Cloning of a Human Type II Phosphatidylinositol 4-Kinase Reveals a Novel Lipid Kinase Family*  

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Shane Minogue‡§, J. Simon Anderson‡, Mark G. Waugh‡§, Maria dos Santos‡, Steven Corless‡, Rainer Cramer‡ and J. Justin Hsuan‡§§  

From the ‡Centre for Molecular Cell Biology, Department of Medicine, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, United Kingdom, and the §Ludwig Institute for Cancer Research, Courtauld Building, 91 Riding House Street, London W1P 8BT, United Kingdom

Phosphoinositide lipids regulate numerous cellular processes in all eukaryotes. The versatility of this phospholipid is provided by combinations of phosphorylation on the 3', 4', and 5' positions of the inositol head group. Two distinct structural families of phosphoinositide (PI) kinases have so far been identified and named after their prototypic members, the PI 3-kinase and phosphatidylinositol (PtdIns) phosphate kinase families, both of which have been found to contain structural homologues possessing PI 4-kinase activity. Nevertheless, the prevalent PtdIns 4-kinase activity in many mammalian cell types is conferred by the widespread type II PtdIns 4-kinase, which has so far resisted molecular characterization. We have purified the human type II isoform from plasma membrane rafts of human A431 epidermoid carcinoma cells and obtained peptide mass and sequence data. The results allowed the cDNA containing the full open reading frame to be cloned. The predicted amino acid sequence revealed that the type II enzyme is the prototypic member of a novel, third family of PI kinases. We have named the purified protein type IIa and a second human isoform, type IIb. The type IIa mRNA appears to be expressed ubiquitously in human tissues, and homologues appear to be expressed in all eukaryotes.

Phosphoinositides have been implicated in a vast range of cellular functions, including receptor signaling, vesicle trafficking, endocytosis and cytoskeletal rearrangement (1, 2). Several distinct metabolic pathways of PtdIns3P phosphorylation exist in eukaryotic cells, producing important effectors such as PtdIns 4,5-bisphosphate, PtdIns 3,4-bisphosphate, and PtdIns 3,4,5-trisphosphate (3). The first step in the phosphorylation of PtdIns in many receptor-dependent phospholipase C (PLC) and PI 3-kinase (PI3K) signaling pathways involves the synthesis of PtdIns 4-phosphate (PtdIns4P) by PtdIns 4-kinase (PtdIns4K) activity (4).

The PI kinase sequences that have been determined so far fall into two families, the PI3K family (5, 6), which includes all the hitherto known PI3K and PtdIns4K sequences (3), and the PtdIns phosphate kinase (PtdInsPK) family (3, 7). All PI3Ks and PtdIns4Ks cloned so far display significant homology within their kinase domains. In contrast, members of the PtdInsPK family have quite distinct sequences. However, recent structural studies have shown that whereas the PtdInsPKs display little primary sequence homology with the PI3K family, they share a common protein fold that is also conserved in many protein kinases (8, 9).

Early chromatographic purification of phosphoinositide kinase activities from bovine brain (4) and cultured rodent fibroblasts (10) identified three fractions containing PtdIns kinase activity, termed types I to III. The type II and III fractions contain different PtdIns4K activities, and the type I fraction was subsequently shown to contain PI3K activity (11). It is now known that the type I PI3K and type III PtdIns4K enzymes have related sequences and belong to the PI3K family (3). However, the fraction containing the type II PtdIns4K (PtdIns4K II) has so far not been characterized at the molecular level despite the fact that in many mammalian cells, the predominant pathway of PtdIns phosphorylation is initiated by this strongly membrane-associated PtdIns4K (3).

Perhaps surprisingly, although members of the PI3K and PtdInsPK families have been readily purified and cloned, numerous attempts to purify the PtdIns4K II enzyme (see Ref. 12 and references therein) have failed to lead to the cDNA being identified. These preparations were probably often impure as PtdIns4K II is labile, difficult to solubilize, and tends to aggregate strongly with other proteins. Indeed, a published cDNA sequence was subsequently found to encode a long-chain fatty acid-CoA ligase (13).

Despite the massive increase in genome data, approaches based on homology to known PtdIns4Ks, such as degenerate polymerase chain reaction (PCR) primers or data base trawling, have also failed to identify the PtdIns4K II sequence. Although three related PtdIns4Ks have so far been cloned (14–18), the failure of such approaches with PtdIns4K II has raised the question of whether the PtdIns4K II enzyme might be a proteolytic fragment or splice variant of the known isozymes or belong to a different structural family altogether. Although there is indirect evidence for the regulation of the PtdIns4K II activity by serine and tyrosine residue phospho-
rylation (19, 20), receptor association (21, 22), heterotrimeric G-proteins (23), and substrate presentation by the PtdIns transfer protein (22), the inability to purify or immunoprecipitate the enzyme has precluded definitive experiments to evaluate its function and regulation. PtdIns4K II has been identified in the plasma membrane, lysosomal, microsomal, transport vesicle, and nuclear compartments (4). More recently this isozyme has been shown to exist in subdomains of the plasma membrane termed non-caveolar membrane rafts (24), in which receptor-dependent PLC signaling also appears to be localized (25). Rafts are typically small, cholesterol-rich membrane domains of low buoyant density, which are generally insoluble in 1% Triton X-100 at 4 °C (reviewed in Ref. 26).

To address questions regarding the pivotal role of this enzyme in many signaling pathways, we set out to purify sufficient human PtdIns4K II to allow the cDNA to be identified.

**EXPERIMENTAL PROCEDURES**

**Type II PtdIns 4-Kinase Purification—**Plasma membrane rafts containing PtdIns4K II were isolated from human A431 epidermoid carcinoma cells, employing similar methods to those described previously (24). Two chromatographic purification steps were selected from the work of Deuel and co-workers (27), adapted to microscale purification and modified using detergent appropriate for cholesterol-rich rafts. A431 cells were cultured to confluence in six 24 × 24-cm dishes in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum. Monolayers were washed in phosphate-buffered saline and then scraped into 12 ml of buffer containing 10 mM Tris-HCl, 10 mM EDTA, 10 mM EGTA, 0.25 M sucrose, and protease inhibitors (Complete™ Mini, EDTA-free; Roche Molecular Biochemicals) prior to Dounce homogenization. The homogenate was centrifuged at 4000 × g for 5 min. Membranes were pelleted from the post-nuclear supernatant by centrifugation at 190,000 × g for 1 h at 4 °C.

Low density membrane rafts were prepared using a modification of the method of Song et al. (28). Briefly, the 190,000 × g membrane pellet was resuspended in 2 ml of 100 mM Na2CO3, pH 11.0, 10 mM EGTA, 10 mM EDTA, 10 mM β-octylglucoside, and 4 mM deoxycholate, containing Complete™ protease inhibitors. The membrane suspension was sonicated on ice (10 × 5-s bursts at 40% maximum power using a VC130PB sonicator [Sonics and Materials Inc.]) and an equal volume of 25 mM MES, pH 6.5, 150 mM NaCl, 10 mM EDTA, and 10 mM EDTA was added. This solution was transferred to a 12.5-ml ultracentrifuge tube and overlaid with 4 ml of 35% sucrose and 4 ml of 5% sucrose in 25 mM MES, pH 6.5, 150 mM NaCl, 10 mM Na2CO3, 10 mM EDTA, and 10 mM EGTA. The discontinuous sucrose gradient was centrifuged at 4 °C for 16 h at 190,000 × g. Rafts were then collected at the 5 and 35% sucrose interface, washed with ~12 ml of 10 mM Tris-HCl, pH 8.0, 10 mM NaCl and repelleted by centrifugation at 4 °C for 1 h at 190,000 × g.

Purified rafts were suspended in 3 ml of base buffer (20 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT) containing Complete™ protease inhibitors and sonicated (10 × 5-s bursts at 40% maximum power) using a VC130PB sonicator. The dispersed rafts were solubilized by the addition of β-octylglucoside and deoxycholate to 100 and 40 mM, respectively, diluted 8-fold with base buffer, and then applied to a MonoQ column (PC 1.6/5; Amersham Pharmacia Biotech). The column was washed in buffer QA and developed with a discontinuous salt gradient in buffer QA (20–189 mM NaCl in 5 min). The peak of PtdIns4K activity was collected at 0–19 min, 189–360 mM NaCl at 19–21 min, and 360–900 mM NaCl at 21–24 min. PtdIns4K activity was collected at 0–19 min, diluted 15-fold with buffer SA (20 mM bis-Tris-HCl, pH 6.0, 50 mM NaCl, 1 mM DTT, 0.1% reduced Triton X-100), and applied to a MiniS column (PC 3.2/5; Amersham Pharmacia Biotech) equilibrated in buffer SA. After washing in buffer SA, the column was developed with a continuous salt gradient in buffer SA (50–900 mM NaCl in 5 min). The peak of PtdIns4K activity was collected at ~2 min.

**Type II PtdIns 4-Kinase Assays—**In-gel assays and product analysis using TLC were performed as described previously (12, 19). Solution assays containing 5 μl of MonoS column fractions or 10 ng of GST-PtdIns4K II were performed in 100-μl volumes containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl2, 0.1% Triton X-100, 1 mM 2-mercaptoethanol, 0.5 mM PtdIns, 0.1 mM EGTA, and 0.2% 2-ME. PtdIns4K activity was measured using the γ-[32P]ATP (1 μCi/assay). Assays were started by the addition of ATP and incubated for 15 min at 37 °C before being terminated by the addition of HCl to 0.5 M. Under these conditions the addition of radi-

![Fig. 1. Analysis of the human type II PtdIns4K preparation.](image-url)
active phosphate to PtdIns was linear (data not shown). The organic phase was extracted (12), and the lipid products were analyzed by TLC separation on Silica 60 plates (Whatman Inc.) in propan-1-ol/CHCl₃/acetic acid (65:35) containing 1% 5 M phosphoric acid followed by autoradiography. Spots containing PtdIns4P were quantitated by scraping and counting in a Beckman LS 6500 liquid scintillation counter.

**Mass Spectrometry**—Proteins were separated by SDS-PAGE and supercritically stained with silver (29). The stained material comigrating with renatured activity was excised and subjected to reduction and alkylation using DTT and iodoacetic acid followed by in-gel digestion using modified trypsin (Promega, Southampton, UK).

Matrix-assisted laser desorption (MALDI) mass spectrometry (MS) was performed on a Reflex III (Bruker Daltonik, Bremen, Germany) reflector time of flight mass spectrometer in the reflector mode with delayed extraction and using a 2,5-dihydroxybenzoic acid matrix. Spectra were internally calibrated using tryptic autolysis product ions of monoisotopic m/z 842.5100 and 2211.1046. The MS-Fit program (ProteinProspector; University of California at San Francisco, San Francisco, CA) was employed for peptide mass mapping.

For electrospray ionization (ESI) liquid chromatography MS/MS, samples were loaded on an Ultimate nano-HPLC (LCPackings, Amsterdam, The Netherlands) via a Famos autosampler (LCPackings). All solvents were HPLC grade (Rathburn, Walkerburn, Scotland, UK). Separation was performed on a 75 μm × 150-mm silica C18 (5-μm particle size) PepMap™ column (LCPackings) with 0.1% formic acid in water as solvent A and 0.08% formic acid in 80% acetonitrile/20% water as solvent B. For equilibration with 5% solvent B, a linear gradient was developed to 40% solvent B in 32 min using a flow rate of 200 nl/min. The nano-HPLC was directly coupled to a Q-TOF ESI mass spectrometer (MicroMass, Manchester, UK) set up for automated liquid chromatography-MS/MS data acquisition using alternating collision energies.

**Isolation of the Type II PtdIns 4-Kinase cDNA**—An antisense oligonucleotide primer (5'-TGCCCTGGGAGCCACCATG) designed to anneal 12 bases to the predicted stop codon of the putative PtdIns4K IIa open reading frame (ORF) was used to prime first strand cDNA synthesis from 0.5 μg of total RNA isolated from A431 cells (30). Reverse transcription was performed in 20 μl using the SuperScript™ Pre-amplification system (Life Technologies, Inc.). A 2-μl aliquot of this reaction was subsequently used to amplify an ~1.4-kilobase band by PCR using the sense primer 5'-AATCCATTTGGGAGGACAGCGCAC-3' and the antisense primer 5'-TGCCCCTGGGAGCCACCATG-3' encompassing the predicted termination codon. PCR was performed using Pfu DNA polymerase (Promega). Cycling conditions were 96°C for 30 s, 66°C for 30 s, and 72°C for 3 min for 40 cycles.

A single PCR product conforming to the predicted size of the putative PtdIns4K IIa ORF was gel purified and 3'A-tailed with DATP and Taq DNA polymerase (Promega) before cloning into the pGEM T-easy vector (Promega) as described by the manufacturer. The identity of the PCR product was confirmed by sequencing.

**Expression and Purification of Recombinant Protein**—A restriction fragment containing the ORF was excised from pGEM T-easy, cloned into the NcoI and SacI sites of pGEX-KG (31), and expressed in the Escherichia coli XL1 strain (Stratagene, Amsterdam, The Netherlands) as a GST fusion protein. Expression of recombinant protein was induced by the addition of isopropyl-β-D-thiogalactopyranoside to 0.1 mM, and cells were harvested after 3 h of growth at 30°C. Bacterial pellets were sonicated in RIPA buffer containing 1 mM EDTA, 1 mM β-mercaptoethanol, and Complete™ protease inhibitors. Lysates were cleared by centrifugation at 20,000 × g for 20 min, after which the recombinant protein was bound to glutathione-Sepharose (Amersham Pharmacia Biotech) as described by the manufacturer.

**Northern Blot Analysis**—A cDNA probe representing the carboxy-terminal 300 amino acids of PtdIns4K IIa was generated by PCR using the sense primer 5'-TTTGGCCGTGGACTGCCTTGCC and the antisense primer 5'-TGCCTCTGGGAGCTACCACCATG. The ~900-base pair (bp) PCR product was gel-purified and labeled with α-[32P]dCTP by random priming as described previously (30). This was then used to probe a human multiple-tissue Northern blot (CLONTECH). The probe was hybridized under high stringency conditions according to the manufacturer’s instructions and then further washed in diethyl pyrocarbonate-treated 0.1 × SSPE (3 M NaCl, 0.2 M NaH₂PO₄, 0.2 mM EDTA), 0.1% SDS for 1 h at 73°C. Following autoradiography, the blot was stripped and reprobed with a human β-actin control probe (CLONTECH) as directed by the manufacturer.

**RESULTS**

**Type II PtdIns 4-Kinase Purification**—The vast majority of the PtdIns4K II in A431 cells is localized within buoyant, non-caveolar membrane rafts (24). Consequently, purified rafts containing PtdIns4K II were solubilized in detergents and further fractionated using sequential anion and cation exchange chromatography. A single peak of activity was obtained at each stage (data not shown). Individual fractions from the MiniS column were analyzed by SDS-PAGE using silver staining (Fig. 1A) and PtdIns4K assays (Fig. 1B). In-gel assays of fraction 3 showed that a band of 52-kDa apparent molecular mass coeluted and comigrated with the bulk of the activity (Fig. 1C).

A much smaller amount of activity was recovered at lower apparent molecular mass as reported previously (12). No other protein appeared to elute with the same profile as the 52-kDa protein or the kinase activity, suggesting the absence of a stoichiometrically associated subunit.

**Mass Spectrometry**—Peptide mass mapping (32) of the 52-kDa protein produced 18 peptide masses. When the mass data were used to search the NCBI non-redundant data base using the MS-fit program they were found to match most closely a partial cDNA encoding a 30.2-kDa fragment of a human pro-
tein (EMBL accession number AL353952). This partial ORF lacked the initiating methionine. Searches of the NCBI human expressed sequence tag database were performed using the 5' region of this cDNA clone to complete the ORF. This revealed an overlapping clone (GenBank™ accession number AW246119), and further searches using AW246119 revealed two more overlapping clones (GenBank™ accession number BE302827 and EMBL accession number AL041898). A clear 1437-bp ORF downstream of an in-frame stop codon and a Kozak consensus sequence (33) was identified from these clones. Furthermore, 14 additional overlapping sequences (GenBank™ accession numbers AA256505, AA495772, AA773590, BE170428, AW770115, A1674489, AI971186, BE300517, AA234669, AI492121, AA458528, and AI332390 and DDBJ accession numbers AK024317 and AK023236) were identified when the NCBI data bases were searched using the sequence assembled from the four clones (AL353952, AW246119, BE302827, and AL041898).

The 1437-bp ORF could account for all 18 tryptic peptide ion masses; these 18 peptides accounted for 37% of the total amino acid sequence (sequence coverage). To confirm this assignment, the 52-kDa protein band was prepared as before, but the gel piece was washed with 80% acetone at −20 °C, and the peptide digest was desalted with a ZipTip™ (Millipore) to improve the

![Fig. 3. Amino acid sequences of human and yeast type II PtdIns4K homologues. A, the predicted amino acid sequence of PtdIns4K IIa showing tryptic peptides identified by MALDI MS (underlined) and ESI MS/MS (bold). The putative leucine zipper sequence is boxed. B, kinase domain alignment for the human PtdIns4K IIa, human PtdIns4K IIb, and the S. cerevisiae type II homologue showing conserved residues. The alignment begins ~30 residues amino-terminal of each putative P-loop and ends at each predicted carboxyl terminus. In this alignment PtdIns4K IIb and S. cerevisiae kinase domains show 68 and 30% residue identity, respectively, with the PtdIns4K IIa kinase domain.

![Fig. 4. Distribution of PtdIns4K IIa mRNA in human tissues. The expression of PtdIns4K IIa mRNA was analyzed using a human multiple-tissue Northern blot (CLONTECH) hybridized with a 32P-labeled fragment of either the PtdIns4K IIa (top) or the ubiquitously expressed human β-actin (bottom) cDNA.](http://www.jbc.org/)

quality of the MS analysis. Analysis of this sample by MALDI-MS (Fig. 2) gave 24 peptides matching the predicted ORF (Fig. 3A) with 44% sequence coverage and a mass accuracy of 100 ppm.

As a separate confirmation of these results, we employed ESI liquid chromatography-MS/MS to analyze the tryptic digest of the first preparation of the 52-kDa protein. This produced sequence information for four peptides, all of which confirmed the sequence assignment. The inset in Fig. 2 shows the MS/MS spectrum of the tryptic peptide Ser460–Arg472, in which virtually the entire series of y-type fragment ions is evident.

The 1437-bp ORF sequence obtained by data base searching was used to design complimentary oligonucleotide primers. These primers were used to isolate the cDNA by PCR with a proof-reading polymerase. Extensive DNA sequencing of independently cloned PCR products further verified the accuracy of the ORF.

Sequence Analysis—The 1437-bp ORF sequence encoded a polypeptide containing 479 amino acid residues and a molecular mass of ~54.0 kDa. One motif was found using MacVector, version 7.0 (Oxford Molecular), a leucine zipper between residues 184 and 205 (Fig. 3A), which may mediate homo- or heterotypic protein-protein interactions. Although the mammalian enzyme is strongly bound to membranes, no transmembrane sequence or any established consensus for acylation was detected. The extended region amino-terminal to the kinase domain showed no homology with any other known protein. Although the enzyme is sensitive to inhibition by low micromolar concentrations of Ca²⁺ (12), no Ca²⁺ binding motif was apparent.

Searches of NCBI data bases for homologous sequences revealed a set of additional sequences from different species including a second human sequence (NCBI accession number 8922869; see Fig. 3B) and a single homologue in Saccharomyces cerevisiae (NCBI accession number 6322361; see Fig. 3B) located on chromosome X. Alignment of over 10 different PtdIns4K II homologues revealed several candidate kinase motifs (34), including a candidate subdomain I P-loop sequence GSSGSY₁₃₉, subdomain II ATP-binding K₁₅₃, subdomain VIb catalytic residues DYIIRN₃₀₇ or DRGNDN₃₁₄, and subdomain VII Mg²⁺ binding motif DNG₃₄₉. No obvious homology was apparent between the region amino-terminal to each putative kinase domain and the corresponding region of the PtdIns4K IIα enzyme. The lack of any close sequence similarity with either the PI3K or PtdInsPK family demonstrated that PtdIns4K IIα and its sequence homologues define a novel, third PI kinase family.

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FIG. 5. Expression and activity of recombinant PtdIns4K IIα. A, the PtdIns4K IIα cDNA was expressed as a GST fusion protein in E. coli. An SDS-PAGE separation stained with Coomassie Brilliant Blue is shown containing vector only (GST) and the GST-PtdIns4K IIα fusion protein before (GST-IIα) and after digestion with thrombin (IIα). The undigested (arrowhead) and digested (arrow) proteins migrated at their expected sizes of ~80 and 54 kDa, respectively. B, PtdIns4K assay of the recombinant GST-PtdIns4K IIα fusion protein (GST-IIα) and GST control (GST). The single product comigrated with an unlabeled iodine-stained PtdIns4P standard (Std) and no other products were detected. The position of the origin is indicated by an arrow. C, the PtdIns4K activity of the recombinant GST-PtdIns4K IIα fusion protein was assayed in the absence (−) and presence (+) of 0.1% Triton X-100. D, sensitivity of the recombinant protein to adenosine. GST-PtdIns4K IIα was assayed in the presence of a range of adenosine concentrations as indicated. E, inhibition of GST-PtdIns4K IIα by monoclonal antibody 4C5G. Recombinant fusion protein was assayed in the presence of the indicated amounts of 4C5G.-32767.

* M. dos Santos and J. J. Hsuan, submitted.
**Northern Blot Analysis**—The PtdIns4K II α mRNA has an apparent size of 6.6 kilobases and appears to be ubiquitously expressed in human tissues (Fig. 4), which is consistent with the purification of this enzyme from numerous different primary tissues (see Ref. 12 and references therein). Expression appeared highest in kidney, brain, heart, skeletal muscle, and placenta and lowest in colon, thymus, and small intestine.

**Expression and Characterization of Recombinant Protein**—The GST fusion protein expressed in bacteria had an apparent molecular mass of ~80 kDa, and removal of the GST polypeptide using thrombin reduced the mass to ~54 kDa (Fig. 5A). The fusion protein possessed PtdIns4K activity, which was undetectable in control preparations containing GST only, and the lipid product comigrated with a PtdIns4P standard (Fig. 5B). When the fusion protein was tested against synthetic phosphoinositide substrates it was able to phosphorylate PtdIns but not PtdIns3P, PtdIns4P, or PtdIns5P (results not shown). Kinetic analysis of the GST fusion protein using the Lineweaver-Burk method gave \( K_m \) values of 28 and 54 \( \mu M \) for ATP and PtdIns, respectively, which fall within the range of previously published values (see Ref. 12 and references therein). The specific activity of the GST fusion protein was ~3.3 nmol/min/mg; this is low compared with the enzyme purified from various primary tissues (see Ref. 12 and references therein) and may reflect differences in post-translational modification or incorrect folding. The recombinate enzyme was activated 32-fold by 0.1% Triton X-100 (Fig. 5C) and inhibited by adenosine with an \( IC_{50} \) of 22 \( \mu M \) (Fig. 5D). Activation by 0.1% Triton X-100 and inhibition by adenosine in the micromolar range are characteristic properties of PtdIns4K II purified from mammalian tissues (4). The GST fusion protein was also inhibited in a dose-dependent manner by the monoclonal antibody 4C5G (Fig. 5E), which is another characteristic property of PtdIns4K II that distinguishes it from the type III PtdIns4K isoforms (35).

**DISCUSSION**

The successful purification of PtdIns4K II in sufficient quantity for sequencing depended on an understanding of the membrane rafting of this enzyme (24). The purification of these rafts and their efficient solubilization allowed the development of a rapid, small-scale purification of the PtdIns4K II enzyme from cultured cells. The mechanism of PtdIns4K II and PI rafting remains unclear, but it appears to be required for receptor-dependent signal transduction (25).

Because of a lack of specific diagnostic reagents, PtdIns4K II activity is typically characterized by its activation by non-ionic detergents and inhibition by adenosine and the monoclonal antibody 4C5G (4, 10, 12, 35). The recombinate enzyme displayed identical properties to those reported for the purified enzyme; GST-PtdIns4KPK IIα was activated by Triton X-100 and inhibited by low concentrations of adenosine and by 4C5G. As the PtdIns4K II α enzyme defines a novel sequence family, it should be carefully distinguished from the previously cloned PtdIns4K α (14), PtdIns4K β (15–17), and type III (18) isoforms, as well as the PI3K and PtdInsPK families. PtdIns4K α, PtdIns4K β, and the type III PtdIns4K belong in the PI3K family. We propose to maintain the type II assignment for PtdIns4K II, at least until the structural and functional range of homologous enzymes is clearer, and to name the human isoform purified in this report and the second isoform identified by data base searches PtdIns4K IIα and PtdIns4K IIβ, respectively. The lack of sequence similarity in the amino-terminal region of the PtdIns4K IIα and PtdIns4K IIβ proteins suggests this region may confer functional specificity, for example by mediating differential localization, allostery, or regulation via post-translational modification.

The existence of a single PtdInsPK II homologue in S. cer-
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