Detection of inositol 1,4,5-trisphosphate kinase in retina

A direct demonstration of phosphorylation of inositol 1,4,5-trisphosphate by ATP

Alan P. TARVER*† and Robert E. ANDERSON*†

*Verna and Marrs McLean Department of Biochemistry and †Cullen Eye Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, U.S.A.

The metabolism of *myo*-inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] consists of two pathways: dephosphorylation by 5-phosphomonoesterase(s) produces inositol 1,4-bisphosphate, and phosphorylation by $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase yields inositol 1,3,4,5-tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$]. The requirements for $\text{Ins}(1,4,5)\text{P}_3$ kinase activity in retina were characterized. Apparent K_m values for ATP and $\text{Ins}(1,4,5)\text{P}_3$ are 1.4 mM and 1.3 μM respectively. A direct demonstration of phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was achieved. Characterization of the ^{32}P -labelled product revealed that it had the expected chromatographic and electrophoretic properties of $\text{Ins}(1,3,4,5)\text{P}_4$.

INTRODUCTION

Inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] causes the release of Ca^{2+} from a portion of the endoplasmic reticulum (Streb *et al.*, 1983; for review, see Berridge & Irvine, 1984). This action of $\text{Ins}(1,4,5)\text{P}_3$ accounts for the observation that an intracellular pool of sequestered Ca^{2+} is hormonally regulated and contributes substantially to the receptor-stimulated increase in intracellular Ca^{2+} (Berridge & Irvine, 1984; Brown & Rubin, 1984). Removal of the phosphate at the 5-position leads to inositol 1,4-bisphosphate [$\text{Ins}(1,4)\text{P}_2$] (Downes *et al.*, 1982; Storey *et al.*, 1984) and inactivation of the Ca^{2+} -mobilizing actions of $\text{Ins}(1,4,5)\text{P}_3$ (Streb *et al.*, 1983).

Phosphorylation is a second pathway of $\text{Ins}(1,4,5)\text{P}_3$ metabolism. Irvine *et al.* (1986) demonstrated that $\text{Ins}(1,4,5)\text{P}_3$ is phosphorylated by ATP at the 3-position. The new product, inositol 1,3,4,5-tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$], appears to be a second messenger which acts in concert with $\text{Ins}(1,4,5)\text{P}_3$ (Irvine & Moor, 1986, 1987; Spät *et al.*, 1987). $\text{Ins}(1,3,4,5)\text{P}_4$ has been proposed to stimulate influx of Ca^{2+} directly into the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool (Irvine & Moor, 1987). This phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ has been observed in numerous hormone-responsive tissues, including brain (Irvine *et al.*, 1986), liver (Irvine *et al.*, 1986; Hansen *et al.*, 1986), parotid (Hawkins *et al.*, 1986), and an insulinoma cell line (Biden & Wollheim, 1986). We report here the characteristics of $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase activity present in the retina. In addition, a direct demonstration of transfer of the γ -phosphate from ATP to $\text{Ins}(1,4,5)\text{P}_3$ by a 3-kinase prepared from rat brain is shown.

MATERIALS AND METHODS

Materials

Nucleotides, triethylamine, trioctylamine and 2,3-bisphosphoglycerate (pentacyclohexylamine salt) were

obtained from Sigma, and 1,1,2-trichloro-1,2,2-trifluoroethane was from Fisher. Trifluoroacetic acid was purchased from Aldrich, and the anion-exchange resins AG 1-X8 (formate form) and AG MP-1 (Cl^- form) were obtained from Bio-Rad. $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ were made by the method of Walseth & Johnson (1979). $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ was kindly given by Dr. Lutz Birnbaumer, and was made by the Baylor Endocrinology Center as described by Walseth & Johnson (1979). $\text{Ins}(1,4,5)\text{P}_3$ was obtained from Boehringer Diagnostics, and was also prepared from human erythrocytes as described by Downes *et al.* (1982) and purified further by h.p.l.c., by a slight modification (Tarver *et al.*, 1987) of the procedure of Hawkins *et al.* (1987). The concentrations of stock solution of inositol polyphosphates were measured by phosphorus determination (Rouser *et al.*, 1970). The concentration of the h.p.l.c.-purified $\text{Ins}(1,4,5)\text{P}_3$ was determined by quantification of inositol as described by Rittenhouse & Sasson (1985). $[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ was obtained from New England Nuclear, and $[2\text{-}^3\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$ was given by New England Nuclear through the courtesy of Dr. Ernest Do. $^{32}\text{P}\text{P}_i$ was from ICN and New England Nuclear, ACS from Amersham, and DEAE-Sephacel from Pharmacia. MonoFluor and Liquiscint were purchased from National Diagnostics. Prosil-28 was obtained from Specialty Chemicals (Gainesville, FL, U.S.A.). All other materials were reagent grade or better.

H.p.l.c. column preparation and use

A 20 cm $\times$ 0.46 cm stainless-steel column of AG MP-1 resin was prepared as described by Axelson *et al.* (1981) by CEL Associates (Houston, TX, U.S.A.). The column was attached to a Spectra-Physics h.p.l.c. apparatus (model 4270) equipped with a 2.0 ml sample loop. A 0.2 μm filter (Rheodyne) and a 5 cm $\times$ 0.46 cm guard column of AG MP-1 resin were placed between the injector and the analytical column. A gradient of

Abbreviations used: $\text{Ins}(1,4)\text{P}_2$, inositol, 1,4-bisphosphate; $\text{Ins}(1,4,5)\text{P}_3$, inositol 1,4,5-trisphosphate; $\text{Ins}(1,3,4,5)\text{P}_4$, inositol 1,3,4,5-tetrakisphosphate; $\text{Ins}(1,4,5,x)\text{P}_3$, $\text{Ins}(1,4,5)\text{P}_3$ phosphorylated at an undetermined position; TEAB, triethylammonium bicarbonate; 5-PME, 5-phosphomonoesterase.

† To whom correspondence should be addressed. Present address: Department of Biochemistry, University of Vermont, College of Medicine, Given Building, Burlington, VT 05405, U.S.A.

trifluoroacetic acid was used to elute inositol polyphosphates. The column was washed with water for 1 min, followed by a linear rise to 50 mM-trifluoroacetic acid over the next 2 min. The trifluoroacetic acid concentration was then raised to 300 mM over 8 min and finally to 500 mM over the succeeding 3 min. This concentration was maintained for another 10 min before returning to water. The flow rate was 2.5 ml/min and the eluate was monitored at 260 nm. Fractions were collected at 0.5 min intervals and counted for radioactivity in 4 ml of ACS. The inositol polyphosphates, i.e. inositol monophosphate, $\text{Ins}(1,4)\text{P}_2$, $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$, were eluted at 7, 9, 12 and 15 min respectively. The adenine nucleotides AMP, ADP and ATP were also completely separated.

Preparation of $\text{Ins}(1,4,5)\text{P}_3$ kinase activity

A high-speed supernatant was prepared from a homogenate of bovine retinas; 30 frozen dark-adapted bovine retinas (Hormel Co., Austin, MN, U.S.A.) were homogenized in 20 ml of ice-cold buffer, consisting of 30 mM-Hepes (pH 7.4), 1 mM-ATP (Mg^{2+} salt) and 0.5 mM- NaH_2PO_4 . The material was centrifuged at 2000 g for 20 min at 4 °C. The pellet was homogenized in 12 ml of fresh buffer and the resulting homogenate was centrifuged as above. The supernatants were combined and centrifuged at 17000 g for 20 min. The resulting supernatant was centrifuged at 120000 g for 60 min. The resulting supernatant was stored at -60 °C. A high-speed supernatant of rat brain synaptosomes was also prepared in nearly the same way, except that the final high-speed supernatant was concentrated 5-fold in a concentrator with a YM-10 membrane (Amicon). The synaptosomes were made as described by Van Rooijen *et al.* (1983). The final ATP concentration in both supernatants was less than 100 μM , because of endogenous ATPase activities. Protein was assayed by the BCA protein assay reagent of Pierce Chemical Co.

Kinase assay

$\text{Ins}(1,4,5)\text{P}_3$ kinase activity was detected by the conversion of $[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ into $[2\text{-}^3\text{H}]\text{Ins}(1,4,5,x)\text{P}_4$. The assay buffer contained 5 μM - $\text{Ins}(1,4,5)\text{P}_3$ (source: Boehringer Diagnostics), 1.5×10^4 – 3.0×10^4 d.p.m. of $[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$, 10 mM-ATP, 15 mM- MgCl_2 , 10 mM-2,3-bisphosphoglyceric acid, 100 mM- NaCl and 100 mM-Hepes (pH 7.4), in a final volume of 400 μl . Assays were conducted at 37 °C and initiated by the addition of protein. The reactions were quenched with an equal volume of ice-cold 1 M-trichloroacetic acid. A 5 min centrifugation in a Microfuge pelleted the protein. The pellet was washed with 400 μl of 0.5 M-trichloroacetic acid. The trichloroacetic acid in the combined supernatants was extracted with water-saturated diethyl ether and neutralized with 1.0 M- NaHCO_3 as described by Berridge *et al.* (1983). The volume was adjusted to 2.5 ml, and a 2 ml sample was analysed by h.p.l.c. on the AG MP-1 column. These assays were conducted in triplicate, and less than 10% variation in results was observed.

Phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$

These assays were conducted under the same conditions as those above, except that the final volume was 50 μl , and the concentrations of $\text{Ins}(1,4,5)\text{P}_3$ (source: h.p.l.c.-purified), ATP, MgCl_2 and 2,3-bisphosphoglyceric acid were 2 μM , 1 mM, 5 mM and 20 mM respec-

Fig. 1. $\text{Ins}(1,4,5)\text{P}_3$ kinase activity of retina as a function of ATP concentration

The total Mg^{2+} concentration was adjusted to be 5 mM in excess of the ATP concentration. These assays were conducted as described in the Materials and methods section, with $\text{Ins}(1,4,5)\text{P}_3$ set at 5 μM . Less than 20% of both substrates were consumed in these assays. Fitting of the data by non-linear least squares with use of a computer program described by Walters & Gilbert (1986) yields an apparent K_m of 1.4 mM for ATP.

tively. About 1×10^4 c.p.m. of $[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and 2.5×10^7 c.p.m. of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ were added; the final specific radioactivities of $[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ were about 100 and 500 c.p.m./pmol respectively. The reaction buffer also contained 1 mg of bovine serum albumin/ml, to minimize the variability of the enzyme activity seen in smaller assay volumes. The reaction was initiated by the addition of 10 μl (30 μg) of rat brain supernatant, and proceeded for 1 h at 37 °C and then for 5 min in boiling water. The remaining ATP was removed by adding 25 units of hexokinase (yeast type C-301; Sigma) and 2 μmol of glucose. This reaction was incubated at 37 °C for 60 min with an addition of 100 nmol of ATP and 800 nmol of glucose at 30 min. The reaction was then incubated in boiling water for 5 min and diluted to 1 ml. The material was loaded on to DEAE-Sephacel (0.5 ml bed volume) and desalted. The column had been pre-equilibrated with 5 ml of 1.0 M-triethylammonium bicarbonate (TEAB), pH 7.8, and 5 ml of 0.1 M-TEAB. The loaded column was washed with 3×2.5 ml of 0.2 M-TEAB and then with 2×0.75 ml of 1.0 M-TEAB. This desalting procedure is a modification of that of Revich *et al.* (1984). Unlabelled ATP (50 nmol) was added to the 1.0 M-TEAB eluate before drying the sample in a vacuum centrifuge (Savant). The desalted material was analysed by h.p.l.c. on a Whatman Partisil strong anion-exchange (SAX) column as described by Batty *et al.* (1985), as modified by Hansen *et al.* (1986). Fractions were collected at 0.4 min intervals and counted for radioactivity in 6 ml of Liquiscint/1 M-HCl/methanol (10:1:1, by vol.).

Purification and analysis of $[2\text{-}^3\text{H}, 3\text{-}^{32}\text{P}]\text{InsP}_4$

Dual-labelled $\text{Ins}(1,3,4,5)\text{P}_4$ was purified by h.p.l.c. on the SAX column by using the gradient just described. Four samples were pooled into 1 ml and injected; the peak of interest was determined by counting radioactivity of one-tenth (60 μl) of each fraction in the region where

Fig. 2. Ins(1,4,5) P_3 kinase activity of retina as a function of Ins(1,4,5) P_3 concentration

Assays were conducted in the presence of constant tracer [$2\text{-}^3\text{H}$]Ins(1,4,5) P_3 . The conversion of Ins(1,4,5) P_3 into Ins(1,4,5, x) P_4 did not exceed 15%, and the formation of Ins P_2 , inositol monophosphate and inositol was less than 2% of total Ins(1,4,5) P_3 . An apparent K_m of 1.3 μM for Ins(1,4,5) P_3 was obtained when the data were analysed in the same way as in Fig. 1.

[$2\text{-}^3\text{H}$]Ins(1,3,4,5) P_4 was eluted. The contents of the three tubes with the greatest radioactivity were combined and diluted to 10 ml, and loaded on a 3.5 ml-bed-volume column of DEAE-Sephacel. The column had been pre-equilibrated before use with 10 ml of 1.0 M-TEAB and then with 10 ml of 0.1 M-TEAB. Ammonium formate and P_i were eluted from the loaded column with 2×5 ml of 0.1 M-TEAB. Ins(1,3,4,5) P_4 was eluted with 2×3 ml of 1.0 M-TEAB and collected in a glass tube treated with Prosil-28. TEAB was removed as described above. The material from two h.p.l.c. runs was dissolved and pooled in 500 μl . A portion of this material was treated with 5-phosphomonoesterase (5-PME) purified from human

platelets (generously given by Dr. Thomas Connolly) under the conditions of Downes *et al.* (1982) with the modification that the buffer contained 1 mg of bovine serum albumin/ml. The reactions were quenched with an equal volume of 1 M-trichloroacetic acid. The protein was pelleted, and the trichloroacetic acid was removed from the supernatant by adding 200 μl of trioctylamine/1,1,2-trichloro-1,2,2-trifluoroethane (1:3, v/v). Reaction products were then analysed by h.p.l.c. on the SAX column or by high-voltage electrophoresis as described by Seiffert & Agranoff (1965). Before high-voltage electrophoresis, the reaction products were desalted on columns (0.5 ml bed volume) of DEAE-Sephacel as described above. The electrophoretograms were autoradiographed overnight. The areas corresponding to Ins P_3 and Ins P_4 were cut out, dissolved in 1 ml of 1 M-HCl and counted for radioactivity in 10 ml of Monofluor.

RESULTS

The Ins(1,4,5) P_3 kinase activity in the retina required ATP and Mg^{2+} for activity. Under the conditions described in the Materials and methods section, the assay is linear up to 20% conversion of substrate into product with respect to time and protein concentration. Optimal activity was observed at 5 mM free Mg^{2+} (results not shown). ATP was the only nucleotide found to support kinase activity: less than 10% of maximal kinase activity is seen in the presence of CTP, UTP or GTP at 10 mM (results not shown). A plot of kinase activity versus ATP concentration reveals that the activity is maximal at 10 mM-ATP and half-maximal at 1.4 mM (Fig. 1). This result is not an artifact of endogenous ATPase activities, because at least 80% of the ATP remained at the end of all of the assays. This dependence of activity on high nucleotide concentration was observed with two different sources of ATP from Sigma (horse muscle and enzymically synthesized) and one from Boehringer Mannheim (results not shown). Ins(1,4,5) P_3 kinase from the retina had an apparent K_m of 1.3 μM for Ins(1,4,5) P_3 , and

Fig. 3. H.p.l.c. analysis of h.p.l.c.-purified [$2\text{-}^3\text{H}$, $3\text{-}^{32}\text{P}$]Ins(1,3,4,5) P_4

A portion of the dual-labelled Ins(1,3,4,5) P_4 purified by h.p.l.c. was analysed by h.p.l.c. on a SAX column as described in the Materials and methods section ($\circ$, $\bullet$). Another portion of the material was treated with purified platelet 5-PME and then analysed ($\square$, $\blacksquare$). These analyses were performed in duplicate. The results of a second experiment yielded results identical with those shown above. $\circ$, $\square$, ^3H ; $\bullet$, $\blacksquare$, ^{32}P . $\triangle$, Elution of [$2\text{-}^3\text{H}$]Ins(1,4,5) P_3 .

Fig. 4. High-voltage electrophoresis of dual-labelled $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$.

The desalted products of the 5-PME reaction were electrophoresed on Whatman no. 1 paper as described in the Materials and methods section, and an autoradiogram of the electrophoretogram is shown. Lane 1 shows the electrophoresis of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and $[\text{P}^{32}]\text{Ins}(1,4,5)\text{P}_3$ (prepared as described by Downes *et al.*, 1982). The electrophoresis of untreated $[2\text{-}^3\text{H}, 3\text{-}^{32}\text{P}]\text{Ins}(1,3,4,5)\text{P}_4$ is shown in lanes 2 and 3. Lanes 4 and 5 are products of hydrolysis of $[2\text{-}^3\text{H}, 3\text{-}^{32}\text{P}]\text{Ins}(1,3,4,5)\text{P}_4$ by 5-PME. A result similar to that shown above was obtained in a second experiment.

activity was maximal (0.33 nmol/min per mg) at $5\text{ }\mu\text{M}$ $\text{Ins}(1,4,5)\text{P}_3$ (Fig. 2). The site of phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ by the kinase activity in the retina was not determined, but it is presumed to be the 3-position.

Analysis of the dual-labelled InsP_4 revealed that it had the characteristics expected of $\text{Ins}(1,3,4,5)\text{P}_4$. Both the ^3H and the ^{32}P labels of the h.p.l.c.-purified product comigrated on rechromatography by h.p.l.c. on the SAX column (Fig. 3) and in the high-voltage electrophoresis system of Seiffert & Agranoff (1965) (Fig. 4). Treatment of this product with purified 5-PME converted both labels into a less-polar product, which migrated with $\text{Ins}(1,4,5)\text{P}_3$ on high-voltage electrophoresis (Fig. 4) and was eluted just before $\text{Ins}(1,4,5)\text{P}_3$ on h.p.l.c. (Fig. 3). The ratio of labels in the major peak of h.p.l.c. or spot of high-voltage electrophoresis did not change with treatment by 5-PME. The amount of phosphate incorporated per mol of $\text{Ins}(1,3,4,5)\text{P}_4$ is 1.0 mol (Table 1).

DISCUSSION

Several lines of evidence presented in this paper are consistent with the proposal that a kinase enzymically transfers the γ -phosphate of ATP to $\text{Ins}(1,4,5)\text{P}_3$ at the 3-position. (1) The appearance of ^{32}P in the region of

Table 1. Molar ratio of phosphate incorporated into product to product formed

$[2\text{-}^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ was incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, and the dual-labelled InsP_4 was isolated and purified as described in the Materials and methods section. Treatment of this product with purified platelet 5-PME yielded a dual-labelled InsP_3 . The stoichiometry of phosphate transfer was calculated from the known specific radioactivities of the ^3H - and ^{32}P -labelled precursors. Less than 10% variation was observed in the analysis of duplicate samples of two experiments.

Product	Phosphate incorporated (mol/mol of product formed)
$\text{Ins}(1,3,4,5)\text{P}_4$	1.04
$\text{Ins}(1,3,4)\text{P}_3$	1.06

InsP_4 required protein, Mg^{2+} , and $\text{Ins}(1,4,5)\text{P}_3$. (2) Little or no detectable ^{32}P label was observed in the region of InsP_4 when $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ or $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ was used as the phosphate donor (A. P. Tarver & R. E. Anderson, unpublished work). (3) The coincidence and ratio of ^{32}P and ^3H labels were maintained after treatment of the h.p.l.c.-purified $[2\text{-}^3\text{H}, 3\text{-}^{32}\text{P}]\text{Ins}(1,3,4,5)\text{P}_4$ with purified 5-PME. (4) No detectable increase of ^{32}P label was observed when unlabelled $\text{Ins}(1,4)\text{P}_2$ or inositol 1-phosphate was substituted for $\text{Ins}(1,4,5)\text{P}_3$ (results not shown). The above results, when taken together with the known substrate specificity of the platelet 5-PME (Connolly *et al.*, 1987) and the results of Irvine *et al.* (1986), suggest that the 3-position of $\text{Ins}(1,4,5)\text{P}_3$ has been phosphorylated. Observations by Balla *et al.* (1987) and Shears *et al.* (1987) have shown that a kinase activity can phosphorylate $\text{Ins}(1,3,4)\text{P}_3$ at the 6-position. Our observation of phosphorylation of $\text{Ins}(1,4,5)\text{P}_3$ is not a result of phosphorylation of dephosphorylated $\text{Ins}(1,3,4,5)\text{P}_4$, because under the conditions similar to those described in the Materials and methods section this kinase activity of rat brain does not phosphorylate $\text{Ins}(1,3,4)\text{P}_3$, nor does it convert $[2\text{-}^3\text{H}]\text{Ins}(1,3,4)\text{P}_3$ into a more polar product (A. P. Tarver, W. E. King & S. E. Rittenhouse, unpublished work).

The presence of $\text{Ins}(1,4,5)\text{P}_3$ kinase in the vertebrate retina and in isolated intact rod outer segments (A. P. Tarver, A. J. Ghalayini & R. E. Anderson, unpublished work) is important, because it can metabolize the second messenger, $\text{Ins}(1,4,5)\text{P}_3$. The concentration of ATP in the photoreceptor cells would probably be enough to sustain about half of the optimal activity. Biernbaum & Bownds (1985) have determined that the concentration of ATP is about 2 mM in the photoreceptors. Decreasing the free Mg^{2+} to the concentration found in brain, 1 mM (Veloso *et al.*, 1973), would decrease the kinase activity by about one-third of the optimal value (A. P. Tarver & R. E. Anderson, unpublished work). This kinase could in theory contribute substantially to the metabolism of $\text{Ins}(1,4,5)\text{P}_3$ when its concentration is below $2\text{ }\mu\text{M}$, because the K_m of the kinase for $\text{Ins}(1,4,5)\text{P}_3$ is more than an order of magnitude lower than the K_m of 5-PME activity of several different tissues (Downes *et al.*, 1982; Connolly *et al.*, 1985; Biden & Wollheim, 1986). Indeed, observations of rapid formation of $\text{Ins}(1,3,4,5)\text{P}_4$ and/or $\text{Ins}(1,3,4)\text{P}_3$ in stimulated tissues, including brain (Batty

et al., 1985), the *Limulus* ventral photoreceptor (Irvine *et al.*, 1985), RINm5F cells (Biden & Wollheim, 1986) and platelets (Tarver *et al.*, 1987) suggest that Ins(1,4,5) P_3 3-kinase phosphorylates at least some of the newly formed Ins(1,4,5) P_3 . Thus Ins(1,4,5) P_3 3-kinase appears to be, like phospholipase C and 5-PME, an important regulatory enzyme of inositide metabolism.

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