Mammalian inositol polyphosphate multikinase synthesizes inositol 1,4,5-trisphosphate and an inositol pyrophosphate

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Using a consensus sequence in inositol phosphate kinase, we have identified and cloned a 44-kDa mammalian inositol phosphate kinase with broader catalytic capacities than any other member of the family and which we designate mammalian inositol phosphate multikinase (mIPMK). By phosphorylating inositol 4,5-bisphosphate, mIPMK provides an alternative biosynthesis for inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. mIPMK also can form the pyrophosphate disphosphoinositol tetrakisphosphate (PP-InsP_4) from InsP_5. Additionally, mIPMK forms InsP_4 from Ins(1,4,5)P_3 and InsP_5 from Ins(1,3,4,5)P_4.

A multiplicity of the inositol phosphates exists in biology. The best known is inositol 1,4,5-trisphosphate (InsP_3), a second messenger that releases intracellular calcium (1), whereas functions of other inositol phosphates have not been rigorously established. Inositol pyrophosphates, diphosphoinositol pentakisphosphate (PP-InsP_3/InsP_7), and bis (diphospho) inositol tetrakisphosphate (bis-PP-InsP_4/InsP_8) contain high energy pyrophosphates that have been suggested to participate in protein phosphorylation (2). InsP_7 and InsP_8 are synthesized by separate InsP_6 and InsP_7 kinases (2). An InsP_6 kinase (InsP_6K) has been purified (2) and two InsP_6 kinases, InsP_6K1 and InsP_6K2, have been cloned (3, 4). InsP_7 kinase has been purified but not cloned (5).

Cloning of InsP_6K revealed a homologous protein in yeast previously designated ArgRIII (also known as Arg-82p), because it influences the transcription of several enzymes regulating arginine metabolism (6, 7). We showed that ArgRIII is a yeast inositol polyphosphate multikinase (yIPMK) that can catalyze the ATP-dependent phosphorylation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 (3, 8). A role for yIPMK in nuclear function was indicated by studies of York and collaborators (9), showing that a mutant yeast deficient in nuclear messenger RNA export lacks a gene that influences inositol phosphate metabolism. We and others (8–10) showed that yIPMK is the gene that determines messenger mRNA export from yeast nuclei. A role for the kinase activity of yIPMK in controlling gene transcription has been proposed (10) but challenged (11).

Despite considerable sequence heterogeneity among the several inositol phosphate kinases that we cloned, four key amino acids are conserved (3). This consensus sequence, P-x-x-x-D-x-K-x-G, is required for the catalytic activity of InsP_3 kinase (InsP_3K) (12). Recently, Mayr and associates (13) established that this domain modulates the catalytic site for phosphate transfer from ATP to the inositol ring. In a search for additional members of the inositol polyphosphate kinase family, we screened expressed sequence tag (EST) databases for proteins containing this consensus sequence. We now report the cloning and characterization of mammalian IPMK (mIPMK). We have discovered an unprecedented substrate specificity whereby mIPMK can synthesize InsP_3 from Ins(4,5)P_2, InsP_4 from Ins(1,4,5)P_3, InsP$_5$ from Ins(1,3,4,5)P_4; and the inositol pyrophosphate diphosphoinositol tetrakisphosphate (PP-InsP_4) from InsP_5. mIPMK provides an alternative pathway for the formation of InsP_3 and diphosphorylated inositol phosphates.

Materials and Methods

Materials. Nonradioactive Ins(1,4)P_2 and Ins(4,5)P_2 were purchased from Sigma. Stock solutions were prepared in 1 mM EDTA. [^{3}H]Ins(1,4)P_2, [^{3}H]Ins(1,4,5)P_3, [^{3}H]Ins(1,3,4,5)P_4, [^{3}H]InsP_6 and [γ-^{32}P]ATP were purchased from NEN. [^{3}H]Ins(1,3,4,5,6)P_5 was prepared by phosphorylating [^{3}H]Ins(1,3,4,5)P_4 by using yIPMK (8), and PP-[^{3}H]InsP_5 and PP-[^{3}H]InsP$_4$ were prepared by phosphorylation of [^{3}H]InsP_6 and [^{3}H]Ins(1,3,4,5,6)P_5, respectively, using the InsP_6K1 (14). All of the radiolabeled inositol phosphates that we synthesized were purified by HPLC and desalted (15). Polyethyleneimine (PEI)-cellulose TLC plates were obtained from J.T. Baker.

Identification and Cloning of Rat IPMK. We used a region of about 40 aa of mouse InsP$_6$K surrounding the consensus sequence P-C-x-x-D-x-K-x-G (3) to screen the EST data bank using the TBLAST program (16). The rat EST UI-R-A1-du-c-04–0-UI (GenBank accession no. AA955187; gene info identifier: 4234270) contained the consensus sequences P-C-x-x-D-x-K-x-G, but had an overall weak similarity with other members of inositol kinase family of enzyme. The EST was purchased from Research Genetics (St. Louis) and used to screen a λZapII Rat hippocampal cDNA library as described (17). The Bluescript plasmid recovered from the positive phage was sequenced on both strands by using a Perkin–Elmer ABI Prism 310 genetic analyzer.

Preparation of Recombinant Protein. The ORF for rat IPMK was PCR-amplified from the phage clone using the following primers: 5′-GCAGGATCCGATGGCCGCCGAGCCCCCAGC-3′ and 5′-GCAGAATTCCCGGGTTACAGACGAGTACAAGGT-3′; the PCR product was subcloned into the *Bam*HI and *Eco*RI sites of the pTrcHisB expression vector (Invitrogen). The methods used to transform *Escherichia coli* (strain BL21), to induce with isopropyl-1-thio-D-galactopyranoside, and to isolate the poly(His)-tagged proteins using

Abbreviations: InsP_3, inositol 1,4,5-trisphosphate; InsP_5, inositol pentakisphosphate; InsP_6, inositol hexakisphosphate; PP-InsP_4, disphosphoinositol tetrakisphosphate; IPMK, inositol polyphosphate multikinase; yIPMK, yeast IPMK; mIPMK, mammalian IPMK; EST, expressed sequence tag.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AY014898).

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Talon resin (CLONTECH) were all according to the manufacturers' recommendations.

Enzyme Assays. Recombinant rat IPMK (10–20 ng) was incubated for various times at 37°C in 20 μl of buffer containing 20 mM Hepes (pH 7.0), 6 mM MgCl, 1 mM DTT, 10 mM ATP, 2 mM NaF, 20 mM phosphocreatine, 1 mM EDTA, and 0.01 mg/ml phosphocreatine kinase (Calbiochem). About 5,000 cpm of the appropriate ^{3}H-labeled inositol phosphate was added. Assays were quenched with ice-cold perchloric acid and neutralized before HLPC analysis (18). Briefly, assays of the activities of recombinant enzymes used HPLC using a 4.6 × 125-mm Partisphere SAX column (Whatman) that was eluted with a gradient generated by mixing buffer A (1 mM Na_2 EDTA) and buffer B [buffer A plus 1.3 M $(NH_4)_2HPO_4$, pH 3.8, with H_3PO_4] as follows: 0–5 min, 0% B; 5–10 min, 0–30% B; 10–60 min, 30–100% B; 60–75 min, 100% B. Fractions (1 ml) were collected and counted by using 5 ml of Ultima-Flo AP LCS-mixture (Packard). The reactions using unlabeled InsP_2 isomer were performed as described above, using 1 μM of the unlabeled InsP_2 isomer and 5 μl of [γ-^{32}P]ATP (3,000 cpm/mmol). The reactions were stopped by addition of 2 μl of TLC running buffer (0.6 M HCl). Reaction mixture (1–3 μl) was spotted onto a PEI-TLC plate. Separation of [γ-^{32}P]ATP and [^{32}P]Ins*P*3 was achieved by incubation of the sheet in a tank of TLC running buffer (3). Sheets were air-dried, and the radioactivity was detected by using Kodak X-Omat film.

Northern Blot Analysis. Total RNA from various rat organs was prepared using LiCl precipitation methods (17). RNA (10–20 μg) was loaded on 1% agarose/formaldehyde/Mops gel and transferred to Hybond N$^+$ nylon membrane (Amersham Pharmacia). ORFs for rat IPMK were labeled with [α-^{32}P]dCTP using oligo labeling as described (17). Hybridization and washing were carried out following the manufacturer's instructions.

***In Situ* Hybridization.** Fresh-frozen rat brain sections (2 to 3 mo old, SD male) were fixed with 4% paraformaldehyde/PBS and permeabilized, prehybridized, and hybridized in 50% formamide/5% SSC with 100 ng/ml unhydrolyzed digoxygenin-labeled probe overnight at 55°C. Sections were washed, blocked, and incubated overnight at 4°C in 4% normal goat serum in TBS with antidigoxygenin-AP antibody (Roche Molecular Biochemicals) at 1:5,000. After washing in TBS, slides were developed with 1 ml of color development solution containing 3.375 mg/ml nitroblue tetrazolium, 3.5 mg/ml 5-bromo-4-chloro-3-indolyl phosphate, and 0.24 mg/ml levamisole, in the dark. The color reaction was allowed to run 48 h at room temperature. The reaction was stopped in ddH_2O, and the slides were sealed in Aquapolymount. Unique probes mIPMK were generated from cDNA corresponding to ORF of mIPMK subcloned in Bluescript SK(+) plasmid (Stratagene), and antisense and sense cRNA probes were generated by T7 and T3 RNA polymerases. Sense control probes used at equal concentration generated no specific signal.

Results

Using the consensus sequence that we characterized in the family of inositol phosphate kinases (3), we identified a rat EST (UI-R-A1-du-c-04–0-UI; GenBank accession no. AA955187; gene info identifier: 4234270). We used this EST to screen a rat hippocampal cDNA library leading to identification and cloning of a full-length cDNA comprising 5,432 nt (sequence deposited in GenBank with the accession no. AY014898). The cDNA cloned contains an ORF of 396 aa (Fig. 1) located in the 5′ region. We have assigned the initiation codon to the ATG at position 115, that is preceded by an in-frame stop codon 30 bases

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IPMK   --MAAEPPALRLRPPGSTGDSPPV---PRLLGGCVPLSHQVAGHMYGKDK 45
IP3KA  -MTLPGHPTGMARPRGAGPCSPGLERAPRRSVGELRLLFEARCAAVAAAA 49
IP6K1  MCVCQTMEVGQYGKNASRAGDRGV----LLEPFIHQVGGHSSMMRYDDHT 46
                  .         .   .

IPMK   VGILQHPDGTVLKQLQPPPRGPRELEFYTMVYAADCADAVLLELRKHLP- 94
IP3KA  AAGEPRARGAKRRGGQVPNGLPRAAPAPVIPQLTVTSEEDVAPASPGPP- 98
IP6K1  VCKPLISREQRFYESLPPEMKEFTPEYKGVVSVCPEGDSDGYINLVAYPY 96
                     *           .     .          *

IPMK   -KYYGVWSPPSAPN---------------------------------- 107
IP3KA  -DREGNWLPAAGSHLQQPRRLSTSSLSSTGSSSLLEDSEDDLLSDSESRS 147
IP6K1  VESETVEQDDTPEREQPRRKHSRRSLHRSGSGSDHKEEKASLSFETSESS 146


IPMK   ------------------------------------------------
IP3KA  RGNVQLETSEDVGQKSHWQKIRTMVNLPVMSPFKKRYSWVQLAGHTGSFK 197
IP6K1  QEAKSPKVELHSHSDVPFQMLDSNSGLSSEKISYNPWSLRCHKQQLSRMR 196


IPMK   ----------------------------------------------DVYLKLE 114
IP3KA  AAGTSGLILKRSSEPEHYCLVRLMADVLRGCVPAFHGVVERDGESYLQLQ 247
IP6K1  SESKDRKLYK---------------------------------FLLLE 211
                                                  .* *.

IPMK   DVTHKFNKPCIMDVKIGRKSYDPFASAEKIQQQVSKYP------------ 152
IP3KA  DLLDGFDGPCVLDCKMGVRTYLEEELTKARERPKLRKDMYKKMLAVDPEA 297
IP6K1  NVVHHFKYPCVLDLKMGTRQHGDDASAEKAARQMRKCE------------ 249
       ..  *  **..* *.* . .     ..   .   .

IPMK   LMEEI--GFLVLGMRVYHLHSDSYETQNQHYGRGLTKETLKEG------- 193
IP3KA  PTEEEHAQRAVTKPRYMQ----WREGISSSTTLGFRIEGIKKADGSCSTD 343
IP6K1  QSTSATLGVRVCGMQVYQLDTGHYLCRNKYYGRGLSIEGFRNA------- 292
             *                          *.  * ...

IPMK   ------------VSKFFHNGFCLRKDAVAASIQKVEKILQWFENQKQLNF 231
IP3KA  FKTTRSREQVTRVFEEFMQGDAEVLKRYLNRLQQIRDTLEISDFFRRHEV 393
IP6K1  ------------LYQYLHNGLDLRRDLFEPILSKLRGLKAVLERQASYRF 330
                  . . . .*           . ..       .

IPMK   YASSLLFVYEGSSQPATTKSNDRTLAGRFLSKGALSDADVLECNNNFHLF 281
IP3KA  IGSSLLFVHD---------------------------------------- 403
IP6K1  YSSSLLVIYDG-------------------KECRS-----ELRLKHVDM 355
         **** ...

IPMK   SSPANGTSVGKSLSKAYSRHRKLYAKKHQSQTSLKVETLEQDNGWKSMSQ 331
IP3KA  ----------------------------------------------------
IP6K1  GLPEVPPPCGPSTSP-------------SSTSLE--------------- 376


IPMK   EHLNGNVLSQLEKVFYHLPAGRQEIAEAEVRMIDFAHVFPSNTVDE---- 377
IP3KA  ----------------------HCHRAGVWLIDFGKTTPLPDGQILDHR 430
IP6K1  -------------------AGPSSPPKVDVRMIDFAHSTFKGFRDDPTVH 407
                                * .*** .

IPMK   ----------GYVYGLKELIAVLRSILDS-- 396
IP3KA  RPWEEGNREDGYLLGLDNLIGILANLAER-- 459
IP6K1  DGPDR-----GYVFGLENLISIMEQVPDENQ 433
            **. ** .** ..  . .
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Fig. 1. Multiple alignment of rat IPMK, mouse IP_6K1, and rat IP_3K. Regions of homology were identified by using the CLUSTAL-W program (32). Manual alignment was subsequently performed to optimize the similarity. * represents an identical amino acid; · represents a conservative amino acid change. In bold are represented the four amino acid domains conserved in this family: the inositol binding site (amino acids 110–150); the SSLL domain (amino acids 232–241); the ATP binding site (amino acids 361–368); and the C terminus (amino acids 377–396).

upstream. The extremely long 3′ untranslated region is characteristic of the inositol phosphate kinase family (19, 20).

The ORF codes for a protein of 44.4 kDa. This protein displays <30% overall homology to InsP_6K1 or 2 and <25% homology to InsP_3KA, B, or C (Fig. 1). Among inositol phosphate kinases, the N-terminal half is generally regarded as regulatory, whereas the catalytic area is confined to the C-terminal portion. The C-terminal half of the gene displays about 40% similarity to InsP_3Ks and InsP_6Ks with four very well-conserved domains (Fig. 1). The inositol phosphate binding (12) and catalytic domains (13) characteristic of all members of this family of enzymes (3, 4) are highly conserved (amino acids 110–152). The ATP binding site previously characterized in InsP_3K (21) is also present in mIPMK (amino acids 361–368). The C-terminal domain (last 19 aa), important for the catalytic activity of InsP_3K (12) as well as the InsP_6Ks (A. Saiardi and S.H.S., unpublished observations), is also well conserved. Finally, we have identified

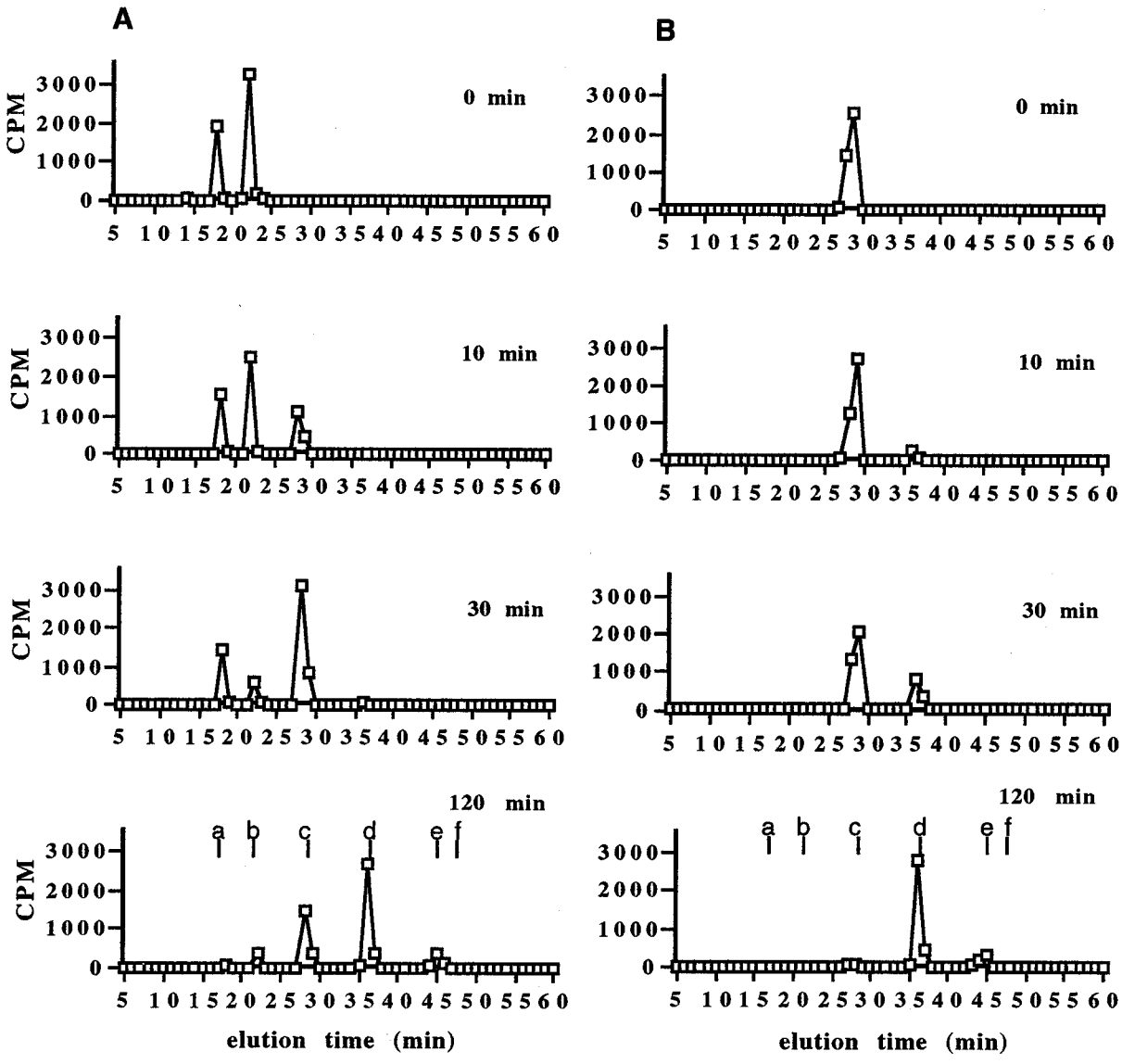

Fig. 2. Phosphorylation of [^{3}H]Ins(1,4,5)P_3 and [^{3}H]Ins(1,3,4,5)P_4 by mIPMK. (*A*) HPLC analysis of assays containing 10 ng of mIPMK incubated with [^{3}H]Ins(1,4,5)P_3 for 0, 10, 30, or 120 min. (*B*) HPLC analysis of assays containing 10 ng of mIPMK incubated with [^{3}H]Ins(1,3,4,5)P_4 for 0, 10, 30, or 120 min. The tritium HPLC standards used to identify the products are: (a) Ins(1,4)P_2; (b) Ins(1,4,5)P_3; (c) Ins(1,3,4,5)P_4; (d) Ins(1,3,4,5,6)P_5; (e) PP-InsP_4; (f) InsP_6. These data are representative of three experiments.

a domain, designated "SSLL" (amino acids 232–241), present in all of the members of this family of enzymes. This region is required for the enzymatic activity of InsP_6Ks (A. Saiardi and S.H.S., unpublished observations).

We examined the enzymatic activity of the protein expressed as His-tag in *E. coli*. The recombinant protein was incubated with a variety of radiolabeled inositol phosphates, and products were separated by Partisphere SAX column chromatography (8, 18). We failed to detect any metabolic activity when enzyme preparations were incubated with [^{3}H]InsP_6 or [^{3}H]InsP_7 (data not shown). We observed considerable enzymatic activity after incubation with [^{3}H]Ins(1,4,5)P_3 (Fig. 2*A*). In the purchased specimen of [^{3}H]Ins(1,4,5)P_3, the InsP_3 peak at 22 min was preceded by an apparent contaminant that elutes very similarly to Ins(1,4)P_2. A peak representing Ins(1,3,4,5)P_4 was detected at 10 min, most prominent at 30 min, and declines at 2 h. The decline of InsP_4 at 2 h was accompanied by the appearance of a peak eluting like the Ins(1,3,4,5,6)P_5 standard, which is the most prominent peak at 2 h. At 2 h, we also observed a new peak corresponding to 10% of the radioactive input with a migration identical to that of PP-InsP_4. We failed to detect radioactive label corresponding to IP_6.

We incubated [^{3}H]Ins(1,3,4,5)P_4 with the enzyme for 10, 30, or 120 min, and observed a time-dependent increase in the formation of a peak with a migration identical to Ins(1,3,4,5,6)P_5. At 120 min, we also observed the formation of PP-InsP_4 (Fig. 2*B*). These findings establish that the conversion of Ins(1,4,5)P_3 to InsP_5 and PP-InsP_4 proceeds through Ins(1,3,4,5)P_4.

The decline of the [^{3}H]Ins(1,4,5)P_3 peak coincides temporally with the appearance of InsP_4, InsP_5, and PP-InsP_4 peaks in a precursor–product relationship. The "contaminant" of [^{3}H]Ins(1,4,5)P_3 also declines with time, although less rapidly than InsP_3 itself. Commercial preparations of InsP_3 preparations are well known to be contaminated with isomers of InsP_2 with levels of InsP_2 increasing with time of storage (S. Shears, personal communication). Of the three isomers of InsP_2 that

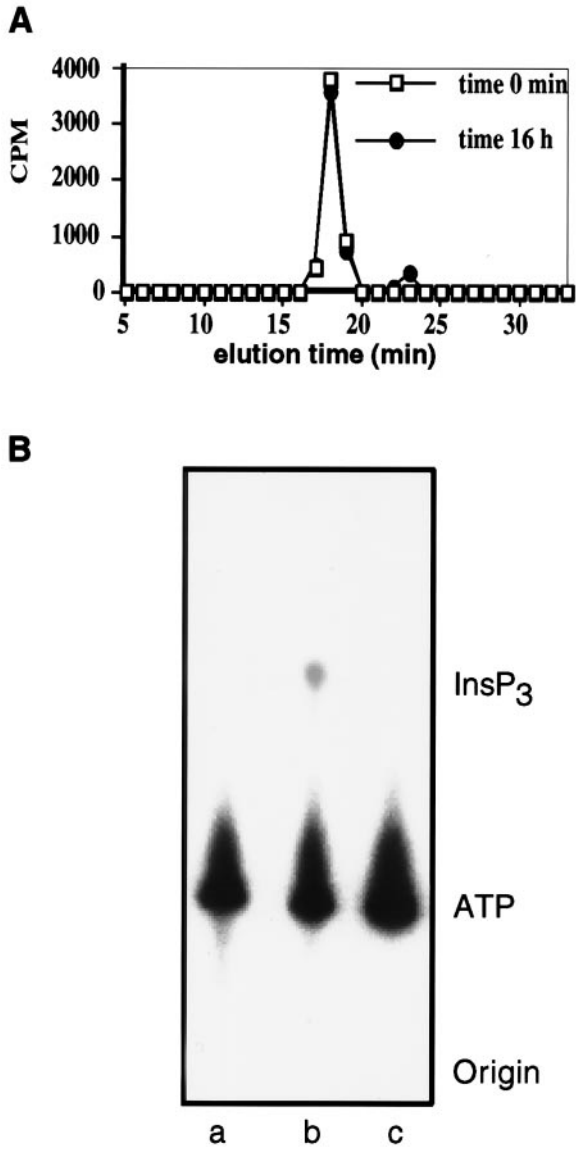

Fig. 3. Phosphorylation of InsP_2 by mIPMK. (*A*) HPLC analysis of assays containing 100 ng of mIPMK incubated with [^{3}H]Ins(1,4)P_2 for 0 min or 16 h. Less than 5% of conversion is visible after overnight incubation at 37°C. (*B*) TLC analysis of reactions using 20 ng of mIPMK, unlabeled InsP_2 isomer, and [γ-^{32}P] ATP, incubated at 37°C for 2 h. Lane a: reaction using Ins(1,4)P_2; lane b: reaction using Ins(4,5)P_2; lane c: reaction without InsP_2. The InsP_3 migration in these TLC conditions was determined by using [^{3}H]Ins(1,4,5)P_3 in a parallel TLC run and analyzed as described (2).

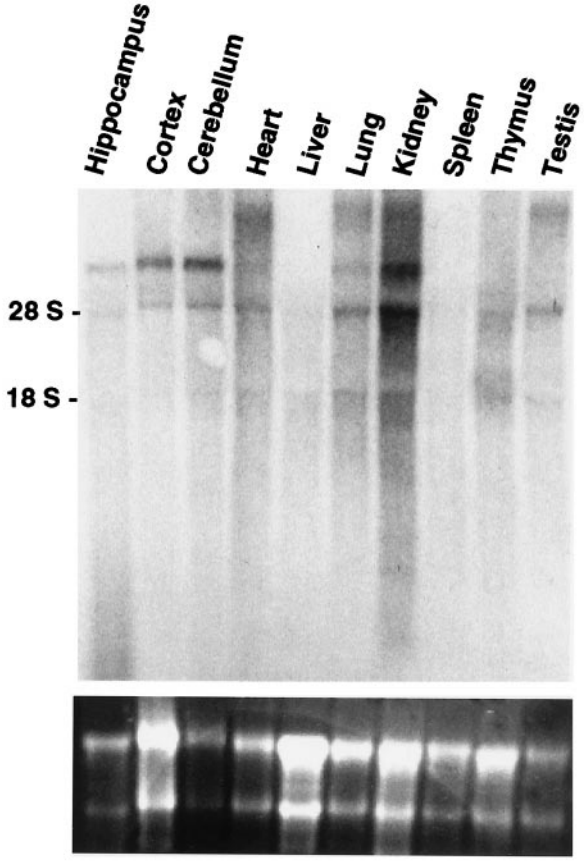

Fig. 4. Northern blot analysis of mIPMK. (*Upper*) Total RNA (20 μg) from different rat tissues was separated on 1% agarose/formaldehyde/Mops gel. After transfer, the blot was hybridized with ORF for mIPMK. (*Lower*) The gel was stained with ethidium bromide to check equivalence of loading.

could be formed from Ins(1,4,5)P_3 [Ins(1,4)P_2, Ins(1,5)P_2, Ins(4,5)P_2], the only radiolabeled one available to us is [^{3}H]Ins(1,4)P_2. To provide maximal opportunity for enzymes to act on Ins(1,4)P_2, we incubated 10 times as much enzyme for 16 h with [^{3}H]Ins(1,4)P_2 (Fig. 3*A*). Only about 4–5% is converted to [^{3}H]InsP_3, implying that Ins(1,4)P_2 is not the constituent of the [^{3}H]InsP_3 preparation that leads to the formation of higher inositol phosphates. To further explore the identity of the InsP_2, we incubated unlabeled Ins(1,4)P_2 and Ins(4,5)P_2 with [γ-^{32}P]ATP and separated products by TLC (Fig. 3*B*). We did not use Ins(1,5)P_2 because samples were not available commercially. Incubation with Ins(4,5)P_2 for 2 h leads to the appearance of radiolabeled InsP_3, whereas no InsP_3 is formed from Ins(1,4)P_2. Thus, we conclude that the additional constituent of [^{3}H]InsP_3 preparations is Ins(4,5)P_2. Most importantly, we have established that the cloned enzyme readily converts Ins(4,5)P_2 to InsP_3, representing a pathway for InsP_3 biosynthesis.

In summary, these experiments demonstrate that the new enzyme has multiple inositol phosphate kinase activities. It can form InsP_3, convert Ins(1,4,5)P_3 to InsP_4, Ins(1,3,4,5)P_4 to InsP_5, and InsP_5 to PP-InsP_4. Because of these multiple kinase activities, we designate the enzyme mIPMK.

We used Northern analysis to evaluate the tissue distribution of mIPMK (Fig. 4). mIPMK is most highly expressed in the kidney with various brain regions being second highest. Within the brain, highest expression occurs in the cerebellum, lowest in the hippocampus, whereas cerebral cortex is intermediate. We detected moderate levels of expression in heart and lung with negligible levels in liver and spleen. We observed three principal bands in the kidney, whereas lower levels of expression in the thymus and testes contained only the two lower bands. These findings suggest that alternative splicing leads to multiple isoforms of mIPMK.

We conducted *in situ* hybridization to examine the cellular localization of mIPMK in the brain (Fig. 5). In the hippocampus, mIPMK expression is confined to neuronal cell bodies with no evidence for expression in glial cells. Levels are most pronounced in the dentate gyrus, whereas moderate levels occur in all layers of the hippocampus. In the cerebellum, mIPMK expression appears exclusively in cell bodies of granule cells with no labeling of Golgi II, stellate, or basket cells and no evident expression in any glial subtype.

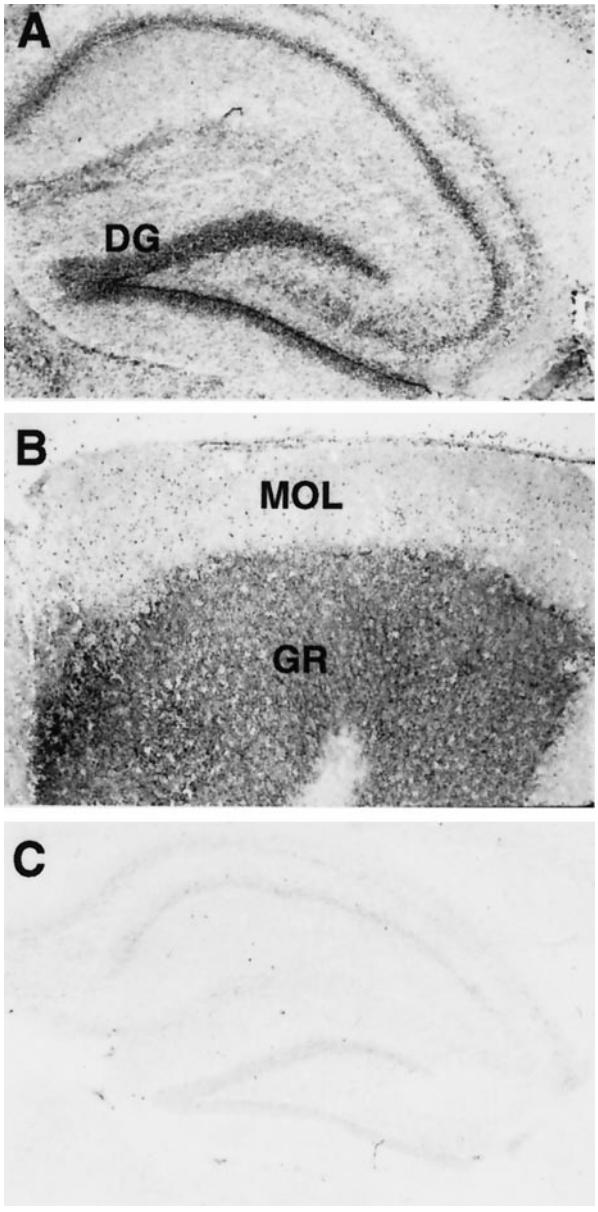

Fig. 5. *In situ* hybridization of mIPMK. Adult rat brain was probed with rat IPMK antisense (*A* and *B*) or sense (*C*). (*A*) In the hippocampus, the mIPMK message was detected principally in the dentate gyrus (DR). (*B*) In the cerebellum, the mIPMK message was localized to the granule cell layer (GR); MCL, molecular cell layer MCL. (*C*) Rat hippocampus probed with sense RNA.

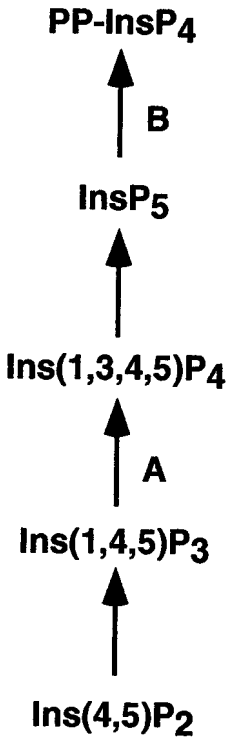

Fig. 6. Catalytic activities of mIPMK. (*A*) Conversion of Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4 is also mediated by InsP_3KA, B, or C (33). (*B*) Formation of PP-InsP_4 can also be mediated by InsP_6K1 and 2 (14).

Discussion

The main finding of our study is the identification of mIPMK, whose range of inositol phosphate kinase activities greatly exceeds that of any previously described enzyme (Fig. 6). mIPMK can form InsP_3 from Ins(4,5)P_2, which may provide a biosynthetic route to InsP_3 formation. The classic pathway for the formation of Ins(1,4,5)P_3 involves the hydrolysis of phosphatidylinositol(4,5)P_2 (PIP_2) to InsP_3 with the liberation of diacylglycerol (1, 22). In circumstances in which Ins(4,5)P_2 levels accumulate, it is conceivable that mIPMK is an important source of InsP_3. This could be relevant to the therapeutic actions of lithium in affective illness. Thus, therapeutic concentrations (1 mM) of lithium are known to inhibit inositol polyphosphate 1-phosphatase (23, 24). These concentrations of lithium also evoke a 10-fold augmentation of Ins(4,5)P_2 levels in brain slices labeled with [^{3}H]inositol, whereas levels of other inositol phosphates are increased much less (25, 26). Whether this effect is related to inhibition of inositol polyphosphate 1-phosphatase or a hitherto unidentified enzyme that removes phosphates at the 4 and 5 positions is unclear.

The ability of mIPMK to convert Ins(4,5)P_2 to InsP_3 may also account for the pronounced calcium-releasing activity of Ins(4,5)P_2 (27). As other isomers of InsP_2 fail to release calcium, we suggest that this calcium-releasing activity reflects conversion to Ins(1,4,5)P_3.

The ability of mIPMK to form an inositol pyrophosphate is also notable, as phosphorylation of another phosphate bond is generally thought to reflect a different type of enzymatic activity than addition of a phosphate to a hydroxyl. The ability of this enzyme to carry out such disparate enzymatic activities suggests that it is a more primitive enzyme than other inositol phosphate kinases. In contrast, InsP_3K and InsP_6K are highly selective. InsP_3K acts only on Ins(1,4,5)P_3, whereas InsP_6K1 and InsP_6K2 are highly selective for InsP_6 and InsP_5 (14).

The wide substrate specificity of mIPMK suggests that it is a more primitive, phylogenetically older protein than InsP_3K and InsP_6K1. In terms of homology, the closest relative of mIPMK is a *Drosophila* gene named CG13688 (FlyBase ID: FBgn0031267) (28) and described as being InsP_3K-like. If the *Drosophila* gene product does display enzyme activity similar to that of mIPMK, then genetic approaches in *Drosophila* might clarify the biological role of this enzyme.

Evolutionary conservation is evident in the existence of mIPMK in yeast. Interestingly, the substrate specificity of yIPMK is more narrow than that of mIPMK (8). yIPMK can convert Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4 and Ins(1,4,5,6)P_4, and the Ins$P$4 to Ins(1,3,4,5,6)P_5 but seems unable to convert InsP_2 to InsP_3 or InsP_5 to inositol pyrophosphates. Because the yeast genome has been completely sequenced and yIPMK has the

highest homology to mIPMK of any yeast gene, we are confident that yIPMK is the yeast homolog of mIPMK, a conclusion supported by their similar, although not identical, substrate specificity. The established role of yIPMK in nuclear export of mRNA suggests that mIPMK may possess a similar function in mammalian systems.

The identification of mIPMK can explain and perhaps simplify a variety of divergent enzymatic functions involving inositol phosphate kinase activities. Thus, the ability of mIPMK to phosphorylate at positions 1, 3, and 6 might account for such activities reported with a variety of substrates that include some that we have not directly examined ourselves. For instance, Shears and associates (29–31) reported enzymatic activity in rat that phosphorylates various inositol phosphates at 1, 3, 5, and 6 positions and might reflect mIPMK activity.

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