Annu. Rev. Biochem. 1992. 61:225–50

INOSITOL PHOSPHATE BIOCHEMISTRY

Philip W. Majerus

Division of Hematology-Oncology, Washington University School of Medicine, St. Louis, Missouri 63110

KEY WORDS: messengers, signal transduction, inositol phospholipids

CONTENTS

PERSPECTIVES 225
CONVENTIONAL PHOSPHATIDYLINOSITOLS 226
PHOSPHOLIPASE C 227
ACTIVATION Of PHOSPHOLIPASE C BY $G_{\alpha}Q$ 227
ACTIVATION OF PLCγ BY TYROSINE PHOSPHORYLATION 229
INOSITOL POLYPHOSPHATE 5-PHOSPHATASE 231
INS(1,4,5)P_3 3-KINASE 232
INOSITOL POLYPHOSPHATE 1-PHOSPHATASE 233
INOSITOL POLYPHOSPHATE 4-PHOSPHATASE 234
INOSITOL POLYPHOSPHATE 3-PHOSPHATASE 235
INOSITOL MONOPHOSPHATASE 236
INOSITOL (1:2 CYCLIC) PHOSPHATE 2 PHOSPHOHYDROLASE AND INOSITOL CYCLIC PHOSPHATES 236
OTHER ENZYMES OF INOSITOL PHOSPHATE METABOLISM 238
INOSITOL PENTAPHOSPHATE AND HEXAPHOSPHATE METABOLISM 238
INOSITOL PHOSPHATE–BINDING PROTEINS 239
PHOSPHATIDYLINOSITOL 3-PHOSPHATE PATHWAY 242

PERSPECTIVES

An enormous literature has followed the last review of inositol phosphate metabolism in *Annual Review of Biochemistry* five years ago (1). At that time,

0066-4154/92/0701-0225$02.00

Berridge proposed a scheme in which phospholipase C (PLC) hydrolyzed phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to yield inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and diacylglycerol. However, the multiple sources of diacylglycerol (2), as well as the many intermediates, enzymes, and routes for formation of inositol phosphates (3), indicate that this signal-transducing pathway is much more complex than described previously. Many new reactions, enzymes, and inositol phosphate–binding proteins have been discovered, and molecular genetic approaches have begun to further define the structures and functions of the proteins involved. Rather than catalog all of the new information, I present selected topics that have yielded new insights for future directions of research. I do not discuss calcium ion mobilization in response to Ins(1,4,5)P_3 as this topic has been reviewed elsewhere (4–7). The main subjects to be discussed are (*a*) mechanisms for triggering phosphatidylinositol turnover and inositol phosphate production; (*b*) characterization of enzymes that metabolize inositol phosphates; (*c*) pathways for metabolism of inositol pentaphosphates and inositol hexaphosphates; (*d*) inositol phosphate–binding proteins, especially the Ins(1,4,5)P_3-binding protein; and (*e*) the newly discovered 3-phosphate-containing inositol phospholipids.

CONVENTIONAL PHOSPHATIDYLINOSITOLS

Inositol-containing phospholipids are ubiquitious components of eukaryotic cells and constitute 2–8% of the total phospholipids. There are three major *myo*-inositol containing lipids: phosphatidylinositol (PtdIns), phosphatidylinositol (4) phosphate [PtdIns(4)P], and PtdIns(4,5)P_2. PtdIns accounts for more than 80% of the total phosphatidylinositols (8, 9).

The pathway for biosynthesis of PtdIns in the endoplasmic reticulum was elaborated in the early 1960s (10, 11). PtdIns(4)P and PtdIns(4,5)P_2 are synthesized sequentially by PtdIns 4-kinase and PtdIns(4)P 5-kinase (12). PtdIns 4-kinase is membrane-associated in most tissues (13–16), and has been purified to homogeneity from bovine uterus (17), A431 cells (18), porcine liver (19), and rat brain (20). The properties of the purified enzymes are very similar.

PtdIns(4)P 5-kinase has been found both in the soluble and particulate fractions of cell homogenates (15, 21, 22). A 53-kDa form of this enzyme has been purified from human erythrocyte membranes (23). There appear to be two distinct forms of PtdIns(4)P 5-kinase in erythrocytes (24). Type II PtdIns(4)P 5-kinase is distributed in both membrane and cytosol and has a molecular weight of 53,000. A second form, Type I PtdIns(4)P 5-kinase, is found only in the membrane fraction of erythrocytes and though not yet purified to homogeneity, appears distinct from the previously characterized 53-kDa Type II enzyme. Antibodies against the cytosolic Type II enzyme

neither inhibit the activity of Type I enzyme nor detect Type I enzyme by immunoblotting.

The reason for multiple isoforms and the physiologic functions of each remain to be determined. Functional characterization of these kinases shows that the Type I kinase has a lower K_m for PtdIns(4)P than does the Type II enzyme. Additionally the Type I PtdIns(4)P 5-kinase phosphorylates PtdIns(4)P in isolated erythrocyte membranes, whereas the Type II PtdIns(4)P 5-kinase has no activity towards membrane PtdIns(4)P.

PHOSPHOLIPASE C

PtdIns-specific PLC enzymes catalyze the hydrolysis of phosphatidylinositols to produce messenger molecules. These enzymes hydrolyze PtdIns, PtdIns(4)P, and PtdIns(4,5)P_2 to yield six water-soluble inositol phosphates corresponding to cyclic 1:2 phosphates and 1 phosphates of inositol mono, bis, and tris phosphates (Figure 1). There are multiple PLC enzymes in mammalian tissues as deduced from direct protein isolation and molecular cloning studies (25, 26). Nine isoforms of PLC identified to date have been categorized into four groups, designated α, β, γ, and δ (6).

None of the PLC isoforms contains a membrane-spanning sequence with the possible exception of PLCα. The phospholipid substrates for these enzymes are all in membrane bilayers; therefore PLC must bind to membranes before hydrolyzing phosphatidylinositols. Reversible membrane binding of PLC is an attractive hypothesis to explain changes in PtdIns turnover upon stimulation of cells by agonists, although such has not been demonstrated experimentally.

ACTIVATION OF PHOSPHOLIPASE C BY $G_\alpha Q$

Numerous studies have demonstrated that GTP and its nonhydrolyzable analogues stimulate PtdIns turnover in permeabilized cells, crude membrane fractions, or partially purified enzyme preparations (28). This has led to speculation that a guanine nucleotide–binding protein (G protein) is involved in coupling between receptors and PLC.

Recently a new class of G protein, designated Gq, that activates the PLCβ1 isoform of PLC has been discovered. Pang & Sternweis (29) isolated $G_\alpha q$ from rat brain by a novel strategy utilizing affinity chromatography on columns containing $\beta\gamma$ subunits. Detergent extracts of brain membranes were allowed to bind to the affinity column in the presence of GDP. When the extract was passed very slowly over the column, the α subunits of the G proteins dissociated from endogenous $\beta\gamma$ and bound to the matrix $\beta\gamma$ subunits. The α subunits of the G proteins were then eluted from the column by

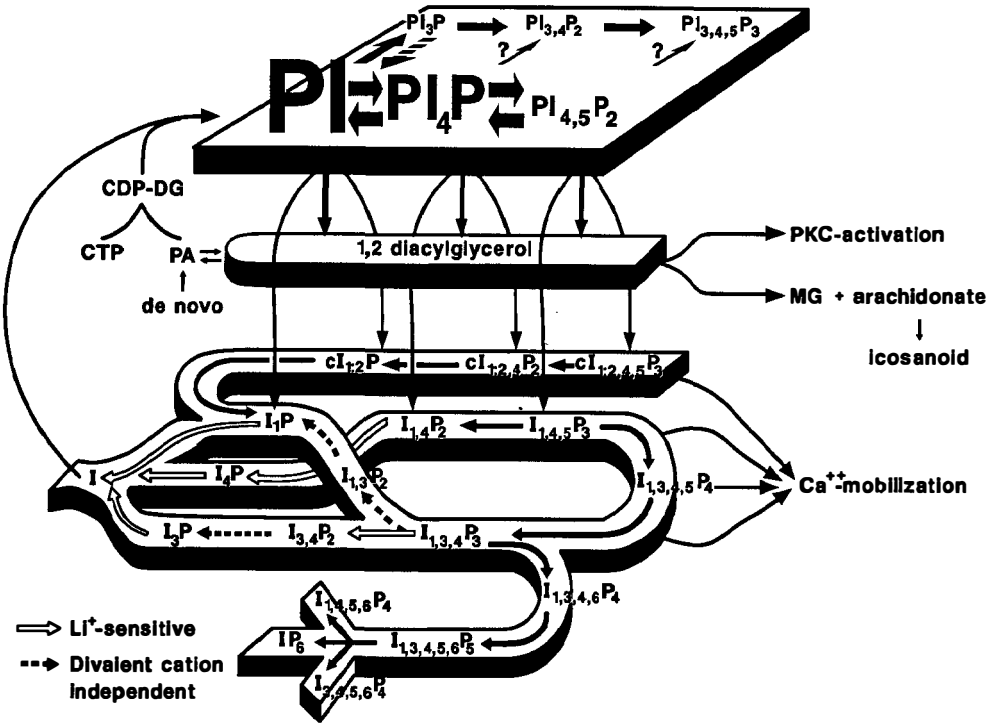

Figure 1 Pathway for inositol phosphate metabolism. In the top level, PI indicates phosphatidylinositol: PI_4P, phosphatidylinositol 4-phosphate; PI_3P, phosphatidylinositol 3-phosphate; $PI_{3,4}P_2$, phosphatidylinositol 3,4-bisphosphate; $PI_{3,4,5}P_3$, phosphatidylinositol 3,4,5-trisphosphate; and $PI_{4,5}P_2$, phosphatidylinositol 4,5-bisphosphate. On the bottom level, I, inositol; P, phosphate. The numbers preceding P refer to positions of phosphates on the inositol ring, and those following P, the number of phosphate groups. PA, phosphatidic acid; CDP-DG, cytidine diphosphate diacylglycerol; PKC, protein kinase C; MG, 2 monoacylglycerol.

addition of the nonhydrolyzable GTP analog, GTPγS. $G_\alpha q$ remained bound to the column under these conditions because it exchanges GDP for GTPγS very slowly, but eluted with aluminum fluoride. Aluminum fluoride binds to GDP on $G_\alpha q$ and thereby mimics GTP, causing dissociation of $G_\alpha q$ from the column. Through use of this method, two novel α subunits were obtained, $G_\alpha q$ and $G_\alpha 11$. Limited sequence information indicated that these proteins correspond to two G protein α subunits that were cloned by Strathmann & Simon (30) using a strategy based on the polymerase chain reaction (PCR). Taylor et al have also isolated $G_\alpha q$ from bovine liver by a different strategy (31). These workers treated liver membranes with GTPγS overnight prior to solubilization with detergents and in this way identified a fraction that could be separated from PLC and that stimulated PLC activity. The isolated G protein was shown to be Gq by immunoblotting with antipeptide antibodies specific for Gq.

The $G_\alpha q$ subunit specifically activates PLCβ1 and has no stimulatory effect on other isoforms of PLC tested, including PLCγ and PLCδ (32). Taylor et al demonstrated a 30-fold stimulation of PLCβ1 by $G_\alpha q$ from approximately 10 μmol/min/mg protein to almost 300 μmol/min/mg protein. The activation was independent of calcium ion concentration. In similar studies, Smrcka et al (33) found that a preparation containing PLCβ1 was markedly stimulated by Gq. However, in this case Gq appeared to lower the affinity of the enzyme for calcium ions. At 0.1 μM free calcium, marked stimulation occurred (~30-fold), whereas at 20 μM calcium only a threefold stimulation occurred. These studies identify at least one isoform of PLC that is activated by a traditional G protein mechanism. Whether other PLC isoforms are similarly activated by other types of G proteins remains to be elucidated.

ACTIVATION OF PLCγ BY TYROSINE PHOSPHORYLATION

Whereas the direct activation $PLC\beta_1$ by Gq-type G proteins has been convincingly demonstrated, the mode of activation of PLCγ by tyrosine protein kinase receptors is not clear. PLCγ has been shown to be phosphorylated on tyrosine residues in cells responding to platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) (34–39). In cells containing EGF receptors and PLC-β, -δ, and -γ, only PLCγ is phosphorylated in response to EGF stimulation of intact cells (34). Although it is clear that PLCγ is a substrate for receptor tyrosine protein kinases, an effect of the phosphorylation on the activity of the enzyme has not been demonstrated. In fact, the magnitude of phosphorylation of PLCγ on tyrosine is variable. For example, in A431 cells, which have large numbers of EGF receptors, up to 50% of PLCγ may be phosphorylated (40); in PDGF-stimulated Balb 3T3 cells less than 5% of PLCγ complexes with receptors and most is not phosphorylated, and much of

the tyrosine-phosphorylated PLCγ is found in the cytosol (41). Kim et al (42) have demonstrated that EGF receptor kinase phosphorylates PLCγ stoichiometrically in vitro on tyrosines 472, 771, 783, and 1254. However, phosphorylation had no effect on PLC activity as measured using PtdIns(4,5)P_2 as substrate at a variety of calcium ion concentrations. In contrast, Nishibe et al (43) immunoprecipitated PLCγ from homogenates of A431 cells, phosphorylated it in vitro with EGF receptor kinase, and found approximately threefold increased PLC activity in the immunoprecipitates. They showed that treatment of these precipitates with a protein tyrosine phosphatase (T-cell phosphatase) both decreased tyrosine phosphate and reduced PLC activity by 50%. However, Goldschmidt-Clermont et al (44) found that PLCγ phosphorylated by the EGF receptor had only 25% increased enzyme activity using PtdIns(4,5)P_2 as substrate. Factors present in the immunoprecipitates (43) may account for the differences in the observed results.

Kim et al (45) have transfected mutant forms of PLCγ with substitution of phenylalanine for tyrosine into NIH 3T3 cells and studied the effects on PLC activity measured in cell homogenates versus inositol phosphate production in response to stimulation of intact cells with PDGF. Thus the former monitors the effects of tyrosine phosphorylation on enzyme activity, whereas the latter measures the effect of tyrosine phosphorylation on the coupling of phosphatidylinositol turnover to PDGF stimulation in intact cells. When either tyrosine 783 or 1254 was mutated to phenylalanine, cellular phosphatidylinositol turnover in response to PDGF was either totally ablated (Tyr783) or attenuated (Tyr1254). Homogenates from both of these mutants expressed the same PLC activity in vitro as those from cells transfected with native PLCγ. This study shows that phosphorylation of PLCγ is necessary for increased phosphatidylinositol turnover in cells stimulated with PDGF. The authors (45) speculate that tyrosine phosphorylation may reverse some inhibitory factor not apparent in enzyme assay of homogenates.

One cytosolic protein that may affect PLCγ activity is the actin-binding protein profilin. Goldschmidt-Clermont (44) showed that profilin binds PtdIns(4,5)P_2, inhibiting the ability of the lipid to be hydrolyzed by unphosphorylated PLCγ. In the presence of 50 μM profilin, PtdIns(4,5)P_2 hydrolysis by PLCγ was reduced fivefold. However, when PLCγ was phosphorylated by the EGF receptor, no inhibition was observed. Interestingly, profilin did not inhibit the action of PLCβ on PtdIns(4,5)P_2. One problem with this study is that the assay conditions used involved substrate vesicles containing inhibitory phospholipids, including phosphatidylserine and/or phosphatidylcholine. Thus, PLC activity in the absence of profilin was only $\sim$0.1% of that obtained under optimal conditions.

Another compound that may affect PLCγ activity is glucosphingolipid. Shayman et al (46) have shown that reduction of cellular glucosphingolipids

with an inhibitor of glucosylceramide synthatase, threo-1-phenyl-2-decanoyl-amino-3-morpholino-1-propanol, increases phosphatidylinositol turnover in response to bradykinin or GTγS in MDCK cells. However, it is not known which PLC isozymes are present in MDCK cells.

INOSITOL POLYPHOSPHATE 5-PHOSPHATASE

There are multiple isoforms of inositol polyphosphate 5-phosphatase enzymes distributed between soluble and particulate fractions of various tissues. The 5-phosphatase acts on three substrates (Figure 1), namely Ins(1,4,5)P_3, Ins-(1,3,4,5)P_4 and cIns(1:2,4,5)P_3 (3).

Two forms of cytosolic 5-phosphatase have been isolated from human platelets (47, 48). Type I enzyme has an apparent molecular weight of 45,000 and requires Mg^{2+}. The K_m of Type I 5-phosphatase for Ins(1,3,4,5)P_4 is 0.5 μM, much lower than that for Ins(1,4,5)P_3 (K_m = 7.5 μM). The V_{max} for Ins(1,3,4,5)P_4 is only 2% of that obtained using Ins(1,4,5)P_3 as substrate (49). Consistent with these findings, low concentrations of Ins(1,3,4,5)P_4 (1 μM) inhibit the breakdown of either Ins(1,4,5)P_3 or cIns(1:2,4,5)P_3 in homogenates. This implies that cellular levels of $InsP_3$ metabolites may be sustained under conditions where Ins(1,3,4,5)P_4 is formed. Type I 5-phosphatase from human platelets is phosphorylated by protein kinase C (50), and this phosphorylation is associated with increased 5-phosphatase activity (50–52). Thus, protein kinase C action tends to reduce $InsP_3$ levels and terminate signalling.

Type II 5-phosphatase from human platelets hydrolyzes the same substrates as the Type I enzyme, although the kinetic parameters are different (K_m values for Ins(1,4,5)P_3 = 24 μM, and for Ins(1,3,4,5)P_4 = 7.5 μM) (48). The Type II enzyme has a molecular weight of 75,000 and is not phosphorylated by protein kinase C. Recently, a cDNA encoding human platelet Type II 5-phosphatase has been isolated by screening a placental λgt11 cDNA library for β-galactosidase fusion proteins that bind to Ins(1,3,4,5)P_4 (53). The sequences derived from the expression clone were used to screen human erythroleukemia and megakaryocyte cell cDNA libraries. The composite cDNA isolated consists of 2381 bp and predicts a protein that includes the amino-terminal amino acid sequence (19 residues) obtained from the platelet 75-kDa Type II 5-phosphatase. The cDNA predicts that the mature enzyme contains 635 amino acids (M_r = 72,891). The recombinant protein expressed in Cos-7 cells has the same intrinsic 5-phosphatase activity as the platelet 5-phosphatase as determined by immunoblotting.

All of the 5' cDNA sequences isolated to date maintain an open reading frame 5' to the predicted amino-terminal sequence of the platelet Type II enzyme. Thus it is possible that the platelet 5-phosphatase is formed by proteolytic processing of a larger precursor. The full length of the transcript

based on Northern blotting is 4.4 kilobases. Antibodies directed against recombinant Type II 5-phosphatase immunoprecipitate Type II enzyme from homogenates of platelets and do not cross-react with Type I enzyme, further supporting the idea that two distinct isoenzymes are present.

There is no sequence homology between Type II 5-phosphatase and other cloned proteins of the phosphatidylinositol pathway, including PLC, Ins-(1,4,5)P_3 3-kinase, inositol monophosphate phosphatase, inositol cyclic 1:2 phosphate 2-phosphohydrolase, inositol polyphosphate 1-phosphatase, or the Ins(1,4,5)P_3-binding protein. In addition no homologies were found when comparisons were made to sequences in the Genbank data base. Sequence analysis of the predicted protein identified several potential phosphorylation consensus peptide sequences (54) for cGMP-dependent protein kinase, proline-dependent kinase, casein kinase I, casein kinase II, mammary gland casein kinase, and glycogen synthetase kinase-3. Consistent with the lack of phosphorylation of Type II 5-phosphatase by protein kinase C in vitro, there are no putative protein kinase C phosphorylation sites.

5-phosphatase enzymes have been partially characterized in several other tissues. The characteristics of these enzymes suggest that additional isoforms of 5-phosphatase are very likely present. Thus molecular weights in excess of 100,000 have been noted as well as a near inability to hydrolyze Ins-(1,3,4,5)P_4 by some isoforms. In particular, two distinct 5-phosphatase enzymes have been identified in rat brain (55, 56) and bovine brain (57). 5-phosphatase activities have also been characterized in extracts from macrophages (58), pancreas (59), erythrocytes (60), and liver (61–63). In hepatocytes, it appears that the particulate 5-phosphatase may be assymetrically localized between canilicular and sinusoidal membranes (64).

INS(1,4,5)P_3 3-KINASE

Another enzyme that utilizes Ins(1,4,5)P_3 as a substrate is Ins(1,4,5)P_3 3-kinase, a widely distributed soluble enzyme that converts Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4 in the presence of Mg^{2+} and ATP (7). The 3-kinase enzyme phosphorylates Ins(1,4,5)P_3 but not cIns(1:2,4,5)P_3 (49, 65). The Ins-(1,4,5)P_3 3-kinase has low K_m for Ins(1,4,5)P_3 (0.2 to 1.5 μM) (66–69). Thus the 3-kinase may compete effectively with the inositol polyphosphate 5-phosphatases for Ins(1,4,5)P_3, since the K_m for the latter enzymes are higher (7–25 μM) (48, 50, 64).

The native enzyme is composed of two catalytic subunits of 53 kDa plus calmodulin. Physiological concentrations of Ca^{2+} stimulate Ins(1,4,5)P_3 3-kinase activity (67–70) via the calmodulin subunit (68, 71–74). Ins(1,4,5)P_3 3-kinase may also be regulated by phosphorylation. Addition of phorbol esters to intact platelets or to a malignant T-cell line causes a twofold increase in 3-kinase activity (52, 75). In hepatocytes, the enzyme is activated by

stimulation of protein kinase A and protein kinase C together (76). In contrast, when isolated Ins(1,4,5)P_3 3-kinase is phosphorylated in vitro with protein kinase C, it is inhibited (77, 78). In vitro phosphorylation of the isolated enzyme by cAMP-dependent protein kinase increased the apparent enzyme activity (77). The site of phosphorylation by protein kinase A was serine 109, whereas multiple sites of phosphorylation by protein kinase C were observed including serine 109 and serine 175.

Results of other studies suggest additional mechanisms for regulation of Ins(1,4,5)P_3 3-kinase. Cellular Ins(1,4,5)P_3 3-kinase activity was increased 6- to 8-fold in cytosolic extracts prepared from vSRC-transformed cells (79). Striking developmental changes in the activity of 3-kinase occur in rat brain, where enzyme activity increases 15-fold from birth to 13 weeks of age (80).

Ins(1,4,5)P_3 3-kinase has been isolated from rat and bovine brain (69, 81, 82). The enzyme is susceptible to proteolysis by calpain, which apparently accounts for the finding of multiple molecular-weight forms ranging from 32 to 53 kDa. A cDNA encoding Ins(1,4,5)P_3 3-kinase has been isolated from a rat liver cDNA library and predicts a protein of 449 amino acids with a molecular weight of 49,853 (83). The protein has no sequence relationship to other enzymes of inositol phosphate metabolism. There are four short sequences from different regions of the molecule that are 20 to 50% identical to sequences in the Ins(1,4,5)P_3-binding protein.

NIH 3T3 cells and CCL39 hamster fibroblasts stably transfected with the 3-kinase cDNA overexpress InsP_3 3-kinase 16- to 18-fold (84). These cells display lower levels of Ins(1,4,5)P_3 and higher levels of Ins(1,3,4,5)P_4 after stimulation with thrombin, PDGF, or bombesin, consistent with increased kinase activity. Blunted calcium mobilization was observed, presumably due to lower levels of Ins(1,4,5)P_3. The previously proposed role for Ins(1,3,4,5)P_4 in calcium mobilization (85) is not supported by this study, since in the 3-kinase-transfected cells the elevated Ins(1,3,4,5)P_4 levels had no effect in promoting calcium ion mobilization. Further study of the physiology of these cells will be required to uncover a function for Ins(1,3,4,5)P_4 or other "downstream" metabolites elevated in these cells.

INOSITOL POLYPHOSPHATE 1-PHOSPHATASE

Inhorn et al (86) discovered a phosphatase in calf brain homogenates that hydrolyzes Ins(1,3,4)P_3 and Ins(1,4)P_2 to Ins(3,4)P_2 and Ins(4)P, respectively. The enzyme was designated inositol polyphosphate 1-phosphatase, although it does not hydrolyze other 1-phosphate-containing inositol polyphosphates. Subsequently, others have found this enzyme in brain and liver (87). The enzyme has been isolated from calf brain and is a monomeric protein of 44 kDa (88, 89). The K_m for Ins(1,4)P_2 is 4 to 5 μM and that for Ins(1,3,4)P_3 is 20 μM. The enzyme requires magnesium ions and is inhibited

at physiological calcium ion concentrations (88, 90). Rabbit polyclonal antisera against 1-phosphatase inhibits hydrolysis (greater than 95%) of Ins(1,4)P_2 in extracts of bovine tissues, suggesting that 1-phosphatase accounts for the majority of Ins(1,4)P_2 hydrolysis (88). Lithium ions inhibit both Ins(1,3,4)P_3 and Ins(1,4)P_2 hydrolysis uncompetitively with a K_i of 0.3 mM and 6.0 mM, respectively (88, 90). The differences in K_i for lithium are consistent with the uncompetitive mode of inhibition, i.e. the inhibitor reacts only with the enzyme-substrate complex (88–91). The inhibitory potency of lithium for Ins(1,3,4)P_3 hydrolysis suggests that inhibition at this step may account for the pharmacologic action of lithium ions used to treat psychiatric disorders, since lithium levels in patients are approximately 1 mM (92).

Bovine brain inositol polyphosphate 1-phosphatase has been digested with cyanogen bromide, and trypsin and peptide fragments were isolated and sequenced (93). The sequence was used to design degenerate oligonucleotide primers that were used to isolate a 1572-bp cDNA with an open reading frame of 400 amino acids, corresponding to a predicted molecular weight of 43,980. The predicted 1-phosphatase amino acid sequence is not similar to any other sequences in Genbank with the exception of slight homology to inositol monophosphate phosphatase (93). In one region of the molecule, 9 of 20 amino acids are identical and in another segment 6 of 15 amino acids are identical. It is of interest that these similar regions are also conserved in three proteins found in *Escherichia coli*. These proteins have pleiotropic effects when mutated but have no other known functions (94). It is possible that they are signalling molecules of *E. coli*.

The cDNA for the 1-phosphatase has been expressed in *E. coli*. The recombinant protein has the same specific activity, affinity for substrates, and inhibition by lithium ions as native inositol polyphosphate 1-phosphatase. These results suggest that the activity of the native protein does not require posttranslational modifications.

The cDNA for bovine brain inositol polyphosphate 1-phosphatase was used to screen a human umbilical vein endothelial cell cDNA library to obtain a 1.75-kilobase cDNA encoding human inositol polyphosphate 1-phosphatase (J. D. York, P. W. Majerus, unpublished observations). The predicted amino acid sequence of the human 1-phosphatase is 82% identical to that of the bovine brain clone.

INOSITOL POLYPHOSPHATE 4-PHOSPHATASE

Inositol polyphosphate 4-phosphatase converts Ins(1,3,4)P_3 to Ins(1,3)P_2 and Ins(3,4)P_2 to Ins(3)P. The enzyme does not require metal ions, is not inhibited by Li^+ (95), and does not hydrolyze the 4-phosphate from other inositol polyphosphates (90, 95, 96). Bansal et al have purified this enzyme 3400-fold from calf brain–soluble extract (97). The isolated enzyme has an

apparent molecular mass of 110 kDa as determined by gel filtration. On SDS polyacrylamide gel electrophoresis the protein migrates at 105 kDa, suggesting that it is monomeric.

The inositol polyphosphate 4-phosphatase has apparent K_m values of 40 and 25 μM for Ins(1,3,4)P_3 and Ins(3,4)P_2, respectively. The maximal velocities for these two substrates are 15 to 20 μM of product/minute/mg protein. Ins(1,3,4)P_3 is a competitive inhibitor of Ins(3,4)P_2 hydrolysis with an apparent K_i of 27 μM, implying that the same active site is involved in hydrolysis of both substrates. The ratio of inositol polyphosphate 1-phosphatase to inositol polyphosphate 4-phosphatase activity in hydrolyzing their common substrate Ins(1,3,4)P_3 varies among tissues. Only 5 to 20% of Ins(1,3,4)P_3 is utilized by inositol polyphosphate 4-phosphatase in various tissues, except in brain, where approximately 70% is metabolized by 4-phosphatase. These observations suggest that Ins(1,3)P_2 or a further metabolite plays a role in neuronal function.

INOSITOL POLYPHOSPHATE 3-PHOSPHATASE

Inositol polyphosphate 3-phosphatase catalyzes the hydrolysis of the 3-position phosphate bond of inositol 1,3-bisphosphate [Ins(1,3)P_2] to form inositol 1-phosphate and inorganic phosphate (95). Two isoforms of this enzyme designated Types I and II have been isolated from rat brain (98). The Type I 3-phosphatase consists of a protein doublet that migrates with a molecular mass of 65,000 upon SDS polyacrylamide gel electrophoresis. The molecular weight of this isoform upon size exclusion chromatography is 110,000, suggesting that the native enzyme is a dimer. The Type II enzyme consists of equal amounts of a molecular weight 65,000 doublet and a molecular weight 78,000 band upon SDS polyacrylamide gel electrophoresis. This isoform has a molecular weight of 147,000 upon size exclusion chromatography, indicating that it is a heterodimer. The 65,000-Da subunits of the two forms of 3-phosphatase appear to be the same based on comigration on SDS polyacrylamide gels and peptide maps generated with *Staphylococcus aureus* protease V8 and trypsin. The peptide map of the 78,000-Da subunit was different from that of the 65,000-Da subunits. The Type II 3-phosphatase catalyzes the hydrolysis of Ins(1,3)P_2 with a catalytic efficiency 1/19th of that measured for the Type I enzyme, suggesting that the presence of the 78-kDa subunit decreases activity. Both isoforms of 3-phosphatase enzyme also hydrolyze phosphatidylinositol 3-phosphate [PtdIns(3)P] to form phosphatidylinositol and inorganic phosphate. The Type II enzyme is more active than Type I enzyme in hydrolyzing Ptd Ins(3)P. Thus far this is the only example in inositol phosphate metabolism of an enzyme that hydrolyzes both a lipid and corresponding water-soluble substrate.

INOSITOL MONOPHOSPHATASE

The other enzyme of inositol phosphate metabolism that is inhibited by Li^+ in vitro is inositol monophosphatase. This enzyme was initially designated as inositol 1-phosphatase, but since it hydrolyzes phosphate groups of all inositol monophosphates with the exception of inositol 2-phosphate, its name has been changed to inositol monophosphate phosphatase (90, 99). Inositol monophosphate phosphatase requires Mg^{2+} for activity and other divalent ions, i.e. Ca^{2+} and Mn^{2+}, are competitive inhibitors (100). Hallcher & Sherman (100) demonstrated that Li^+ inhibits inositol monophosphate phosphatase uncompetitively with an apparent K_i of 0.8 μM; thus this enzyme is another potential target for the action of lithium as a therapeutic agent. Inositol monophosphate phosphatase has been purified to homogeneity from bovine and rat brain (101, 102). The enzyme (M_r = 55,000) is a dimer of identical subunits (100–102). A cDNA encoding inositol monophosphatase was recently isolated and predicts a protein of 277 amino acids. The recombinant protein has been expressed in *E. coli* and found to have the same activity and inhibition by Li^+ as native monophosphatase (103). The protein is not homologous to any other proteins in the Genbank data base with the exception of inositol polyphosphate 1-phosphatase and the bacterial proteins mentioned above. Modification of monophosphatase by the arginine-specific reagent phenylglyoxal results in enzyme inactivation, suggesting that an arginine is in the active site of this enzyme. One mole of arginine is modified per mole of protein in the process of enzyme inactivation (104).

INOSITOL (1:2 CYCLIC) PHOSPHATE 2-PHOSPHOHYDROLASE AND INOSITOL CYCLIC PHOSPHATES

The metabolism of inositol cyclic phosphates is distinct from that of the more widely studied noncyclic counterparts. They are produced by PLC and subsequently metabolized by distinct enzymes (Figure 1). The existence of inositol cyclic phosphates in vivo has been documented in a wide variety of cells and tissues (105–115). It is not clear what determines the ratio of cyclic versus noncyclic phosphates produced upon stimulation of cells with various agonists. In general, prolonged stimulation of cells or tissues tends to produce higher levels of cyclic phosphates, in part because these metabolites are metabolized slowly (109, 112). For example, inositol polyphosphate 5-phosphatase hydrolyzes cIns(1:2,4,5)P_3 very poorly compared to Ins(1,4,5)P_3 (100- to 200-fold less), which may explain the accumulation of cyclic trisphosphate in cells (116). A 4-phosphatase in bovine brain hydrolyzes cIns(1:2,4)P_2 and requires magnesium ions (V. S. Bansal, P. W. Majerus,

unpublished observations). This phosphatase differs from inositol polyphosphate 4-phosphatase.

Inositol cyclic (1:2) phosphate 2-phosphohydrolase (cyclic hydrolase) is the only enzyme known to hydrolyze the cyclic phosphate bond, and this enzyme only utilizes inositol cyclic (1:2) phosphate [cIns(1:2)P] as a substrate. Since all inositol cyclic phosphates are finally metabolized by this enzyme, it is likely that it regulates cellular levels of these molecules. Both Zn^{2+} and Ins(2)P are potent inhibitors, while Mn^{2+} and acidic phospholipids stimulate the enzyme (117–119). Cyclic hydrolase also catalyzes the hydrolysis of a second substrate glycerophosphoinositol to form glycerol and inositol 1-phosphate (118). As in the case of hydrolysis of inositol cyclic phosphates (117), the glycerophosphoryl derivatives with additional monoester phosphate groups in the 4 or 4 and 5 positions are not substrates for cyclic hydrolase.

Cyclic hydrolase has been isolated from human placenta and characterized (117, 118) and shown to be identical to lipocortin III (119). Lipocortin III is one of eight related proteins that bind lipids and calcium ions (120–122) but have no previously known physiological functions (123, 124), and have recently been renamed *annexins*. In order to investigate possible functions for inositol cyclic phosphates, Ross et al have transfected a cDNA encoding cyclic hydrolase into 3T3 cells (125). 3T3 cells that overexpress cyclic hydrolase (3–10-fold) have decreased cellular levels of cIns(1:2)P, indicating that alterations in the cellular levels of the enzyme directly alter cellular cIns(1:2)P levels. Cells with increased cyclic hydrolase activity grow to a lower density at confluence (4-fold) than untransfected cells or cells transfected with vector alone. Similar results were obtained when the growth properties of several different cell types with varying endogenous cyclic hydrolase activity (100-fold range) were examined. Those cells with high levels of cyclic hydrolase had correspondingly low cIns(1:2)P levels and grew to a lower density at confluence than cells expressing low levels of enzyme (4-fold range) (125). Cyclic hydrolase levels have also been found to be lower within a given cell line during active cell growth than when cells are confluent. These studies suggest that cyclic hydrolase is antiproliferative and could be an example of an antioncogene. Whether cyclic hydrolase can reverse or prevent the transformed phenotype remains to be determined.

While cyclic hydrolase is important in determining cellular cIns(1:2)P levels, another way to vary this metabolite is through alterations in PLC activity. Camilli et al have shown that an essential virulence factor in the intracellular pathogen *Listeria monocytogenes* is a PLC enzyme (126). This enzyme produces only cIns(1:2)P and diacylglycerol from PtdIns. Thus it is possible that overproduction of either or both of these metabolites is required to produce disease.

OTHER ENZYMES OF INOSITOL PHOSPHATE METABOLISM

Several other enzymes involved in $InsP_4$ metabolism have been partially characterized. Ins(1,3,4,6)P_4 is formed by a soluble 6-kinase that metabolizes Ins(1,3,4)P_3 (127–130). The 6-kinase is widely distributed and is not affected by either calcium or lithium ions (129). A 3-phosphatase that converts Ins(1,3,4,5)P_4 to Ins(1,4,5)P_3 has been described in cell extracts (131–135). It has been suggested that this enzyme may actually be involved in $InsP_5$ and $InsP_6$ metabolism, since these substances are potent inhibitors of this 3-phosphatase (64).

INOSITOL PENTAPHOSPHATE AND HEXAPHOSPHATE METABOLISM

The metabolism of inositol phosphates containing 5 and 6 phosphate esters is not fully understood. Further, it appears that there are differences among organisms in the particular isomers of $InsP_5$ present. There are six possible $InsP_5$ isomers, most of which have been found in various plant species and *Dictyostelium* (136). In animal cells, the major $InsP_5$ isomer is Ins-(1,3,4,5,6)P_5, although other isomers including traces of (D,L)Ins(1,2,4,5,6)P_5 and (D,L)Ins(1,2,3,4,5)P_5 have been found in HL60 cells and NG1 15 401L-C3 cells (136, 137). In animal cells, the major pathway for $InsP_5$ synthesis appears to be that outlined in Figure 1. Thus, Ins(1,3,4,5)P_4 is isomerized to Ins(1,3,4,6)P_4 via sequential action of inositol polyphosphate 5-phosphatase and Ins(1,3,4)P_3 6-kinase. Ins(1,3,4,6)P_4 is converted to Ins-(1,3,4,5,6)P_5 by a 5-kinase (128, 130, 139). Ins(1,3,4,5,6)P_5 is further metabolized to three compounds: $InsP_6$, Ins(3,4,5,6)P_4, and Ins(1,4,5,6)P_4 (the latter two compounds are an enantiomeric pair and thus not separable by most conventional means) (127–130, 138, 140).

The mass of $InsP_5$ and $InsP_6$ in HL60 cells has been measured as 35 μM and 50 μM, respectively (137). These levels are approximately 100-fold greater than those of other inositol phosphate metabolites. In HL60 cells stimulated with formyl-methionyl-leucyl-phenylalanine (FMLP), the levels of $InsP_5$ and $InsP_6$ rise within two minutes to 50 μM and 60 μM, respectively. Thus, these compounds appear to undergo accelerated synthesis in response to agonists. In studies of $InsP_5$ and $InsP_6$ turnover, as measured by changes in [^{3}H] inositol incorporation into these compounds in response to agonists, conflicting results have been obtained, suggesting increased, decreased, or no change in turnover. Much of the variation in these results may be accounted for by the fact that isotope incorporation can reflect either changes in mass, specific activity, or a combination of the two. Many investigators assume that

labelling of tissue-culture cells with [^{3}H]inositol for 48 to 72 hours yields steady-state labelling wherein all metabolites of inositol have the same specific activity. This appears not to be the case in some instances and is never proven without mass measurements.

In HL60 cells labelled with [^{3}H]Inositol for 48 hours (137), the specific activity of Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4, and InsP_5 was similar, yet upon stimulation with FMLP there was a rapid rise in specific activity of these compounds, thus indicating that they were not in equilibrium with precursor inositol phosphate metabolites. The specific activity of (D,L)Ins(3,4,5,6)P_4 was approximately half that of InsP_5 in unstimulated HL60 cells, implying that this metabolite turns over very slowly. Upon stimulation with FMLP, labelling of (D,L)Ins(3,4,5,6)P_4 increased rapidly, suggesting accelerated turnover of this metabolite. Similar results were found in bombesin-stimulated AR4-2J cells (140).

The pathway for metabolism of these compounds in avian erythrocytes appears to be quite different. Stephens & Downes have analyzed InsP_4 and InsP_5 metabolism by labelling erythrocytes with [^{3}H]Inositol and $^{32}PO_4$ (139). They analyzed the incorporation of $^{32}PO_4$ into various monoester phosphates of Ins(1,3,4,5,6)P_5 and Ins(3,4,5,6)P_4 in a protocol similar to that used by Cunningham et al (141) to determine pathways of 3-phosphate containing inositol phospholipid metabolism (see below). In this analysis the most highly labelled phosphate is the last added to the molecule. They found that the 1-position phosphate of Ins(1,3,4,5,6)P_5 is most highly labelled, suggesting that its precursor is Ins(3,4,5,6)P_4 rather than Ins(1,3,4,6)P_4 as shown in Figure 1. In fact, the overall scheme they propose is Ins(4,6)P_2 → Ins(3,4,6)P_3 → Ins(3,4,5,6)P_4 → Ins(1,3,4,5,6)P_5. This pathway predominates despite the fact that avian erythrocytes have the enzymes to form both Ins(1,3,4,5)P_4 and Ins(1,3,4,6)P_4. It will be interesting to analyze the InsP_4 and InsP_5 isomers of animal cells by this same strategy.

The functions of InsP_4, InsP_5, and InsP_6 in signalling remain a mystery. InsP_5 and InsP_6 have been proposed to be extracellular agonists that stimulate neuronal excitability and reduce heart rate and blood pressure (142). It is unclear, however, how these compounds could exit cells. InsP_5 does reduce the affinity of hemoglobin for oxygen in erythrocytes of birds (143). Proteins that bind InsP_4, InsP_5, and InsP_6 have been identified recently, as described below.

INOSITOL PHOSPHATE–BINDING PROTEINS

The action of Ins(1,4,5)P_3 in calcium ion mobilization has been validated through identification and isolation of an Ins(1,4,5)P_3-binding protein that serves as a calcium channel across various membranes (144, 145). The

isolated Ins(1,4,5)P_3-binding protein has a subunit polypeptide chain of 260 kDa upon SDS polyacrylamide gel electrophoresis, but a molecular weight of approximately 1 million upon size exclusion chromatography, thus implying that the native molecule is a tetramer (144). Cross-linking studies of the isolated protein also suggest a tetramer (146). The binding protein had a K_d for Ins(1,4,5)P_3 of ~10 nM. Other inositol phosphates displace bound Ins-(1,4,5)P_3 poorly (1000-fold higher concentration).

The Ins(1,4,5)P_3-binding protein has been reconstituted into lipid vesicles and shown to directly mediate the transfer of calcium across the lipid bilayer (147). Photo-affinity labelling and direct binding studies have shown that the protein contains an ATP-binding site and further that ATP affects the flux of calcium (146, 148).

The distribution of the Ins(1,4,5)P_3-binding protein in brain is highly enriched in purkinje cells, where 10 to 100 times greater concentration of this protein is present than in other cells (148, 149). Mikoshiba and coworkers noted that the enrichment in purkinje cells and other properties of the Ins-(1,4,5)P_3-binding protein suggested that it was likely to be similar or identical to a protein that they had previously characterized as abundant in purkinje cells and nearly lacking in cerebella of mice with various mutations in which purkinje cells are deficient or absent (150). These workers utilized monoclonal antibodies to isolate a cDNA encoding the Ins(1,4,5)P_3-binding protein (151). The full-length transcript is approximately 10 kilobases and encodes a protein of 2749 amino acids with a molecular weight of 313,000. The predicted protein contains an ATP-binding motif and several putative transmembrane domains near the carboxyl terminus of the molecule. The majority of the protein is thought to reside in the cytoplasm of the cell with the carboxyl terminus in the lumen of the endoplasmic reticulum (i.e. outside the cell).

These authors concluded that there might be as many as seven membrane-spanning domains contained within the molecule. Based on studies with monoclonal antibodies, they concluded that the number of membrane-spanning domains was odd and therefore the amino terminus and carboxyl terminus were on opposite sides of the membrane. They and others also noted a striking homology between the Ins(1,4,5)P_3-binding protein and the ryanodine receptor (152). The homology is particularly strong in the carboxyl-terminal membrane-spanning domains. One puzzling difference is that the ryanodine receptor has an even number of membrane-spanning domains and the carboxyl terminus is on the cytoplasmic face of the membrane. Both the Ins(1,4,5)P_3-binding protein and ryanodine receptor appear to be tetramers of similar structure as determined by electron microscopy, and both are involved in the movement of calcium across cell membranes.

A partial cDNA encoding the purkinje cell Ins(1,4,5)P_3-binding protein had been isolated previously by obtaining cDNA clones encoding proteins

uniquely expressed in mouse purkinje cells. This was achieved by using a library of normal mouse cerebellar cDNA subtracted with cDNA sequences from the cerebella of mice with mutations causing purkinje cell degeneration (153, 154). It is interesting that the predicted mass of the monomeric Ins-(1,4,5)P_3-binding protein is 313 kDa (plus some carbohydrates since the molecule is a glycoprotein), but by SDS polyacrylamide gel electrophoresis the molecular weight appears to be 260 kDa (155, 156). Recently a cDNA for rat brain Ins(1,4,5)P_3-binding protein was reported (157).

Several different forms of Ins(1,4,5)P_3-binding protein cDNA have been identified (158). Nakagawa et al found five different subtypes in various tissues during mouse brain development that they postulate arise by alternatively spliced exons. They used an S1 nuclease protection assay of RNA from different tissues and from mouse brain at different times after birth. They found that a "short-form" RNA with a deletion of 15 amino acids predominates in adult brain and accounts for 20–80% of the mRNA in other adult tissues. Danoff et al (159) found the same short form in rat brain by a PCR assay, but curiously found the opposite developmental correlation in that the short form was in fetal brain while the long form predominates in adult tissues. The significance of the different forms is unknown, and the lack of similarity of distribution in closely related species may suggest that the variant forms are not functionally important. Alternatively, the PCR assay (159) may not accurately reflect quantities of mRNA in various tissues.

While most of the physiological studies have suggested that calcium release is primarily from endoplasmic reticulum, localization of the Ins(1,4,5)P_3-binding protein by immunohistochemical methods suggests a potentially wider distribution of calcium-mobilizing sites. Ross et al (155), utilizing immunoelectron microscopy of rat cerebellar sections, have shown that the Ins(1,4,5)P_3-binding protein is distributed over much of the endoplasmic reticulum, but is also present over most of the nuclear membrane and in some areas of the cis golgi. This localization has suggested a possible nuclear site for calcium mobilization, which might mediate cellular responses involving cell growth or proliferation. Recently Ins(1,4,5)P_3 was shown to mediate calcium release from isolated nuclei (160, 161).

Evidence for other inositol phosphate–binding proteins has been obtained. A distinct Ins(1,3,4,5)P_4-binding protein (K_d = 5 nM) has been solubilized and partially purified from porcine and rat cerebellar membranes (162–165). Donie et al have purified a porcine cerebellar Ins(1,3,4,5)P_4-binding protein approximately 20,000-fold (165). The purified protein has a mass of 42,000 daltons as estimated by SDS polyacrylamide gel electrophoresis. It binds 0.2 mol of Ins(1,3,4,5)P_4/mol of protein. The Ins(1,3,4,5)P_4-binding protein shows an approximately 100 times greater affinity for Ins(1,3,4,5)P_4 than for Ins(1,4,5)P_3, the converse of that observed for the Ins(1,4,5)P_3-binding protein.

Theibert et al (164) recently isolated two Ins(1,3,4,5)P_4-binding proteins from rat brain that show similar binding specificities to that found by Donie & Reiser (165); however, the molecular weights were quite different (182,000 and a dimer of 174,000 and 84,000). The specificity of the various $InsP_4$-binding proteins for particular $InsP_4$ isomers has not been defined, and some preparations bind $InsP_5$ quite well.

Specific binding proteins for $InsP_6$ have also been identified (166, 167). $InsP_6$ binds to isolated cerebellar membranes with an apparent K_d of 60 nM. Displacement by $InsP_5$ required approximately 1 μM. Binding sites were found in rat brain membrane fractions from mitochondria, myelin, and synaptosomes and represented at least 1% of membrane protein (167). An apparently different $InsP_6$-binding protein has been purified from rat brain membranes by affinity chromatography on an Ins(1,3,4,5)P_4 derivative affinity column (164). It differs in that it binds $InsP_5$ almost as well as $InsP_6$. $InsP_6$-binding sites have also been found in brain membranes by autoradiographic staining of brain sections using [^{3}H]$InsP_6$ (166). It was determined that $InsP_6$-binding sites were particularly enriched in cerebellum, choroid plexis, and in the nucleus of the solitary tract. This distribution mimics that of the regions of brain mediating pharmacologic activities of $InsP_6$ (142). Definition of the functions of these proteins awaits further study.

PHOSPHATIDYLINOSITOL 3-PHOSPHATE PATHWAY

Phosphatidylinositols containing phosphate esters in the 3 position of inositol represent a recently discovered pathway of phosphatidylinositol metabolism (168). These include PtdIns(3)P, phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2], and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] (see Figure 1). PtdIns(3)P is present in cells under all conditions and serves as a precursor for the polyphosphorylated 3-phosphate-containing phosphatidylinositols (141). The last two mentioned compounds are formed transiently in many cells in response to agonists and growth factors (169). Whitman et al (170) first identified PtdIns(3)P in transformed fibroblasts and proposed that the 3-phosphate-containing lipids serve a function in controlling cell proliferation. However, they are also found in normal cells and in nongrowing cells such as neutrophils, platelets, and brain, indicating that they serve a function other than, or in addition to, stimulating cell proliferation (reviewed in reference 26).

The 3-phosphate-containing phosphatidylinositols are present in very small amounts in cells. In fact the mass of these lipids has not been measured in any system. Based on incorporation of trace radiolabelled inositol or phosphate, they represent only 1 to 2% of the levels of the previously known non-3-phosphate-containing phosphatidylinositol polyphosphates (171–177). The 3-phosphate-containing lipids are not hydrolyzed by PLC enzymes (178,

179). Given this lack of reactivity and the small amounts of these compounds, it is unlikely that they serve as precursors of water-soluble messenger molecules. The water-soluble inositol phosphates that would be derived from these lipids via PLC action, namely Ins(1,3)P_2, Ins(1,3,4)P_3, and Ins(1,3,4,5)P_4, are all present in cells and are formed by other reactions as described above. The levels of these metabolites in stimulated cells, based on estimates from radiolabelling, are 10 to 50 times greater than the estimated levels of 3-phosphate-containing phosphatidylinositols. Therefore, it is likely that the 3-phosphate-containing lipids either function as lipids per se or they are converted to other as-yet-unidentified compounds.

Many agonists that evoke classical PtdIns turnover also stimulate the production of 3-phosphate-containing phosphatidylinositols. In quiescent NIH 3T3 cells (169) or unstimulated platelets (175, 176), it is possible to label PtdIns(3)P with [^{3}H] inositol or $^{32}PO_4$, indicating that there is turnover or synthesis of PtdIns(3)P in a basal state. After stimulation of platelets with thrombin, neutrophils with FMLP (174), or NIH 3T3 cells with PDGF, there is a rapid increase in labelling of both PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, suggesting that the formation of the polyphosphorylated lipids is the functionally significant event. A question that remains to be addressed is whether all agonists that stimulate PtdIns turnover activate both 3-phosphate- and PLC-mediated inositol lipid turnover.

The pathway for formation of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 is uncertain. Carpenter et al (180) have isolated a PtdIns 3-kinase that phosphorylates PtdIns(4)P and PtdIns(4,5)P_2 in vitro. However, labelling of intact cells suggests a different pathway. Cunningham et al (141) labelled human platelets with $^{32}PO_4$ for brief periods and stimulated them with thrombin. The relative specific activities of the phosphate group at each position of the 3-phosphate-containing phosphatidylinositols were determined by degrading the deacylated and deglycerated compounds with specific inositol phosphate phosphatases. As labelling reaches a steady state, all phosphates are equally labelled. Prior to a steady state, the order of phosphate addition is determined by the relative specific activity of each phosphate. In this analysis, the last phosphate added reaches steady state first and therefore is identified by demonstrating that it has the highest specific activity. These experiments clearly indicated that the major pathway in thrombin-stimulated platelets is PtdIns $\rightarrow$ PtdIns(3)P $\rightarrow$ PtdIns(3,4)P_2 $\rightarrow$ PtdIns(3,4,5)P_3.

This pathway for synthesis was also found in NIH 3T3 cells (181) by determining the specific activity of each phosphate in PtdIns(3,4,5)P_3 after brief PDGF stimulation of $^{32}PO_4$-labelled cells. The phosphate in position 5 was most highly labelled, followed by 4, then 3, and finally 1. Further support for this pathway comes from the discovery of a PtdIns(3)P 4-kinase in homogenates of platelets, erythrocytes, and human erythroleukemia cells that forms PtdIns(3,4)P_2 from PtdIns(3)P (182). Stephens et al (183) studied the

pathway for PtdIns(3,4,5)P_3 synthesis in FMLP-stimulated neutrophils. They labelled cells with [^{3}H] inositol and $^{32}PO_4$ and analyzed the relative specific activity of $^{32}PO_4$ in each position of PtdIns(3,4,5)P_3. They found that the 3-position was most highly labelled and thus suggested that PtdIns(3,4,5)P_3 arises from PtdIns(4,5)P_2. It is difficult to reconcile this study with those of Cunningham et al (141, 181). The main differences in procedure are that Stephens et al used crude extracts as sources of the phosphatases to degrade the compounds and used a different cell type. Discounting possible errors because of the crude enzymes, it is possible that 3-phosphate-containing lipids may be synthesized by different routes under varied conditions in different cells.

PtdIns 3-kinase has recently been isolated from bovine thymus (184). Two discrete types of 3-kinase enzyme were isolated. Type I is a monomeric protein of 110 kDa, while Type II is a heterodimeric form with subunits of 110 kDa and 85 kDa. Peptide mapping analysis indicated that the 110-kDa proteins were the same, while the 85-kDa protein was different. This suggests that the enzyme catalytic unit is the 110-kDa protein and that the 85-kDa subunit is a regulatory unit. The specific activity of Type I enzyme was fivefold greater than that of the Type II PtdIns 3-kinase, indicating that the monomer is an active type and the heterodimer is less active. The PtdIns(3)P 3-phosphatase enzymes described above are analogous, since they show varied activity in that the Type I enzyme is less active than the heterodimeric Type II enzyme. The Type II PtdIns 3-kinase has also been isolated from rat liver (180).

Recently, a cDNA encoding the 85-kDa regulatory subunit has been obtained by three different laboratories (185–187). The 85-kDa regulatory subunit of PtdIns 3-kinase is phosphorylated on both tyrosine and serine (180). This protein forms complexes with receptors that stimulate PtdIns(3)P synthesis in a manner similar to that described above for PLCγ (188–193). PtdIns 3-kinase forms complexes with wild-type but not a mutant form of middle T antigen. The mutant, which encodes phenylalanine in place of tyrosine at the major site of phosphorylation by pp60$^{c\text{-}src}$, retains the ability to form complexes with *src,* and activates its tyrosine kinase. The incidence and spectrum of tumors induced by cells with this mutant middle T antigen is reduced, implying that tyrosine phosphorylation is needed for recruitment of PtdIns 3-kinase into a complex that is in turn important for oncogenesis (194). The significance of these complexes in activating PtdIns 3-phosphate-containing phospholipid turnover is as yet uncertain.

An additional uncertainty is the messenger role that could be served by the 3-phosphate-containing inositol phospholipids. PtdIns(4,5)P_2 has been shown to promote actin polymerization in vitro by causing dissociation of gelsolin-actin complexes and association of profilin-actin complexes (195–198). Pro-

filin inhibits actin polymerization by binding G-actin, while gelsolin severs actin filaments. PtdIns(4,5)P_2 binds profilin with high affinity ($K_d < 0.1$ μM) with a stoichiometry of 5 moles PtdIns(4,5)P_2/mole profilin (44, 199). PtdIns(4,5)P_2 also binds to gelsolin. When platelets are stimulated by thrombin, there is rapid polymerization of actin, causing them to extend pseudopods and spread on surfaces. These events occur at a time when PtdIns(4,5)P_2 levels fall transiently and then return to basal levels. This pattern of change is not consistent with the proposed role for PtdIns(4,5)P_2 in binding profilin to promote actin polymerization. Dadabay et al (200) found no correlation between changes in PtdIns(4,5,)P_2 and actin polymerization in EGF-stimulated A431 cells. In contrast, PtdIns(3,4,5)P_3 levels do rise rapidly upon thrombin stimulation, at least as measured by incorporation of $^{32}PO_4$ (175). Perhaps PtdIns(3,4,5)P_3 binds profilin or gelsolin to serve this proposed function. Eberle et al (201) have shown that the time course of actin polymerization and subsequent depolymerization in neutrophils in response to FMLP stimulation parallels the rise and fall of PtdIns(3,4,5)P_3 in these same cells. When ligand binding is reversed with an antagonist, F-actin rapidly depolymerizes and ^{32}P-labelled PtdIns(3,4,5)P_3 disappears. If these patterns of labelling actually reflect changes in mass, then PtdIns(3,4,5)P_3 may control transient actin polymerization. Mass quantities of PtdIns(3,4,5)P_3 are needed to determine whether this molecule binds profilin as well or better than PtdIns(4,5)P_2. It is possible that proliferative responses to growth factors require changes in actin polymerization and associations between actin and cellular cytoskeletal elements (202). Future studies along the lines of those described for PLC should provide a much clearer picture of the role of the 3-phosphate-containing inositol lipids.

ACKNOWLEDGMENTS

I am very grateful to Theo Ross and Linda Pike for their help in writing this article. I thank Kevin Caldwell, Anne Bennett Jefferson, John York, and Kim Aiken for reading the manuscript and for suggestions of ways to clarify it.

This research was supported by Grants HL 14147 (Specialized Center for Research in Thrombosis), HL 16634, and Training Grant HL 07088 from the National Institutes of Health and Monsanto.

Literature Cited

1. Berridge, M. J. 1987. *Annu. Rev. Biochem.* 56:59–193
2. Pelech, S. L., Vance, D. E. 1989. *Trends Biochem. Sci.* 14:28–30
3. Majerus, P. W., Connolly, T. M., Bansal, V. S., Inhorn, R. C., Ross, T. S., Lips, D. L. 1988. *J. Biol. Chem.* 263:3051–54
4. Rana, R. S., Hokin, L. E. 1990. *Physiol. Rev.* 70:115–61
5. Berridge, M. J., Irvine, R. F. 1989. *Nature* 341:97–205
6. Putney, J. W., Takemura, H., Hughes, A. R., Horstman, D. A., Thastrup, O. 1989. *FASEB J.* 3:1899–905
7. Irvine, R. F., Moor, R. M., Pollock, W.

K., Smith, P. M., Wreggett, K. A. 1988. *Philos. Trans. R. Soc. London Ser. B* 320:281–98
8. Majerus, P. W., Neufeld, E. J., Wilson, D. B. 1984. *Cell* 37:701–3
9. Majerus, P. W., Connolly, T. M., Deckmyn, H., Ross, T. S., Bross, T. E., et al. 1986. *Science* 234:1519–26
10. Agranoff, B. W., Bradley, R. M., Brady, R. O. 1958. *J. Biol. Chem.* 233:1077–83
11. Paulus, H., Kennedy, E. P. 1960. *J. Biol. Chem.* 235:1303–11
12. Brockerhoff, H., Ballou, C. E. 1962. *J. Biol. Chem.* 237:1764–66
13. Smith, C. D., Wells, W. W. 1983. *J. Biol. Chem.* 258:9368–73
14. Campbell, C. R., Fishman, J. B., Fine, R. E. 1985. *J. Biol. Chem.* 260:10948–51
15. Hawthorne, J. N. 1983. *Biosci. Rep.* 3:887–904
16. Kurosawa, M., Parker, C. W. 1986. *J. Immunol.* 136:616–22
17. Porter, F. D., Li, Y. S., Deuel, T. F. 1988. *J. Biol. Chem.* 263:8989–95
18. Walker, D. H., Dougherty, N., Pike, L. J. 1988. *Biochemistry* 27:6504–11
19. Hou, W. M., Zhang, Z. L., Tai, H. H. 1988. *Biochim. Biophys. Acta* 959:67–75
20. Yamakawa, A., Takenawa, T. 1988. *J. Biol. Chem.* 263:17555–60
21. Lundberg, G. A., Jergil, B., Sundler, R. 1986. *Eur. J. Biochem.* 161:257–62
22. Vandongen, C. J., Kok, J. W., Schrama, L. H., Oestreicher, A. B., Gispen, W. H. 1986. *Biochem. J.* 253:859–64
23. Ling, L. E., Schulz, J. T., Cantley, L. C. 1989. *J. Biol. Chem.* 264:5080–88
24. Bazenet, C. E., Ruano, A. R., Brockman, J. L., Anderson, R. A. 1990. *J. Biol. Chem.* 265:18012–22
25. Bansal, V. S., Majerus, P. W. 1990. *Annu. Rev. Cell Biol.* 6:41–67
26. Majerus, P. W., Ross, T. S., Cunningham, T. W., Caldwell, K. K., Jefferson, A. B., Bansal, V. S. 1990. *Cell* 63:459–65
27. Deleted in proof
28. Fain, J. N., Wallace, M. A., Wojcikiewicz, R. J. H. 1988. *FASEB J.* 2:2569–74
29. Pang, I. H., Sternweis, P. C. 1990. *J. Biol. Chem.* 265:18707–12
30. Strathmann, M., Simon, M. I. 1990. *Proc. Natl. Acad. Sci. USA* 87:9113–17
31. Taylor, S. J., Smith, J. A., Exton, J. H. 1990. *J. Biol. Chem.* 265:17150–56
32. Taylor, S. J., Chae, H. Z., Rhee, S. G., Exton, J. H. 1991. *Nature* 350:516–18
33. Smrcka, A. V., Hepler, J. R., Brown, K. O., Sternweis, P. C. 1990. *Science* 251:804–7
34. Nishibe, S., Wahl, M. I., Rhee, S. G., Carpenter, G. 1989. *J. Biol. Chem.* 264:10335–38
35. Wahl, M. I., Nishibe, S., Suh, P. G., Rhee, S. G., Carpenter, G. 1989. *Proc. Natl. Acad. Sci. USA* 86:1568–72
36. Margolis, B., Rhee, S. G., Felder, S., Mervic, M., Lyall, R., et al. 1989. *Cell* 57:1101–7
37. Downing, J. R., Margolis, B. L., Zilberstein, A., Ashmun, R. A., Ullrich, A., et al. 1989. *EMBO J.* 8:3345–50
38. Meisenhelder, J., Suh, P. G., Rhee, S. G., Hunter, T. 1989. *Cell* 57:1109–22
39. Kumjian, D. A., Wahl, M. I., Rhee, S. G., Daniel, T. O. 1989. *Proc. Natl. Acad. Sci. USA* 86:8232–36
40. Todderud, G., Wahl, M. I., Rhee, S. G., Carpenter, G. 1990. *Science* 249:296–98
41. Kumjian, D. A., Branstein, A., Rhee, S. G., Daniel, T. O. 1991. *J. Biol. Chem.* 266:3973–80
42. Kim, J. W., Sim, S. S., Kim, U. H., Nishibe, S., Wahl, M. I., et al. 1990. *J. Biol. Chem.* 265:3940–43
43. Nishibe, S., Wahl, M. I., Hernandez-Sotomayor, S. M. T., Tonks, N. K., Rhee, S. G., Carpenter, G. 1990. *Science* 250:1253–56
44. Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G., Pollard, T. D. 1991. *Science* 251:1231–33
45. Kim, H. K., Kim, J. W., Zilberstein, A., Margolis, B., Kim, J. G., Schlessinger, J., Rhee, S. G. 1991. *Cell* 65:435–41
46. Shayman, J. A., Mahdiyoun, S., Deshmukh, G., Barcelon, F., Inokuchi, J. I., Radin, N. S. 1990. *J. Biol. Chem.* 265:12135–38
47. Connolly, T. M., Bross, T. E., Majerus, P. W. 1985. *J. Biol. Chem.* 260:7868–74
48. Mitchell, C. A., Connolly, T. M., Majerus, P. W. 1989. *J. Biol. Chem.* 264:8873–77
49. Connolly, T. M., Bansal, V. S., Bross, T. E., Irvine, R. F., Majerus, P. W. 1987. *J. Biol. Chem.* 262:2146–49
50. Connolly, T. M., Lawing, W. J., Majerus, P. W. 1986. *Cell* 46:951–58
51. Molina y Vedia, L. M., Lapetina, E. G. 1986. *J. Biol. Chem.* 261:10493–95
52. King, W. G., Rittenhouse, S. E. 1989. *J. Biol. Chem.* 264:6070–74
53. Ross, T. S., Jefferson, A. B., Mitchell,

C. A., Majerus, P. W. 1991. *J. Biol. Chem.* 266:20283–89
54. Kemp, B. E., Pearson, R. B. 1990. *Trends Biochem. Sci.* 15:342–46
55. Hansen, C. A., Johanson, R. A., Williamson, M. T., Williamson, J. R. 1987. *J. Biol. Chem.* 262:17319–26
56. Takimoto, K., Okada, M., Nakagawa, H. 1989. *J. Biol. Chem.* 106:684–90
57. Erneux, C., Lemos, M., Verjans, B., Vanderhaeghen, P., Delvaux, A., Dumont, J. E. 1989. *Eur. J. Biochem.* 181:317–22
58. Kukita, M., Hirata, M., Koga, T. 1986. *Biochim. Biophys. Acta* 885:121–28
59. Rana, R. S., Sekar, M. C., Hokin, L. E., MacDonald, M. J. 1986. *J. Biol. Chem.* 261:5237–40
60. Downes, C. P., Mussat, M. C., Michell, R. H. 1982. *Biochem. J.* 203:169–77
61. Storey, D. J., Shears, S. B., Kirk, C. J., Michell, R. H. 1984. *Nature* 312:374–76
62. Seyfred, M. A., Farrell, L. E., Wells, W. W. 1984. *J. Biol. Chem.* 259:13204–8
63. Joseph, S. K., Williams, R. J. 1985. *FEBS Lett.* 180:150–54
64. Shears, S. B. 1991. *Pharmacol. Ther.* 49:79–104
65. Morris, A. J., Murray, K. J., England, P. J., Downes, C. P., Michell, R. H. 1988. *Biochem. J.* 251:157–63
66. Irvine, R. F., Letcher, A. J., Heslop, J. P., Berridge, M. J. 1986. *Nature* 320:631–34
67. Biden, T. J., Wollheim, C. B. 1986. *J. Biol. Chem.* 261:11931–34
68. Ryu, S. H., Lee, S. Y., Lee, K. Y., Rhee, S. G. 1987. *FASEB J.* 1:388–93
69. Johanson, R. A., Hansen, C. A., Williamson, J. R. 1988. *J. Biol. Chem.* 263:7465–71
70. Daniel, J. L., Dangelmaier, C. A., Smith, J. B. 1988. *Biochem. J.* 253:789–94
71. Biden, T. J., Peter-Riesch, B., Schlegel, W., Wollheim, C. B. 1987. *J. Biol. Chem.* 262:3567–71
72. Yamaguchi, K., Hirata, M., Kuriyama, H. 1987. *Biochem. J.* 244:787–91
73. Kimura, Y., Hirata, M., Yamaguchi, K., Koga, T. 1987. *Arch. Biochem. Biophys.* 257:363–69
74. Morris, A. J., Downes, C. P., Hardin, T. K., Michell, R. H. 1987. *Biochem. J.* 248:489–93
75. Imboden, J. B., Pattison, G. 1987. *J. Clin. Invest.* 79:1538–41
76. Biden, T. J., Altin, J. G., Karajalainen, A., Bygrave, F. L. 1988. *Biochem. J.* 256:697–701
77. Sim, S. S., Kim, J. W., Rhee, S. G. 1990. *J. Biol Chem.* 265:10367–72
78. Lin, A. N., Barnes, S., Wallace, R. W. 1990. *Biochem. Biophys. Res. Commun.* 170:1371–76
79. Johnson, R. M., Wasilenko, W. J., Mattingly, R. R., Weber, M. J., Garrison, J. C. 1989. *Science* 246:121–24
80. Moon, K. H., Lee, S. Y., Rhee, S. G. 1989. *Biochem. Biophys. Res. Commun.* 164:370–74
81. Lee, S. Y., Sim, S. S., Kim, J. W., Moon, K. H., Kim, J. H., Rhee, S. G. 1990. *J. Biol. Chem.* 265:9434–40
82. Takazawa, K., Passareiro, H., Dumont, J. E., Ereneux, C. 1989. *Biochem. J.* 261:483–88
83. Choi, K. Y., Kim, H. K., Lee, S. Y., Moon, K. H., Sim, S. S., et al. 1990. *Science* 248:64–66
84. Bala, T., Sim, S. S., Iida, T., Choi, K. Y., Catt, K. J., Rhee, S. G. 1991. *J. Biol. Chem.* 266:24719–26
85. Irvine, R. F., Moore, M. 1986. *Biochem. J.* 240:917–20
86. Inhorn, R. C., Bansal, V. S., Majerus, P. W. 1987. *Proc. Natl. Acad. Sci. USA* 84:2170–74
87. Shears, S. B. 1989. *Biochem. J.* 260:313–24
88. Inhorn, R. C., Majerus, P. W. 1988. *J. Biol. Chem.* 263:14559–65
89. Gee, N. S., Reid, G. G., Jackson, R. G., Barnaby, R. J., Ragan, C. I. 1988. *Biochem. J.* 253:777–82
90. Inhorn, R. C., Majerus, P. W. 1987. *J. Biol. Chem.* 262:15946–52
91. Hansen, C. A., Inubushi, T., Williamson, M. T., Williamson, J. R. 1988. *Biochim. Biophys. Acta* 1001:134–44
92. Berridge, M. J., Downes, C. P., Hanley, M. R. 1989. *Cell* 59:411–19
93. York, J. D., Majerus, P. W. 1990. *Proc. Natl. Acad. Sci. USA* 87:9548–52
94. Newald, A. F., York, J. D., Majerus, P. W. 1991. *FEBS Lett.* 294:16–18
95. Bansal, V. S., Inhorn, R. C., Majerus, P. W. 1987. *J. Biol. Chem.* 262:9444–47
96. Howell, S., Barnaby, R. J., Rowe, T., Ragan, C. I., Gee, N. S. 1989. *Eur. J. Biochem.* 183:169–72
97. Bansal, V. S., Caldwell, K. K., Majerus, P. W. 1990. *J. Biol. Chem.* 265:1806–11
98. Caldwell, K. K., Lips, D. L., Bansal, V. S., Majerus, P. W. 1991. *J. Biol. Chem.* 266:18378–86
99. Ackermann, K. E., Gish, B. G., Hon-

char, M. P., Sherman, W. R. 1987. *Biochem. J.* 242:517–24
100. Hallcher, L. M., Sherman, W. R. 1980. *J. Biol. Chem.* 255:10896–901
101. Takimoto, K., Okada, M., Matsuda, Y., Nakagawa, H. 1985. *J. Biochem.* 98:363–70
102. Gee, N. S., Ragan, C. I., Watling, K. J., Aspley, S., Jackson, R. G., et al. 1988. *Biochem. J.* 249:883–89
103. Diehl, R. E., Whiting, P., Potter, J., Gee, N., Ragan, C. I., et al. 1990. *J. Biol. Chem.* 265:5946–49
104. Jackson, R. G., Gee, N. S., Ragan, C. I. 1989. *Biochem. J.* 264:419–22
105. Koch, M. A., Diringer, H. 1974. *Biochem. Biophys. Res. Commun.* 58:361–67
106. Binder, H., Weber, P. C., Siess, W. 1985. *Anal. Biochem.* 148:220–27
107. Dixon, J. F., Hokin, L. E. 1985. *J. Biol. Chem.* 260:16068–71
108. Dixon, J. F., Hokin, L. E. 1987. *Biochem. Biophys. Res. Commun.* 149:1208–13
109. Dixon, J. F., Hokin, L. E. 1987. *J. Biol. Chem.* 262:13892–95
110. Shayman, J. A., Auchus, R. J., Morrison, A. R. 1986. *Biochim. Biophys. Acta* 886:171–75
111. Ishii, H., Connolly, T. M., Bross, T. E., Majerus, P. W. 1986. *Proc. Natl. Acad. Sci. USA* 83:6397–400
112. Sekar, M. C., Dixon, J. F., Hokin, L. E. 1987. *J. Biol. Chem.* 262:340–44
113. Graham, R. A., Meyer, R. A., Szwergold, B. S., Brown, T. R. 1987. *J. Biol. Chem.* 262:35–37
114. Hughes, A. R., Takemura, H., Putney, J. W. Jr. 1988. *J. Biol. Chem.* 263:10314–19
115. Tarver, A. P., King, W. G., Rittenhouse, S. E. 1987. *J. Biol. Chem.* 262:17268–71
116. Connolly, T. M., Wilson, D. B., Bross, T. E., Majerus, P. W. 1986. *J. Biol. Chem.* 261:122–26
117. Ross, T. S., Majerus, P. W. 1986. *J. Biol. Chem.* 261:11119–23
118. Ross, T. S., Majerus, P. W. 1991. *J. Biol. Chem.* 266:851–56
119. Ross, T. S., Tait, J. F., Majerus, P. W. 1990. *Science* 248:605–7
120. Tait, J. F., Sakata, M., McMullen, B. A., Miao, C. H., Funakoshi, T., et al. 1988. *Biochemistry* 27:6268–76
121. Pepinsky, R. B., Tizard, R., Mattaliano, R. J., Sinclair, L. K., Miller, G. T., et al. 1988. *J. Biol. Chem.* 263:10799–811
122. Kaetzel, M. A., Hazarika, P., Dedman, J. R. 1989. *J. Biol. Chem.* 264:14463–70
123. Crompton, M. R., Moss, S. E., Crumpton, M. J. 1988. *Cell* 55:1–3
124. Haigler, H. T., Fitch, J. M., Schlaepfer, D. D. 1989. *Trends Biochem. Sci.* 14:48–51
125. Ross, T. S., Whiteley, B., Graham, R. A., Majerus, P. W. 1991. *J. Biol. Chem.* 266:9086–92
126. Camilli, A., Goldfine, H., Portnoy, D. A. 1991. *J. Exp. Med.* 173:751–54
127. Balla, T., Guillemette, G., Baukal, A. J., Catt, K. J. 1987. *J. Biol. Chem.* 262:9952–55
128. Stephens, L. R., Hawkins, P. T., Barker, C. J., Downes, C. P. 1988. *Biochem. J.* 253:721–23
129. Hansen, C. A., Dahl, S. V., Huddell, B., Williamson, J. R. 1988. *FEBS Lett.* 236:53–56
130. Shears, S. B. 1989. *J. Biol. Chem.* 264:19879–86
131. Cunha-Melo, J. R., Dean, N. M., Ali, H., Beavan, M. A. 1988. *J. Biol. Chem.* 263:14245–50
132. Doughney, C., McPherson, M. A., Dormer, R. L. 1988. *Biochem. J.* 251:927–29
133. Hoer, D., Kwiatkowski, A., Seib, C., Rosenthal, W., Schultz, G., Oberdisse, E. 1988. *Biochem. Biophys. Res. Commun.* 154:668–75
134. Hodgson, M. E., Shears, S. B. 1990. *Biochem. J.* 267:831–34
135. McIntosh, R. P., McIntosh, J. E. A. 1990. *Biochem. J.* 268:141–45
136. Stephens, L. R., Hawkins, P. T., Stanley, A. F., Moore, T., Poyner, D. R., et al. 1991. *Biochem. J.* 275:485–99
137. Pittet, D., Schlegel, W., Lew, D. P., Monod, A., Mayr, G. W. 1989. *J. Biol. Chem.* 264:18489–93
138. Balla, T., Hunyady, L., Baukal, A. J., Catt, K. J. 1989. *J. Biol. Chem.* 264:9386–90
139. Stephens, L. R., Downes, C. P. 1990. *Biochem. J.* 265:435–52
140. Menniti, F. S., Oliver, K. G., Nogimori, K., Obie, J. F., Shears, S. B., Putney, J. W. 1990. *J. Biol. Chem.* 265:11167–76
141. Cunningham, T. W., Lips, D. L., Bansal, V. S., Caldwell, K. K., Mitchell, C. A., Majerus, P. W. 1990. *J. Biol. Chem.* 265:21676–83
142. Vallejo, M., Jackson, P., Lightman, S., Hanley, M. R. 1987. *Nature* 330:656–58
143. Isaacks, R. E., Harkens, D. R. 1980. *Am. Zool.* 20:115–29
144. Supattapone, S., Worley, P. F., Baraban, J. M., Snyder, S. H. 1988. *J. Biol. Chem.* 263:1530–34
145. Ferris, C. D., Huganir, R. L., Supatta-

pone, S., Snyder, S. H. 1989. *Nature* 342:87–89
146. Maeda, N., Kawasaki, T., Nakade, S., Yokota, N., Taguchi, T., et al. 1991. *J. Biol. Chem.* 266:1109–16
147. Ferris, C. D., Huganir, R. L., Snyder, S. H. 1990. *Proc. Natl. Acad. Sci. USA* 87:2147–51
148. Worley, P. F., Baraban, J. M., Colvin, J. S., Snyder, S. H. 1987. *Nature* 325:159–61
149. Nunn, D. L., Potter, B. V., Taylor, C. W. 1990. *Biochem. J.* 265:393–98
150. Mikoshiba, K., Okano, H., Tsukada, Y. 1985. *Dev. Neurosci.* 7:179–87
151. Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., Mikoshiba, K. 1989. *Nature* 342:32–38
152. Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., et al. 1989. *Nature* 339:439–45
153. Nordquist, D. T., Kozak, C. A., Orr, H. T. 1989. *J. Neurosci.* 8:4780–89
154. Mignery, G. A., Sudhof, T. C., Takei, K., De Camilli, P. 1989. *Nature* 342:192–95
155. Ross, C. A., Meldolesi, J., Milner, T. A., Satoh, T., Supattapone, S., Snyder, S. H. 1989. *Nature* 339:468–70
156. Maeda, N., Niinobe, M., Mikoshiba, K. 1990. *EMBO J.* 9:61–67
157. Mignery, G. A., Newton, C. L., Archer, B. T., Sudhof, T. C. 1990. *J. Biol. Chem.* 265:12679–85
158. Nakagawa, T., Okano, H., Furuichi, T., Aruga, J., Mikoshiba, K. 1991. *Proc. Natl. Acad. Sci. USA* 88:6244–48
159. Danoff, S. K., Ferris, C. D., Donath, C., Fischer, G. A., Minemitsu, S., et al. 1991. *Proc. Natl. Acad. Sci. USA* 88:2951–55
160. Malviya, A. N., Rogue, P., Vincendon, G. 1990. *Proc. Natl. Acad. Sci. USA* 87:9270–74
161. Nicotera, P., Orenius, S., Nilsson, T., Derggren, P. O. 1990. *Proc. Natl. Acad. Sci. USA* 87:6858–62
162. Donie, F., Hulser, E., Reiser, G. 1990. *FEBS Lett.* 268:194–98
163. Theibert, A. B., Supattapone, S., Ferris, C. D., Danoff, S. K., Evans, R. K., Snyder, S. H. 1990. *Biochem. J.* 267:441–45
164. Theibert, A. B., Estevez, V. A., Ferris, C. D., Danoff, S. K., Barrow, R. K., et al. 1991. *Proc. Natl. Acad. Sci. USA* 88:3165–69
165. Donie, F., Reiser, G. 1991. *Biochem. J.* 275:453–57
166. Hawkins, P. T., Reynolds, D. J., Poyner, D. R., Hanley, M. R. 1990. *Biochem. Biophys. Res. Commun.* 167:819–27
167. Nicoletti, F., Bruno, V., Cavallar, S., Copani, A., Sortino, M. A., Canono, P. L. 1990. *Mol. Pharmacol.* 37:689–93
168. Carpenter, C. L., Cantley, L. C. 1990. *Biochemistry* 29:11147–56
169. Serunian, L. A., Auger, K. R., Roberts, T., Cantley, L. C. 1990. *J. Virol.* 64:4718–25
170. Whitman, M., Downes, C. P., Keeler, M., Keller, T., Cantley, L. 1988. *Nature* 332:644–46
171. Varticovski, L., Drucker, B., Morrison, D., Cantley, L., Roberts, T. 1989. *Nature* 342:699–702
172. Auger, K. R., Serunian, L. A., Soltoff, S. P., Libby, P., Cantley, L. C. 1989. *Cell* 57:167–75
173. Lips, D. L., Majerus, P. W., Gorga, F. R., Young, A. T., Benjamin, T. L. 1989. *J. Biol. Chem.* 264:8759–63
174. Traynor-Kaplan, A. E., Thompson, B. L., Harris, A. L., Taylor, P., Omann, G. M., Sklar, L. A. 1989. *J. Biol. Chem.* 264:15668–73
175. Kucera, G. L., Rittenhouse, S. E. 1990. *J. Biol. Chem.* 265:5345–48
176. Sultan, C., Breton, M., Mauco, G., Grondin, P., Plantavid, M., Chap, H. 1990. *Biochem. J.* 269: 831–34
177. Stephens, L. R., Hawkins, P. T., Downes, C. P. 1989. *Biochem. J.* 259:267–76
178. Lips, D. L., Majerus, P. W. 1989. *J. Biol. Chem.* 264:19911–15
179. Serunian, L. A., Haber, M. T., Fukui, T., Kim, J. W., Rhee, S. G., et al. 1989. *J. Biol. Chem.* 264:17809–15
180. Carpenter, C. L., Duckworth, B. C., Auger, K. R., Cohen, B., Schafferhausen, B. S., Cantley, L. C. 1990. *J. Biol. Chem.* 265:19704–11
181. Cunningham, T. W., Majerus, P. W. 1991. *Biochem. Biophys. Res. Commun.* 175:568–76
182. Yamamoto, K., Graziani, A., Carpenter, C., Cantley, L. C., Lapetina, E. G. 1990. *J. Biol. Chem.* 265:22086–89
183. Stephens, L. R., Hughes, K. T., Irvine, R. F. 1991. *Nature* 351:33–39
184. Shibasaki, F., Homma, Y., Takenawa, T. 1991. *J. Biol. Chem.* 266:8108–14
185. Otsu, M., Hiles, I., Gout, I., Fry, J., Ruiz-Larrea, F., Panayotou, G., et al. 1991. *Cell* 65:91–104
186. Escobedo, J. A., Navankasattusas, S., Kavanaugh, W. M., Milfay, D., Fried, V. A., Williams, L. T. 1991. *Cell* 65:75–82
187. Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., et al. 1991. *Cell* 65:83–90

188. Whitman, M., Downes, C. P., Keeler, M., Keller, T., Cantley, L. 1988. *Nature* 332:644–46
189. Fukui, Y., Kornbluth, S., Jong, S.-M., Wang, L.-H., Hanafusa, H. 1989. *Oncogene Res.* 4:283–92
190. Auger, K. R., Serunian, L. A., Soltoff, S. P., Libby, P., Cantley, L. C. 1989. *Cell* 57:167–75
191. Varticovski, L., Drucker, B., Morrison, D., Cantley, L., Roberts, T. 1989. *Nature* 342:699–702
192. Coughlin, S. R., Escobedo, J. A., Williams, L. T. 1989. *Science* 243:1191–94
193. Ullrich, A., Schlessinger, J. 1990. *Cell* 61:203–12
194. Talmage, D. A., Freund, R., Young, A. T., Dah, J., Dawe, C. J., Benjamin, T. L. 1989. *Cell* 59:55–65
195. Larsson, H., Lindberg, U. 1988. *Biochim. Biophys. Acta* 953:95–105
196. Lassing, I., Lindberg, U. 1985. *Nature* 314:472–74
197. Lassing, I., Lindberg, U. 1988. *J. Cell. Biochem.* 37:255–67
198. Janmey, P. A., Stossel, T. P. 1989. *J. Biol. Chem.* 264:4825–31
199. Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J., Pollard, T. D. 1990. *Science* 247:1575–78
200. Dadabay, C. Y., Patton, E., Cooper, J. A., Pike, L. J. 1991. *J. Cell Biol.* 112:1151–56
201. Eberle, M., Traynor-Kaplan, A. E., Sklar, L. A., Norgauer, J. 1990. *J. Biol. Chem.* 265:16725–28
202. Bockus, B. J., Stiles, C. D. 1984. *Exp. Cell Res.* 153:186–97