dimers released from heterotrimeric G-proteins of the G_i family also activate PLC-β2 and PLC-β3 [18–20]. PLC-ε is a recently identified isoenzyme containing a CDC25 Ras-GEF (guanine nucleotide exchange factor) domain at its N-terminus and two tandem RA (Ras-associating) domains at its C-terminus. Rho and Ras family GTPases activate PLC-ε by two distinct mechanisms [21–25].

We report here the identification of two members of a new class of PLC enzyme and describe the molecular cloning and characterization of one of these proteins, PLC-η2. This enzyme is activated by G$\beta\gamma$ subunits of heterotrimeric G-proteins.

EXPERIMENTAL

Materials

Inositol-free medium was purchased from ICN Biochemicals (Costa Mesa, CA, U.S.A.). ^{3}H-Labelled phosphoinositide substrates were prepared from [^{3}H]inositol-labelled turkey erythrocytes as described previously [15]. The 1 kb DNA ladder was purchased from Promega (Madison, WI, U.S.A.).

The SuperScript™ First-Strand Synthesis System for RT-PCR (reverse transcriptase-PCR) was obtained from Invitrogen (Carlsbad, CA, U.S.A.). The Marathon-Ready™ cDNA kit was obtained from ClonTech BD Biosciences (Mountain View, CA, U.S.A.), which includes Marathon-Ready human retina cDNA (0.1 ng/μl in Tricine/EDTA buffer).

Abbreviations used: AS, antisense; DMEM, Dulbecco's modified Eagle's medium; EF, elongation factor; EST, expressed sequence tag; ORF, open reading frame; PH, pleckstrin homology; PLC, phospholipase C; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase-PCR; S, sense; SH, Src homology; TIM, triose phosphate isomerase; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; UTR, untranslated region.

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The gene-specific primers and adaptor primers used for cloning of human PLC-η2 were as follows: CCATCCTAATACGACTCA-CTATAGGGC (adaptor primer, P1), ACTCACTATAGGGCT-CGAGCGGC (adaptor primer, P2), GGACATCATCTTGTA-GAAGGCACAGA (P3), CGTGGAGTCTGGGGAGGATGCCG (P4) and GCCTCTTCCATCCCTTCTAGGTACA (P5). The gene-specific primers used for RT-PCR of the C-terminus of mouse PLC-η2 were as follows: CAAAAGCCAGAAGCCAA-GTC [21S (sense)], AGGGATTGAGACCTGCGAGT (22S), CTCGCAGGTCTCAATCCCTA [22AS (antisense)], ATCT-CAGGAAGGGTCCCAGT (23S), TCTTCCAGAAGTCCCCT-CAG (23AS), AGGGTATCCCCTCTTGGAGA (21cS) and TCTCCAAGAGGGGATACCCT (21cAS). A specific PLC-η2 antibody was generated against the peptide sequence KGSKL-KKAASVEEGDEG within the X–Y linker region of human PLC-η2 (Quality Controlled Biochemicals, Hopkinton, MA, U.S.A.).

Identification of the novel PLC-η sequences

The basic local alignment search tool (BLAST) was applied to screen the non-redundant NCBI database (http://www.ncbi.nlm.nih.gov) for novel sequences with homology with the sequence in the conserved catalytic core of known PLC isoenzymes. Two unique sequences were identified and submitted to the BLAT search engine on the Santa Cruz genome browser (http://www.genome.ucsc.edu). The position of each of these unique sequences was identified in the human genomic sequence, and six human ESTs (expressed sequence tags), which mapped to the same genomic region as one of these novel genes (designated PLC-η2), were obtained from the A.T.C.C. (Manassas, CA, U.S.A.) and sequenced. The protein sequence corresponding to the ORF (open reading frame) represented by one EST, BU178908, was submitted to the SMART website (http://www.smart.embl-heidelberg.de) for protein domain prediction.

5′-RACE (rapid amplification of cDNA ends)-PCR

A gene-specific primer (P3 or P5) and P1 adaptor primer were used to amplify the 5′-end of human PLC-η2 using human retina cDNA (Marathon RACE kit; Clontech BD Biosciences) as a template. Gene-specific primers (P4 and P5) were used to amplify a 1 kb product as an internal positive control for the presence of PLC-η2 transcript. PCR products from RACE reactions were gel-extracted, cloned into pCR2.1-TOPO vector (Invitrogen) and transformed into TOP10-competent cells (Invitrogen). Plasmids were isolated from bacterial cultures with QIAprep Spin Miniprep kit (Qiagen) and sequenced at the University of North Carolina DNA sequencing facility.

Construction of human PLC-η2 cDNA

A chimaera of EST1 and EST2 was constructed by ligating the two sequences at an internal BamHI site present in both ESTs. This chimaeric sequence is referred to as construct 1′-23, and contains exon 1′ and exons 2–23. A second human PLC-η2 cDNA sequence was then constructed by joining the 5′-RACE PCR product with the EST1/EST2 chimaera at the unique BglII restriction site (see Figure 1). This sequence is referred to as construct 1–23, and contains exon 1 and exons 2–23. A third construct corresponding to exon 2–23 with an exogenously added ATG start codon was also constructed. All three constructs were cloned into pcDNA4, which incorporates a His_6 tag at the C-terminus.

RT-PCR

Individual mouse tissues (brain, eye and lung) were resuspended in TRIzol® reagent (Invitrogen). Following addition of chloroform, the aqueous and organic phases were separated by centrifugation at 14 000 ***g*** at 4 °C for 15 min. The aqueous portion containing RNA was combined with propan-2-ol to precipitate RNA, and the sample was centrifuged at 14 000 ***g*** at 4 °C for 10 min. The RNA pellet was then washed in 1 ml of 75 % ethanol, dried and resuspended in DEPC (diethyl pyrocarbonate)-treated distilled H_2O. The concentration of total RNA was determined by measuring its absorbance at 260 nm. Up to 5 μg of total RNA was used in a reverse transcription reaction with oligo(dT) primer and 1 μl of 10 mM dNTPs mix, which was heated to 65 °C for 5 min and chilled on ice for at least 1 min. A mixture of 10× reverse transcription buffer, 25 mM $MgCl_2$, 0.1 M dithiothreitol and RNaseOUT ribonuclease inhibitor (Invitrogen) was added to the above RNA mix and incubated at 42 °C for 2 min. An aliquot (1 μl) of reverse transcriptase (Invitrogen) was added and the reaction was allowed to proceed at 42 °C for 50 min. Reverse transcription was terminated by incubation at 70 °C for 15 min. An aliquot (1 μl) of *Escherichia coli* RNase H was used to degrade the template RNA and leave first-strand cDNA. Of this cDNA, 2 μl was then used in each PCR reaction for amplification using the gene-specific primers 21S, 22S, 22AS, 23S, 23AS, 21cS and 21cAS.

In vitro PLC assay

PLC-η2 (1–23, 1′–23 or 2–23) or PLC-β2 was transiently expressed in COS-7 cells. Cells were harvested 48 h after transfection using hypotonic buffer with protease inhibitors [leupeptin, aprotinin, TPCK (*N*-tosyl-L-phenylalanine chloromethyl ketone) and PMSF]. The soluble fraction was obtained by centrifugation at 100 000 ***g*** for 30 min at 4 °C. The total protein concentration of the soluble fraction was determined by Bio-Rad protein assay. PLC assays included 5 nmol of PtdIns(4)P or PtdIns(4,5)P_2 (Avanti Polar Lipids, Alabaster, AL, U.S.A.) and 10 000 c.p.m. [^{3}H]PtdIns(4)P (prepared from [^{3}H]inositol-labelled turkey erythrocytes, as described previously [15]) in a final buffer composition of 10 mM Hepes/NaOH (pH 7.4), 120 mM KCl, 10 mM NaCl, 2 mM EGTA, 5.8 mM $MgSO_4$, 0.5 % (w/v) cholate and 100 μM free calcium in a final volume of 50 μl. Assays were incubated at 30 °C for 10 min and were terminated by adding 200 μl of 10 % (w/v) trichloroacetic acid and 100 μl of 10 mg/ml BSA. [^{3}H]Ins(1,4)P_2 was quantified by liquid-scintillation counting of the soluble fraction after centrifugation of the reaction mixture.

In vivo PLC assay

COS-7 cells were seeded in 96-well plates at a density of 8000 cells/well and maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10 % (v/v) fetal bovine serum at 37 °C in an atmosphere of 90 % air/10 % CO_2. PLC-β1, PLC-β2, PLC-ε, or PLC-η2 (2–23) plasmid DNA was transfected into COS-7 cells in the absence or presence of expression vectors for Gα subunits, G$\beta\gamma$ subunits or small GTPases using Fugene 6 (Roche) transfection reagent according to the manufacturer's protocol. The medium was changed to inositol-free DMEM containing 1 μCi of [^{3}H]inositol/well 24 h after transfection. After an additional 12 h incubation, [^{3}H]inositol phosphate accumulation was initiated by addition of LiCl to a final concentration of 10 mM. The reaction was stopped after 60 min by aspiration of the medium and the addition of 50 mM ice-cold formic acid. [^{3}H]Inositol phosphates were quantified as described previously [26].

Western blots

COS-7 cell lysates co-expressing PLC-η2 and G$\beta_1\gamma_2$ were collected in 20 mM Hepes/NaOH buffer, pH 7.4, containing protease inhibitors (TPCK, PMSF, aprotinin and leupeptin). The soluble

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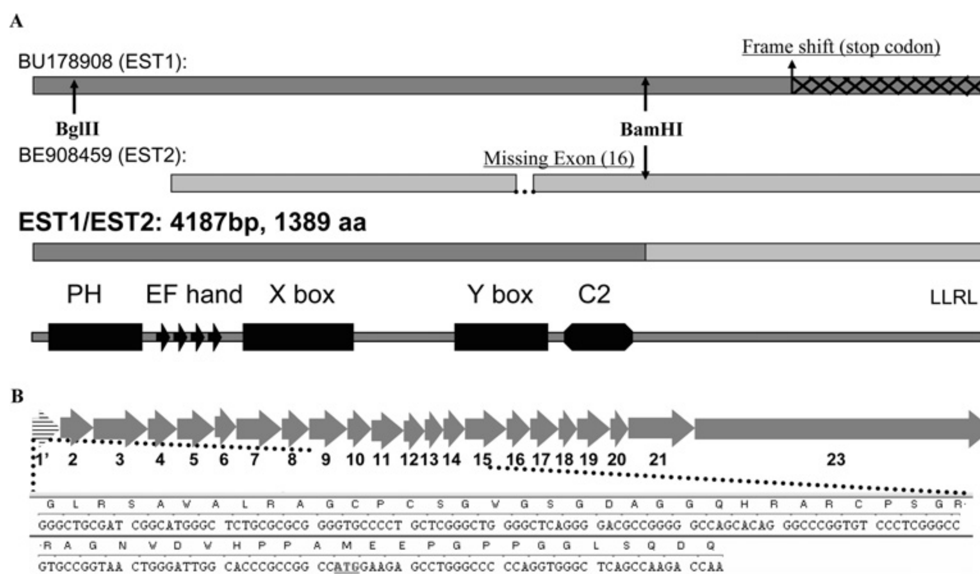

Figure 1 Identification of a new PLC isoenzyme, PLC-η2

(**A**) EST chimaera construction and predicted canonical domains of PLC-η2. A BLAST search of the non-redundant NCBI DNA database was conducted, and two novel sequences were identified with homology with sequences in the conserved catalytic core of known PLC isoenzymes. One of these genes was mapped to human chromosome 1p36.32, and two ESTs (BU178908 and BE908459) encompassed most of the coding sequence of this gene. Because both ESTs contain a sequence error, they were combined at a common BamHI site to yield a chimaeric cDNA of 4189 bp, representing a 1389-amino-acid protein. The predicted sequence contains the canonical PH domain, four EF hands, an X and Y catalytic TIM barrel, and a C2 domain of a PLC isoenzyme. A PDZ-binding motif, LLRL, is present at the C-terminus of the chimaeric sequence. (**B**) Analysis of the first exon of the EST chimaera. Analysis of the genomic sequence revealed that PLC-η2 is composed of at least 23 exons. Inspection of the first exon of the EST chimaera revealed no in-frame stop codon upstream of the first ATG (shown emboldened and underlined), suggesting that extra sequence exists upstream of the chimaera. aa, amino acids.

fraction was obtained by centrifugation at 100 000 ***g*** for 30 min at 4 °C. The total protein concentration of the soluble fraction was determined by Bio-Rad protein assay. Equivalent samples were loaded, and immunoreactivity of PLC-η2 was detected by polyclonal anti-His antibody.

Whole cell lysates from 1321N1 human astrocytoma cells were collected in 20 mM Hepes/NaOH buffer, pH 7.4, containing protease inhibitors (TPCK, PMSF, aprotinin and leupeptin). The soluble fraction was obtained by centrifugation at 13 000 ***g*** for 20 min at 4 °C. Equivalent samples of the whole cell lysates and the soluble fraction were loaded, and immunoreactivity of PLC-η2 was detected by polyclonal PLC-η2 antibody with or without the specific peptide used for PLC-η2 antibody generation. COS-7 cell lysates expressing PLC-η2(2–23) was used as a positive control.

RESULTS

With the goal of identifying novel PLC isoenzymes, we conducted BLAST searches against the non-redundant NCBI DNA database to identify novel sequences with homology with sequences in the conserved catalytic core of known PLC isoenzymes. Two unique sequences were identified and submitted to the BLAT search engine on the Santa Cruz genome browser. The sequences mapped to the human genome at positions 1p36.32 and 3q25.31. One of these, PLC-η1 (3q25.31), was recently reported by Hwang et al. [27], and therefore is not the focus of this manuscript. Our studies focused on the gene that mapped to 1p36.32, designated PLC-η2.

Although no apparent full-length genes were indicated in the 1p36.32 subregion (bp positions 2 435 000–2 470 000), several predictive algorithms suggested the existence of ORFs. Additionally, many ESTs mapped to this subregion, and six of these ESTs were obtained and sequenced. Two of these, BU178908 (EST1) and BE908459 (EST2), contained nearly full-length sequences of PLC-η2, with two exceptions: first, a deletion of 146 bp in exon 23 in BU178908 (not observed in other ESTs) resulted in a frame-shift and consequently introduced an early stop codon; and secondly, there was a missing exon (exon 16) in BE908459 (present in all other ESTs; Figure 1A). Moreover, five other ESTs and mRNAs identified from the University of California, Santa Cruz (UCSC) genome browser also matched the PLC-η2 sequence from exon 13 to the end of the gene, strongly suggesting that the two exceptions mentioned above are artifacts. Thus sequences of BU178908 and BE908459 were combined at a unique BamHI site to form a cDNA (EST1/EST2 chimaera) encoding a 1389-amino-acid protein (Figure 1A). The protein sequence of the EST1/EST2 chimaera was submitted to the SMART website, which predicted the presence of a PH domain, four EF-hand domains [the latter two were predicted by a 3D-PSSM algorithm (three-dimensional position-specific scoring matrix)], a catalytic TIM (triose phosphate isomerase) barrel and a C2 domain. Thus the unique sequence of PLC-η2 comprises the canonical domains of a PLC isoenzyme. PLC-η2 contains a long C-terminal region, within which no obvious domain is predicted. However, a class II PDZ-binding motif, LLRL, is present at the C-terminus of PLC-η2 (Figure 1A) [28,29], which is conserved in the mouse PLC-η2 sequence, as well as in both human and mouse sequences of PLC-η1.

Further analysis of the genomic sequence revealed that PLC-η2 comprises at least 23 exons, designated here 1′ to 23 (Figure 1B). Exon 1′ is designated as such for the reason explained below in Figure 2. A more detailed illustration of the 3′-end of PLC-η2, including alternatively spliced exon 22, is shown below in Figure 4. Since most mammalian genes contain a stop codon in frame with the start methionine in the 5′-UTR (untranslated region) of the gene sequence, we analysed the first exon of this EST chimaera further. Absence of a stop codon in frame with the start methionine (Figure 1B) suggested that additional coding

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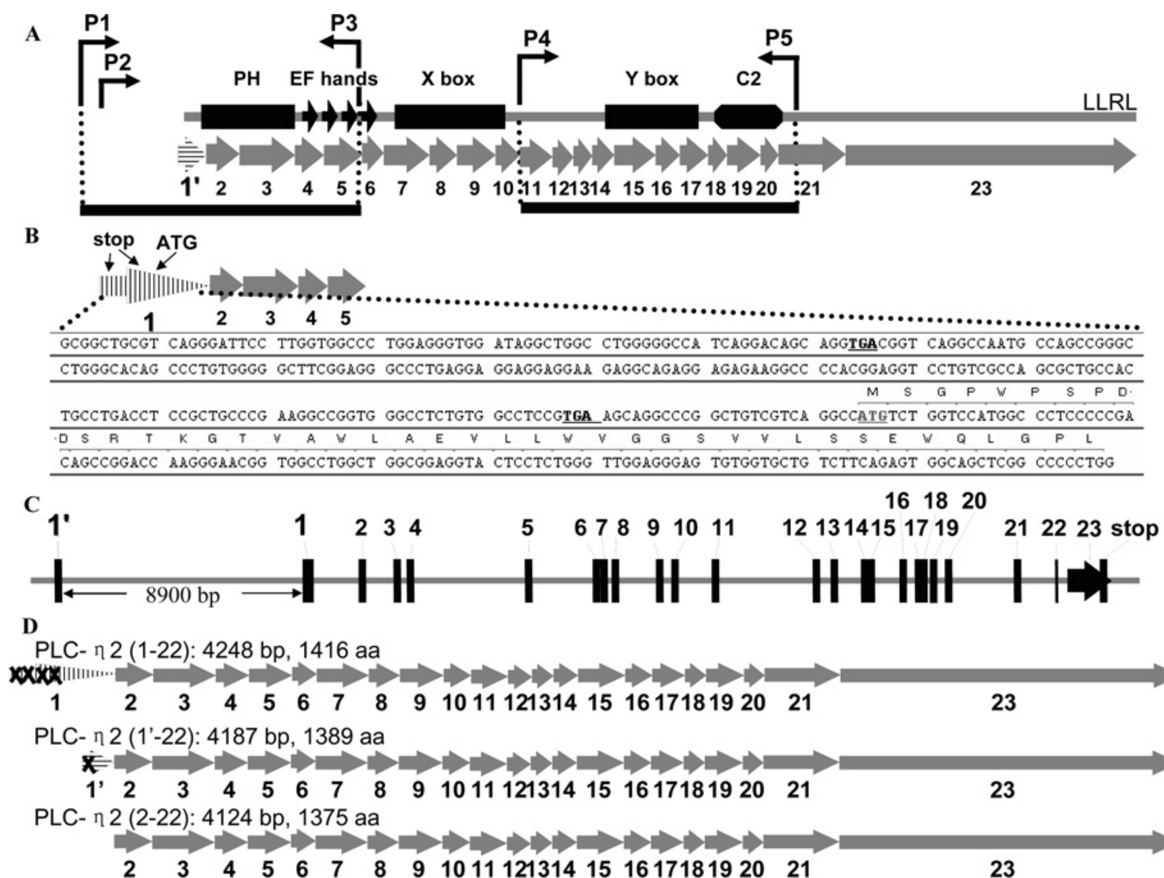

Figure 2 Identification of the first exon of human PLC-η2

(**A**) Amplification of a sequence at the 5′-end of PLC-η2. 5′-RACE PCR was used to clone the 5′-end of PLC-η2 using the P1 adaptor primer and the gene-specific primer P3. Gene-specific primers P4 and P5 were used to yield an approx. 1 kb product as an internal positive control. (**B**) Sequence of a new exon of PLC-η2. Human retina cDNA was used as a template, and a 1 kb product was generated that corresponded to a new first exon and exons 2–5. Analysis of the genomic sequence identified a start ATG and two in-frame stop codons residing in the 5′-UTR region. This newly identified exon was termed exon 1, and the first exon in the chimaera was termed exon 1′. (**C**) Genomic organization of the PLC-η2 gene. The horizontal bar represents the genomic sequence 1p36.32 (bp 2 435 000–2 470 000). The vertical bars represent exons of PLC-η2. Exon 1′ (present in the EST BU178908) lies 8900 bp 5′ to exon 1 (identified from 5′-RACE PCR) in the genomic sequence. (**D**) Three constructs of PLC-η2 using different exons at the N-terminus. Three PLC-η2 constructs are graphically represented in this Figure. PLC-η2(1–23) represents exon 1 and exons 2–23. PLC-η2(1′–23) represents exon 1′ and exons 2–23. PLC-η2(2–23) represents exons 2–23. In exon 1 and exon 1′, non-coding sequences are represented by hatched symbols. All three constructs were cloned into pcDNA4 vector with a C-terminal His_6 tag. aa, amino acids.

sequence might exist 5′ to the first exon in the chimaera. Therefore, 5′-RACE PCR was used to clone the 5′ end of PLC-η2. A gene-specific primer (P3) and a RACE adaptor primer (P1) were used to amplify the 5′-end of the gene using human retina cDNA as a template (Figure 2A). PCR resulted in amplification of an approx. 1 kb fragment corresponding to a new exon (designated exon 1) and exons 2 to 5 (Figure 2B). Further analysis of this newly identified first exon revealed two upstream stop codons in frame with a start methionine, suggesting that this ATG is the correct start site (Figure 2B). A construct corresponding to exon 1 and exons 2–23 was generated by joining the newly identified 5′-end with the chimaeric sequence at the common BglII site to form a 4248 bp cDNA encoding a 1416-amino-acid protein predicted to be 155 kDa. Analysis of the genomic sequence at 1p36.32 revealed that the first exon from the EST chimaera (designated exon 1′) lies 8900 bp upstream of the first exon identified by RACE PCR (Figure 2C). In addition, an identical 5′-sequence, encompassing exon 1 to exon 5, was cloned from a human kidney cDNA library (results not shown). Mammalian expression vectors (in pcDNA4 with a His_6 tag at the C-terminus) were generated for three different constructs (1–23, 1′–23 and 2–23) of PLC-η2. These are graphically illustrated in Figure 2(D).

The amino acid sequence of human PLC-η2(1–23) was aligned with its mouse orthologue in Figure 3. This mouse orthologue of PLC-η2 is 77.7 % identical in overall sequence with human PLC-η2, 92.4 % identical in sequence from the PH to the C2 domain, and 66.4 % identical in the C-terminal tail. The first exon (exon 1) of human PLC-η2 is well conserved in its mouse orthologue. However, since no stop codons are present upstream of the equivalent methionine in mouse PLC-η2, the coding sequence of the first exon in mouse PLC-η2 potentially begins at a methionine present 75 residues 5′ to the first methionine of human PLC-η2. Human PLC-η2 is 41.3 % identical in overall sequence with human PLC-η1, 66.9 % identical in sequence from the PH to the C2 domain, and 17.4 % in the C-terminal tail sequence (Figure 3). Therefore the PLC-η1 and PLC-η2 sequences are well conserved in their catalytic core, but PLC-η1 apparently contains additional sequence in the C-terminal region beyond the C2 domain. The PDZ-binding domain, LLRL, is conserved in both PLC-η1 and PLC-η2 from fugu (the Japanese puffer fish) to human, suggesting a potential interaction site for other signalling proteins.

The form of human PLC-η2 identified and characterized in the present study, and the mouse sequence of PLC-η2 illustrated in Figure 3, both differ from the mouse sequence recently reported

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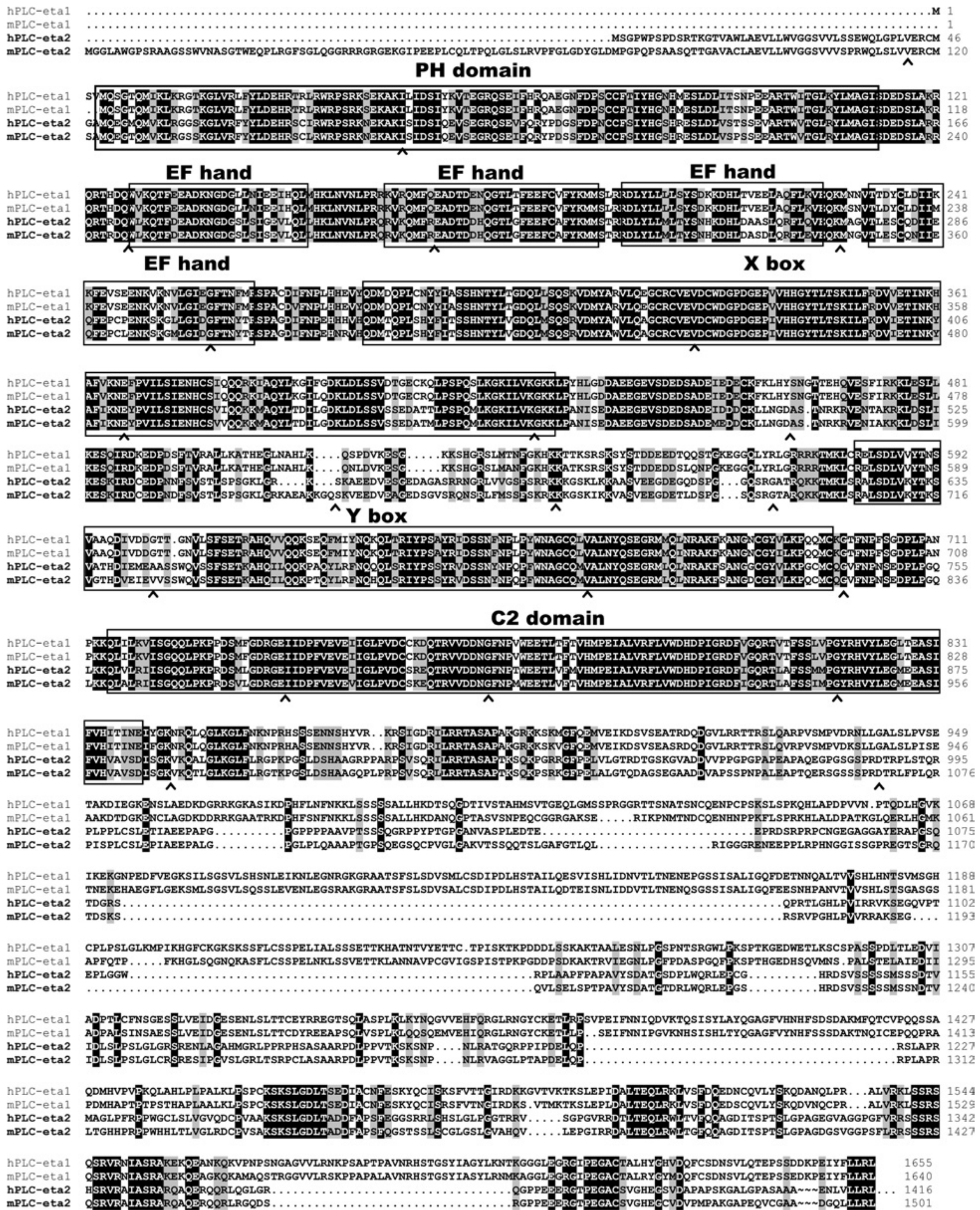

Figure 3 Sequence alignment of human and mouse PLC-η2 with human PLC-η1

Both human and mouse protein sequences of PLC-η2 (accession number: human DQ176850, mouse DQ176851) were aligned with those of PLC-η1 (accession number: NM_014996, NM_183191). Conserved residues are highlighted in black, and similar residues are highlighted in grey. Domains of PLC-η2 are boxed and labelled. Each exon border is marked with a caret (^).

by Nakahara et al. [30] within the C-terminus. This difference is due to alternative splicing, and Figure 4(A) illustrates five predicted splice variants at the C-terminus of the mouse gene. For example, 21a/23 refers to a splice form containing exon 21a and exon 23, and both human and mouse PLC-η2 sequences shown in Figure 3 are 21a/23 splice forms. Other potential splice

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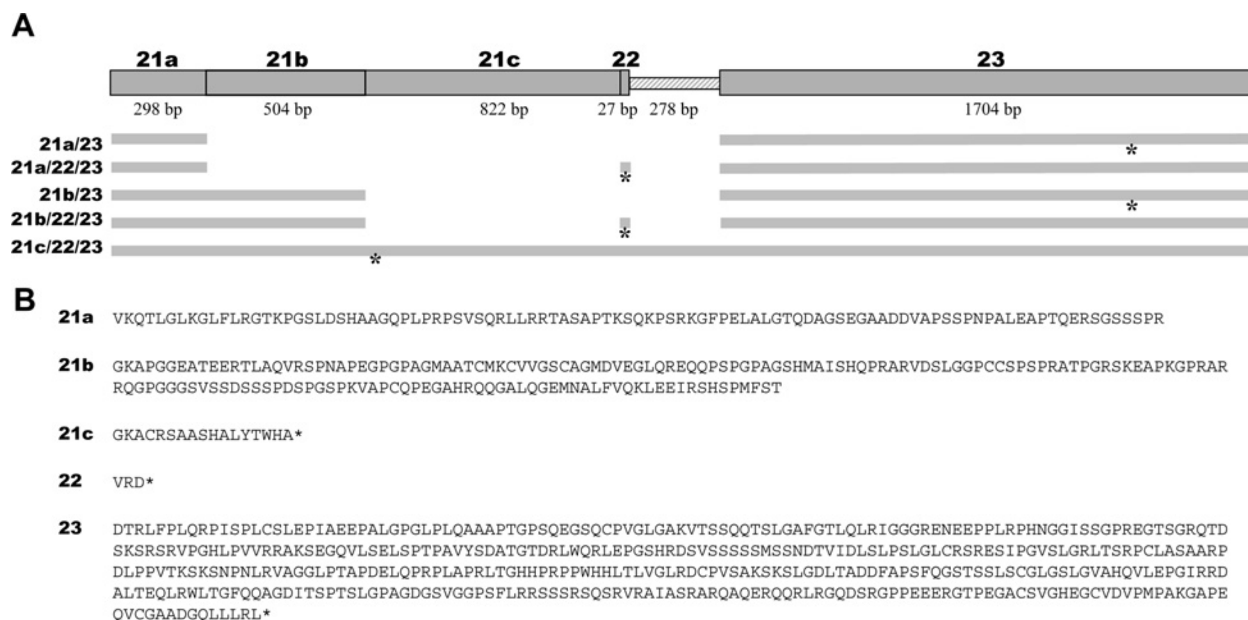

Figure 4 Diagram of splice variants of mouse PLC-η2 at the C-terminus

(**A**) Mouse PLC-η2 C-terminal exon–intron structure and predicted splice variants. Three alternative splice forms occur at exon 21 (21a, 21b or 21c). The absence or presence of exon 22 also represents a splicing event. The sizes of the cDNA sequence of each exon are indicated, and five predicted splice variants and their alternative use of exons are graphically illustrated. In-frame stop codons are marked using asterisks (*). The solid horizontal grey bars represent individual exons. The hashed horizontal bar represents the intron between exon 22 and exon 23. (**B**) The amino acid sequence encoded by each exon (exon 21 to exon 23) is shown.

Table 1 Protein sizes and mouse tissue distribution of five splice variants

aa, amino acids; ?, unknown.

	Protein (aa)		Molecular mass (kDa)		
Splice form	Human	Mouse	Human	Mouse	Tissue distribution (mouse)
21a/23	1416	1501	154.7	164.3	Eye, lung
21a/22/23	989	1070	110.2	119.2	Eye
21b/23	1583	1669	171.7	181.4	?
21b/22/23	1156	1238	127.2	136.3	Eye, brain
21c22/23	1211	1252	132.8	137.7	Eye, brain

forms include 21a/22/23, 21b/23, 21b/22/23 and 21c/22/23. Both exon 22 and exon 21c encode in-frame stop codons, which would result in smaller proteins. The amino acid sequence encoded by each exon is listed in Figure 4(B).

Differences between the form of human PLC-η2 identified and studied here and that reported from mouse by Nakahara et al. [30] prompted us to examine mouse RNA for the potential existence of multiple forms, including exon usage that would encode the sequence illustrated in Figure 3. Primers designed to each C-terminal exon were applied in RT-PCR analyses of cDNA reverse-transcribed from total RNA extracted from mouse eye, brain and lung to distinguish potential splice forms mentioned above. Table 1 summarizes protein sizes and mouse tissue distribution of five splice variants.

Only the 21a/23 splice form (no other variants) was observed in mouse lung (Figure 5). RT-PCR analyses of mouse brain cDNA revealed the presence of the 21b/22/23 splice form, as determined with gene-specific primers (i.e. 21S and 23AS; 21S and 22AS). This 21b/22/23 form was reported by Nakahara et al. [30]. The 21c/22/23 form was not observed in cDNA from mouse brain, eye or lung using the above primers, but gene-specific primers directed towards exon 21c (21S and 21cAS; 21cS and 22AS) revealed the presence of the 21c/22/23 form in mouse eye and brain. In contrast, four splice forms were observed in mouse eye. The potential splice form 21b/23 was not observed in our RT-PCR analysis. In summary, both the mouse sequence reported by Nakahara et al. [30] and the human PLC-η2 sequence studied here appear to be legitimately spliced forms of mouse PLC-η2.

Similar to mouse PLC-η2, all of the above splice forms are possible in humans. Our results indicate that the human PLC-η2 reported here is equivalent to the mouse PLC-η2 21a/23 splice form. With the goal of identifying expressed forms of native human PLC-η2, we generated PLC-η2-specific antisera against a conserved sequence (KGSKLKKAASVEEGDEG) in the catalytic core of PLC-η2 that is unique to this isoenzyme. These antisera readily detected a species of approx. 155 kDa in COS-7 cells expressing recombinant human PLC-η2(2–23) (Figure 6). This immunoreactive species was not observed in empty vector-transfected COS-7 cells (results not shown). A similar 155 kDa immunoreactive species was also observed natively expressed in 1321N1 human astrocytoma cells. A specific peptide used for PLC-η2 antibody generation blocked the immunoreactivity associated with the approx. 155 kDa species expressed in COS-7 cells transfected with an expression vector for human PLC-η2, as well as the immunoreactive species expressed natively in 1321N1 cells. These results with antisera against human PLC-η2 are consistent with expression of both a recombinant and a natively expressed 155 kDa protein corresponding to the 21a/23 splice form of PLC-η2. Exon-specific primers also indicated the presence of multiple splice forms in RT-PCR analyses of cDNA reverse-transcribed from total RNA extracted from human 1321N1 cells and SH-SY5Y human neuroblastoma cells (results not shown).

The potential activity of PLC-η2 as a functional PLC was tested *in vitro*. PLC-η2 (1–23, 1′–23 or 2–23) or PLC-β2 was transiently expressed in COS-7 cells, and the soluble fraction was isolated from cell lysates. PLC activity was quantified as described in

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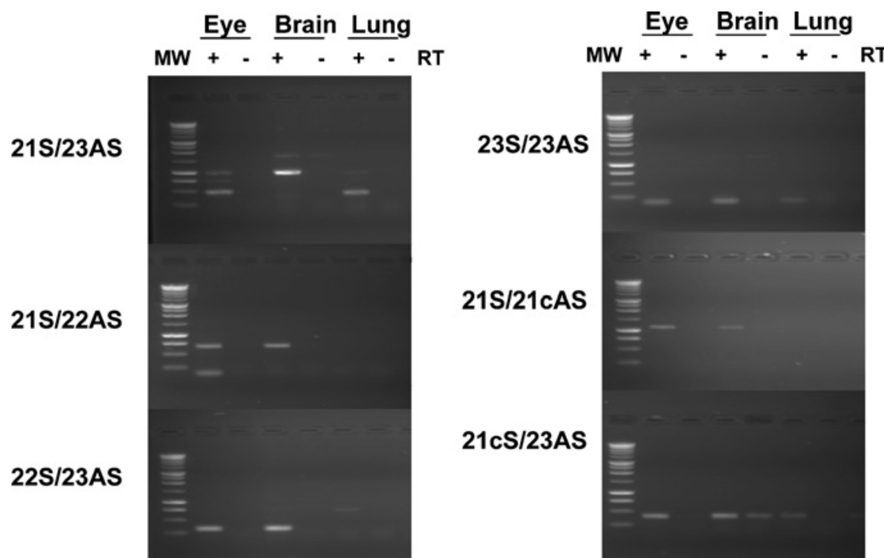

Figure 5 RT-PCR analysis of the carboxyl terminus of PLC-η2 in mouse tissues

The RT-PCR reactions were carried out as described in the Experimental section. The DNA ladder consists of 13 double-stranded, blunt-end fragments with sizes of 250/253, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000, 6000, 8000 and 10 000 bp. RT-PCR of RNA from mouse eye (lanes 2 and 3) resulted in amplification of the following specific cDNA fragments: 463 bp and 971 bp using the 21S/23AS primer pair; 179 bp and 687 bp using 21S/22AS; 332 bp using 22S/23AS; 187 bp using 23S/23AS; 945 bp using 21S/21cAS; and 411 bp using 21cS/23AS. RT-PCR of RNA from mouse brain (lanes 4 and 5) resulted in amplification of the following specific cDNA fragments: 971 bp using 21S/23AS; 687 bp using 21S/22AS; 332 bp using 22S/23AS; 187 bp using 23S/23AS; 945 bp using 21S/21cAS; and 411bp using 21cS/23AS. RT-PCR of RNA from mouse lung (lanes 6 and 7) resulted in amplification of 463 bp using 21S/23AS and 187 bp using 23S/23AS. Each of the above PCR products was sequenced and confirmed to encode C- terminal sequence of PLC-η2. The positions of molecular-mass markers (MW) is shown in the leftmost lane of either gel.

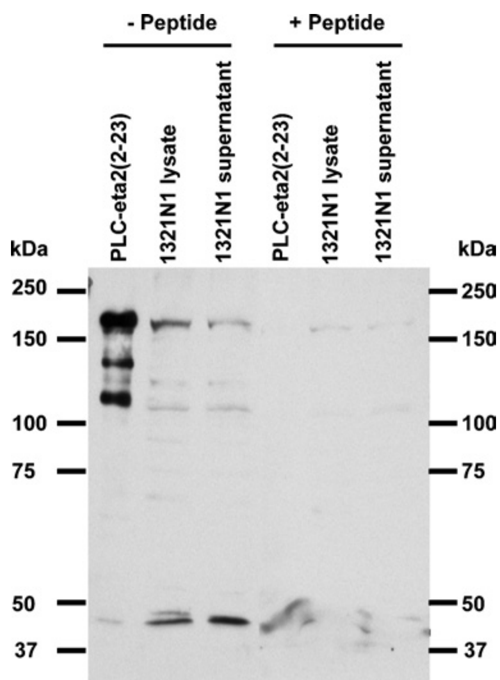

Figure 6 Expression of native human PLC-η2 in 1321N1 human astrocytoma cells

Endogenous expression of human PLC-η2 was assessed in whole cell lysates and soluble fractions prepared from 1321N1 human astrocytoma cells. Immunoblots were developed using PLC-η2 antibody in the presence or absence of a specific peptide, as described in the Experimental section. Lysate from COS-7 cells expressing PLC-η2(2–23) was used as a positive control.

the Experimental section in the presence of [^{3}H]PtdIns(4)P and 0.5 % cholate and increasing amounts of total cytosolic protein. A 5–10-fold increase in phospholipase activity was observed in homogenates of cells expressing either PLC-η2(1′–23) or PLC-η2(2–23) (Figure 7A). An approx. 2-fold increase was observed for the cytosolic fraction obtained from COS-7 cells expressing PLC-η2(1–23) (Figure 7A). Similar results were obtained using [^{3}H]PtdIns(4,5)P_2 as substrate (results not shown). Western blots revealed that similar amounts of PLC-η2(1–23), PLC-η2(1′–23), PLC-η2(2–23) and PLC-β2 were expressed under the conditions used in these experiments (results not shown). The concentration of cholate (0.5 %) used in these assays is optimal for quantification of the activity of PLC-β2, and therefore we tested PLC-η2 phospholipase activity at this cholate concentration. However, under these assay conditions, PLC-β2 exhibited enzyme activities that were approximately twice those of PLC-η2(1′–23) or PLC-η2(2–23). In contrast, higher activities of the PLC-η2 constructs relative to that of PLC-β2 were observed in the absence of detergent, or with lower concentrations of cholate (Figure 7B). Taken together, these results illustrate that PLC-η2 functions as an active PLC against phosphoinositide substrate, and suggest that the basal activities of the PLC-η2(1′–23) and PLC-η2(2–23) constructs are greater than that of PLC-η2(1–23).

To determine whether PLC-η2 is activated by G-proteins, we co-expressed PLC-η2(2–23) with GTPase-deficient mutants of various Gα subunits and Ras superfamily GTPases, and measured [^{3}H]inositol phosphate accumulation, as described in the Experimental section. PLC-β1, PLC-β2 and PLC-ε, which are known downstream effectors of certain G-proteins [15,19,21], were also co-expressed with corresponding G-proteins as positive controls. The construct PLC-η2(2–23) was used in these assays because it elicits robust lipase activity (Figure 8), and contains a sequence common to all constructs. Expression of PLC-η2(2–23) alone increased basal [^{3}H]inositol phosphate accumulation as much as 2-fold. Under the conditions tested, wild-type Gα_q activated PLC-β1, and the GTPase-deficient mutants of Gα_{12} (Q226L) and Gα_{13} (Q229L) activated PLC-ε (Figure 8A). As expected, expression of RhoA, RhoB or RhoC significantly increased [^{3}H]inositol phosphate accumulation in COS-7 cells

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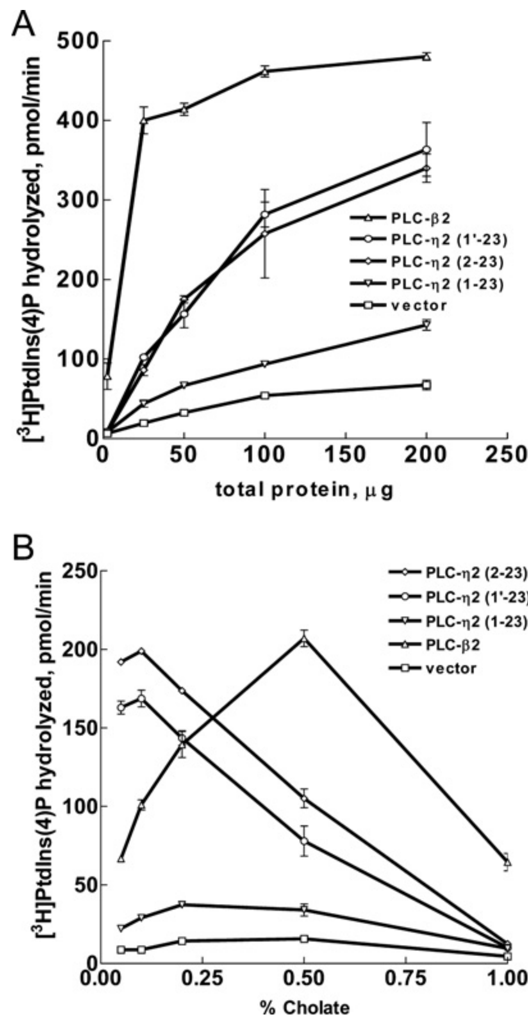

Figure 7 PLC-η2 is an active PLC enzyme

Quantification of phospholipase activity at various protein concentrations. COS-7 cells transiently expressing PLC-β2 or PLC-η2 (1–23, 1′–23 or 2–23) were lysed and the soluble fraction was obtained to test the lipase activity of each PLC directly in the presence of substrate [^{3}H]PtdIns(4)*P* and 0.5 % cholate as described in the Experimental section. The results are presented as means ± S.E.M. for triplicate determinations. (**B**) Quantification of phospholipase activity at various cholate concentrations. Samples were prepared as in (**A**), and activity was measured with 200 μg of COS-7 lysate expressing the indicated PLC enzyme and the indicated concentration of cholate.

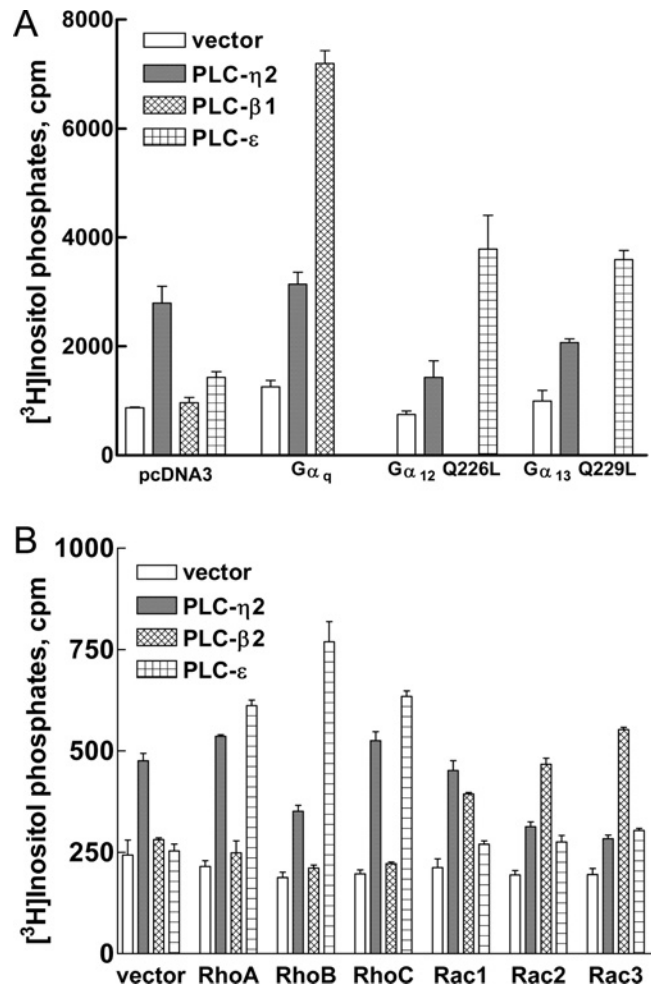

Figure 8 Screen of potential G-protein activators of PLC-η2

PLC-η2(2–23) was co-expressed with either wild-type or GTPase-deficient mutants of various Gα subunits (**A**) or Ras-superfamily GTPases (**B**), and [^{3}H]inositol phosphate accumulation was measured as described in the Experimental section. PLC-β1, PLC-β2 or PLC-ε was co-expressed with certain G-proteins as positive controls. The results are presented as means ± S.E.M. for triplicate determinations, and are representative of results from two separate experiments.

expressing PLC-ε, whereas expression of Rac1, Rac2 or Rac3 activated PLC-β2 (Figure 8B). In contrast, no activation of PLC-η2(2–23) was observed following co-expression with any of the Gα subunits (wild-type Gα_q, Gα_{11}, Gα_{14} and G$\alpha_{15/16}$, and Gln → Leu mutants of Gα_q, Gα_{11}, Gα_{14}, G$\alpha_{15/16}$, Gα_{12}, Gα_{13}, Gα_s, Gα_{olf}, Gα_{i1}, Gα_{i2}, Gα_{i3}, Gα_{oA}, Gα_{oB}, Gα_{rod} and Gα_{cone}) or Ras-superfamily GTPases (RhoA, RhoB, RhoC, Rac1, Rac2, Rac3, Cdc42, Cdc42p, TCL, TC10, RhoG, RhoD, Rnd1, Rap1A, Rap1B, Rap2A and Rap2B) tested (Figure 8, and results not shown). Thus, although activation of PLC-η2(2–23) may occur downstream of certain Gα subunits or Ras-family GTPases, no evidence for such activation was revealed under the conditions used in our assays.

The capacity of G$\beta\gamma$ to activate PLC-η2 was also examined in COS-7 cells. As illustrated in Figure 9, co-expression of increasing amounts of PLC-η2(2–23) with G$\beta_1\gamma_2$ resulted in marked elevation of [^{3}H]inositol phosphates compared with cells expressing PLC-η2(2–23) alone. The extent of activation of PLC-η2(2–23) was at least as large as that observed with PLC-ε under the same conditions. Expression of soluble PLC-η2(2–23), corresponding to a 155 kDa band on the immunoblot, did not increase as a consequence of expression of G$\beta\gamma$ (Figure 9, inset), indicating that the increased activity of PLC-η2(2–23) is a result of G$\beta\gamma$-promoted activation, rather than being due to a change in expression of PLC-η2(2–23).

DISCUSSION

The work described here illustrates the existence of a new isoform of PLC termed PLC-η2. This isoenzyme contains the conserved domains found in the catalytic core of all PLC isoenzymes, and also contains a long C-terminal region. Co-expression of PLC-η2 with G$\beta_1\gamma_2$ resulted in a robust increase in inositol lipid hydrolysis, and therefore this isoenzyme may in part function downstream of G-protein-coupled receptors.

Most of the coding sequence of PLC-η2 was constructed from two ESTs, the validity of which was confirmed in the C-terminal half of the isoenzyme by the existence of multiple mRNAs in the database exhibiting identical sequences. Our 5′-RACE analyses

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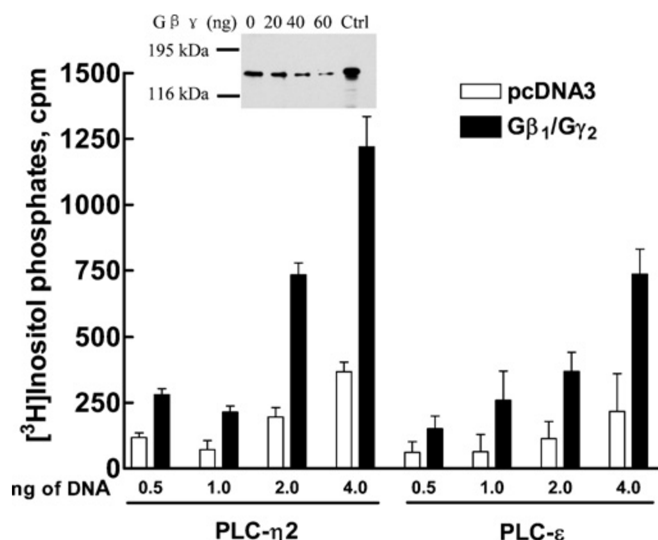

Figure 9 G$\beta\gamma$ dimer promotes activation of PLC-η2

The indicated amount of expression vector for PLC-η2(2–23) (left panel) or PLC-ε (right panel) was co-transfected with 30 ng of expression vector for Gβ_1 and 30 ng of expression vector for Gγ_2 in COS-7 cells. [^{3}H]Inositol phosphate accumulation was quantified as described in the Experimental section. Data are presented as means $\pm$ S.E.M. for triplicate determinations, and are representative of results from at least five separate experiments. Inset: the level of PLC-η2 in COS-7 cells co-expressed with the indicated amounts of Gβ_1 and Gγ_2 DNA was assessed in immunoblots using anti-His antibody.

using either human retina or kidney cDNA identified an upstream exon (exon 1) not present in available ESTs or mRNA data. Indeed, the first exon (exon 1′) present in EST1 (BU178908) exists far upstream (8900 bp) of the coding sequence we amplified by 5′-RACE. The sequence amplified from both retinal and kidney cDNA is encoded by a new exon (exon 1) with two stop codons in the 5′ UTR in-frame with a start methionine, and by exon 2 to exon 5. Thus an isoenzyme transcribed from exon 1 to exon 23 is at least one form of this novel PLC expressed in human tissues.

Although no functional data were reported, Stewart et al. [31] recently recognized a sequence for PLC-η2 in the available human DNA database. The 5′-end of the gene proposed by Stewart and co-workers potentially represents a splice variant of PLC-η2, since it differs markedly at the first exon from that confirmed in our 5′-RACE analyses of human cDNA. However, it is unclear from our analyses of genomic structure how this splice variant would be formed. Moreover, although the 5′-end of PLC-η2 encoded by exon 1 (Figure 2) was amplified from two sources of human cDNA (retina and kidney), we have failed to amplify the 5′ sequence suggested by Stewart and co-workers applying various primers constructed on the basis of the unique 5′-end of PLC-η2 reported by Stewart and co-workers (results not shown).

Alternative splicing also occurs in the C-terminus of PLC-η2. Indeed, the form of mouse PLC-η2 recently cloned and studied by Nakahara et al. [30] differs in exon usage in the C-terminal region from the human PLC-η2 we have cloned and studied. Although no apparent domains or obvious tertiary structure exists in this portion of the isoenzyme, our observation of multiple modes of exon usage and tissue-specific expression of C-terminal variants of PLC-η2 suggest important functionality in the C-terminal region of the isoenzyme. For example, mouse lung exclusively expresses the 21a/23 form, suggesting that the C-terminal region may be important for the enzyme to function or interact with other proteins in epithelial tissues. This form ends with the potential PDZ domain-binding motif LLRL at its extreme C-terminus, which ostensibly confers specific protein–protein interactions on lung PLC-η2. Mouse brain expresses both the 21b/22/23 and 21c/22/23 splice forms. The former variant has an in-frame ston codon in exon 22, which renders exon 23 an untranslated sequence. The latter form contains an in-frame stop codon in exon 21c, which renders both exons 22 and 23 untranslated. These two forms may play important roles in neuronal function. Mouse eye expresses four alternative splice forms (21a/23, 21a/22/23, 21b/22/23 and 21c/22/23), and it will be important to establish whether, for example, these exhibit different functional activities, interact with different regulatory proteins, are expressed in different development stages, are expressed in different pools of retinal cells, and/or are localized to different subcellular compartments.

PLC activity was observed with three different constructs of PLC-η2, and the activities observed both *in vitro* and *in vivo* are of the order of that quantified with PLC-β2 and other PLC isoenzymes under similar conditions. Interestingly, the PLC-η2 constructs comprising exons 2–23 or 1′–23 exhibited severalfold-higher phospholipase activity than the PLC-η2(1–23) construct. We believe that these data reflect true differences in the activity of these isoenzyme forms, since similar amounts of soluble and supposedly fully folded protein were expressed and compared among the three constructs. Thus one interpretation of these results is that the presence of exon 1 leads to a decrease in lipase activity in both *in vitro* and *in vivo* measurements of enzyme activity. No precedent exists for autoinhibition of a PLC enzyme, and potential mechanisms whereby the sequence provided by exon 1 would inhibit lipase activity are not obvious.

Sequence analyses of PLC-η2 revealed all of the canonical domains of a PLC isoenzyme with an additional long C-terminus that contains a PDZ domain-binding motif, LLRL. Members of the PLC-β family containing PDZ domain-binding motifs at their C-termini have been shown to interact with PDZ domain-containing proteins, including NHERF (the Na^+/H^+ exchanger regulatory factor) [32,33]. NHERF acts as a scaffolding protein that augments the signal from a G-protein-coupled receptor, PTH1R (parathyroid hormone 1 receptor), to PLC-β enzymes in response to parathyroid hormone [34,35]. Whether this motif in PLC-η2 has unique functionality and whether it serves as an interaction site for an upstream activator, a downstream interacting protein, a scaffold protein-binding site, or some other function needs to be determined.

Activation of PLC-η2(2–23) by Gα subunits of heterotrimeric G-proteins or by Ras-superfamily GTPases was not observed under the conditions of our assays. Although predictable activation of PLC-β and PLC-ε isoenzymes by G-proteins was observed, our results do not unequivocally rule out the possibility that PLC-η2 lies downstream of signalling pathways promoted by these or other Gα subunits or Ras GTPases. On the other hand, our experiments revealed robust activation of PLC-η2 by G$\beta\gamma$, and this isoenzyme, like PLC-β2, PLC-β3 [18–20] and PLC-ε [36], apparently functions downstream of G-protein-coupled receptors that release G$\beta\gamma$ through activation of heterotrimeric G-proteins, particularly those of the G_i family. An important current goal is to purify PLC-η2 to homogeneity and to establish whether G$\beta\gamma$ or possibly other G-proteins activate this isoenzyme by a direct mechanism.

Protein sequences in the catalytic core are well conserved between human PLC-η2 and human PLC-η1 (Figure 3). Hwang and co-workers [27] have recently reported the identification of human PLC-η1, in which the exon arrangement is similar to that of human PLC-η2 in our studies. Based on the sequence alignment illustrated in Figure 3, PLC-η1 sequence lacks the first exon, and therefore its coding sequence begins with the PH domain.

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Sequences of PLC-η1 diverge from those of PLC-η2 in the C-terminus in that PLC-η1 contains four inserted regions resulting in an additional 300 amino acids (Figure 3). BLAST analysis of the C-terminal tails of both PLC-η1 and PLC-η2 yields no homology with any other proteins in the human database. The significance of this difference needs further investigation, and whether the extra sequence in PLC-η1 renders different modes of regulation will be explored further.

We have not carried out a systematic and rigorous analysis of the presence of PLC-η2 in human tissues. However, we know from our PCR analyses that the distribution is relatively broad, since PLC-η2 RNA is expressed in both human kidney and retina, as well as in mouse brain, eye and lung. PLC-η2 is natively expressed as an approx. 155 kDa immunoreactive species in 1321N1 human astrocytoma cells. This size corresponds to the molecular size of the PLC-η2(2–23) construct utilized in our characterization of enzymatic activities of human PLC-η2.

Summary

In conclusion, a second member of the subclass of PLC isoenzymes designated as PLC-η has been identified, cloned, and shown to be activated by G$\beta\gamma$ subunits of heterotrimeric G-proteins. At least 13 different PLC isoenzymes now are known to exist in mammalian tissues, but our knowledge of the relative roles played by these signalling proteins remains rudimentary. The activities of these isoenzymes clearly are regulated in multiple subcellular compartments by multiple mechanisms. Moreover, we increasingly realize that various membrane phosphoinositides play important roles in cell signalling in addition to the historically considered production of Ins(1,4,5)P_3 and diacylglycerol from PtdIns(4,5)P_2. Thus it is imperative that we begin to unravel the functions of PLC-η2 and other PLC isoenzymes at the cellular level.

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REFERENCES

1 Michell, R. H. (1975) Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta. **415**, 81–147
2 Berridge, M. J. and Irvine, R. F. (1987) Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu. Review Biochem. **56**, 159–193
3 Lemmon, M. A. (2003) Phosphoinositide recognition domains. Traffic **4**, 201–213
4 Toker, A. (2002) Phosphoinositides and signal transduction. Cell Mol. Life Sci. **59**, 761–779
5 Irvine, R. F. (2002) Nuclear lipid signaling. Sci. STKE **150**, RE13
6 Ostrom, R. S. and Insel, P. A. (2004) The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. Br. J. Pharmacol. **143**, 235–245
7 Delmas, P., Crest, M. and Brown, D. A. (2004) Functional organization of PLC signaling microdomains in neurons. Trends Neurosci. **27**, 41–47
8 Rhee, S. G. (2001) Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. **70**, 281–312
9 Saunders, C. M., Larman, M. G., Parrington, J., Cox, L. J. and Royse, J. (2002) PLC ζ: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. Development **129**, 3533–3544
10 Katan, M., Rodriguez, R., Matsuda, M., Newbatt, Y. M. and Aherne, G. W. (2003) Structural and mechanistic aspects of phospholipase C-γ regulation. Adv. Enzyme Regul. **43**, 77–85
11 Wahl, M. I., Nishibe, S., Suh, P. G., Rhee, S. G. and Carpenter, G. (1989) Epidermal growth factor stimulates tyrosine phosphorylation of phospholipase C-II independently of receptor internalization and extracellular calcium. Proc. Natl. Acad. Sci. U.S.A. **86**, 1568–1572
12 Meisenhelder, J., Suh, P. G., Rhee, S. G. and Hunter, T. (1989) Phospholipase C-γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases *in vivo* and *in vitro*. Cell **57**, 1099–1122
13 Rhee, S. G. and Choi, K. D. (1992) Regulation of inositol phospholipid-specific phospholipase C isozyme. J. Biol. Chem. **267**, 12393–12396
14 Singer, A. U., Waldo, G. L., Harden, T. K. and Sondek, J. (2002) A unique fold of phospholipase C-β mediates dimerization and interaction with Gαq. Nat. Struct. Biol. **9**, 32–36
15 Waldo, G. L., Boyer, J. L., Morris, A. J. and Harden, T. K. (1991) Purification of an ALF4- and G-protein $\beta\gamma$-subunit-regulated phospholipase C-activating protein. J. Biol. Chem. **266**, 14217–14225
16 Smrcka, A. V., Hepler, J. R., Brown, K. O. and Sternweis, P. C. (1991) Regulation of polyphosphoinositide-specific phospholipase C by purified Gq. Science **251**, 804–807
17 Taylor, S. J., Chae, H. Z., Rhee, S. G. and Exton, J. H. (1991) Activation of the β1 isozyme of phospholipase C by α-subunits of the Gq class of G-proteins. Nature (London) **350**, 516–518
18 Camps, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J. and Gierschik, P. (1992) Isozyme-selective stimulation of phospholipase C-β2 by G protein $\beta\gamma$-subunits. Nature (London) **360**, 684–686
19 Boyer, J. L., Waldo, G. L. and Harden, T. K. (1992) $\beta\gamma$-Subunit activation of G-protein-regulated phospholipase C. J. Biol. Chem. **267**, 25451–25456
20 Blank, J. L., Brattain, K. A. and Exton, J. H. (1992) Activation of cytosolic phosphoinositide phospholipase C by G-protein $\beta\gamma$ subunits. J. Biol. Chem. **267**, 23069–23075
21 Song, C., Hu, C. D., Masago, M., Kariyai, K., Yamawaki-Kataoka, Y., Shibatohge, M., Wu, D., Satoh, T. and Kataoka, T. (2001) Regulation of a novel human phospholipase C, PLCε, through membrane targeting by Ras. J. Biol. Chem. **276**, 2752–2757
22 Lopez, I., Mak, E. C., Ding, J., Hamm, H. E. and Lomasney, J. W. (2001) A novel bifunctional phospholipase C that is regulated by Gα12 and stimulated the Ras/mitogen-activated protein kinase pathway. J. Biol. Chem. **276**, 2758–2765
23 Kelley, G. G., Reks, S. E., Ondrako, J. M. and Smrcka, A. V. (2001) Phospholipase C-ε: a novel Ras effector. EMBO J. **20**, 743–754
24 Wing, M. R., Snyder, J. T., Sondek, J. and Harden, T. K. (2003) Direct activation of phospholipase C-ε by Rho. J. Biol. Chem. **278**, 41253–41258
25 Seifert, J. P., Wing, M. R., Snyder, J. T., Gershburg, S., Sondek, J. and Harden, T. K. (2004) RhoA activates purified phospholipase C-ε by a guanine nucleotide-dependent mechanism. J. Biol. Chem. **279**, 47992–47997
26 Brown, H. A., Lazarowski, E. R., Boucher, R. C. and Harden, T. K. (1991) Evidence that UTP and ATP regulate phospholipase C through a common extracellular 5′-nucleotide receptor in human airway epithelial cells. Mol. Pharmacol. **40**, 648–665
27 Hwang, J. I., Oh, Y. S., Shin, K. J., Kim, H., Ryu, S. H. and Suh, P. G. (2005) Molecular cloning and characterization of a novel phospholipase C, PLC-η. Biochem. J. **389**, 181–186
28 Chung, J. J., Shikano, S., Hanyu, Y. and Li, M. (2002) Functional diversity of protein C-termini: more than zipcoding? Trends Cell Biol. **12**, 146–150
29 Kang, B. S., Cooper, D. R., Devedjiev, Y., Derewenda, U. and Derewenda, Z. S. (2003) Molecular roots of degenerate specificity in syntenin's PDZ2 domain: reassessment of the PDZ recognition paradigm. Structure **11**, 845–853
30 Nakahara, M., Shimozawa, M., Nakamura, Y., Irino, Y., Morita, M., Kudo, Y. and Fukami, K. (2005) A novel phospholipase C, PLCη2, is a neuron-specific isozyme. J. Biol. Chem. **280**, 29128–29134
31 Stewart, A. J., Mukherjee, J., Roberts, S. J., Lester, D. and Farquharson, C. (2005) Identification of a novel class of mammalian phosphoinositol-specific phospholipase C enzymes. Int. J. Mol. Med. **15**, 117–121
32 Tang, Y., Tang, J., Chen, Z., Trost, C. and Flockerzi, V. (2000) Association of mammalian trp4 and phospholipase C isozymes with a PDZ domain-containing protein, NHERF. J. Biol. Chem. **275**, 37559–37564
33 Hwang, J. I., Heo, K., Shin, K. J., Kim, E. and Yun, C. (2000) Regulation of phospholipase C-β3 activity by Na^+/H^+ exchanger regulatory factor 2. J. Biol. Chem. **275**, 16632–16637
34 Mahon, M. J., Donowitz, M., Yun, C. C. and Segre, G. V. (2002) Na^+/H^+ exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signaling. Nature (London) **417**, 858–861
35 Oh, Y. S., Jo, N. W., Choi, J. W., Kim, H. S. and Seo, S. W. (2004) NHERF2 specifically interacts with LPA2 receptor and defines the specificity and efficiency of receptor-mediated phospholipase C-β3 activation. Mol. Cell. Biol. **24**, 5069–5079
36 Wing, M. R., Houston, D., Kelley, G. G., Der, C. J., Siderovski, D. P. and Harden, T. K. (2001) Activation of phospholipase C-ε by heterotrimeric G protein betagamma-subunit. J. Biol. Chem. **276**, 48257–48261

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