Phosphatidylinositol transfer protein (PITP) is essential for phospholipase C signaling and for constitutive and regulated vesicular traffic. PITP has a single lipid-binding site that can reversibly bind phosphatidylinositol (PI) and phosphatidylcholine (PC) and transfer these lipids between membrane compartments in vitro. The role of the carboxyl terminus was examined by comparing wild-type PITPa with PITPa in which 5, 10, and 20 amino acids were deleted from the C terminus. Δ5- and Δ10-PITP had reduced PI and PC transfer activities compared with wild-type PITP, with the effect on PI transfer being more marked than that on PC transfer. Δ20-PITP was inactive at all concentrations tested. All three truncated mutants were unable to restore G-protein-mediated phospholipase Cβ stimulation in HL-60 cells. Δ5- and Δ10-PITP, but not Δ20-PITP, inhibited the signaling function of wild-type protein without any effect on lipid transfer in vitro. We conclude that (a) the carboxyl terminus of PITP plays a critical role in phospholipase C signaling; (b) the transfer activity is not the only determining factor that dictates the restorative function of PITP in inositol lipid signaling; and (c) the dominant inhibitory effects of Δ5- and Δ10-PITP on wild-type PITP in phospholipase C signaling suggest the existence of a receptor for PITP.

The First 5 Amino Acids of the Carboxyl Terminus of Phosphatidylinositol Transfer Protein (PITP) α Play a Critical Role in Inositol Lipid Signaling

Shuntaro Hara*, Phil Swigart, David Jones, and Shamshad Cockcroft†§

From the Department of Physiology, University College London, London WC1E 6JJ, United Kingdom

Phosphatidylinositol transfer protein (PITP) is a ubiquitous and abundant cytosolic protein that was originally identified because of its ability to transfer phosphatidylinositol (PI) and phosphatidylcholine (PC) between membrane bilayers in vitro (1, 2). Two isoforms of PITP (α and β) that show different lipid binding properties have been identified in mammalian cells. PITPa has a single lipid-binding site that can reversibly bind PI and PC, with a 16-fold higher affinity for PI than for PC (3, 4). In vitro, PITPa can transfer PI, PC, and PG to a lesser extent (5), whereas PITPβ can transfer sphingomyelin in addition (6). In cells, both PITPa and PITPβ participate in phospholipase C (PLC)-mediated signaling (7–9) and in vesicular traffic (10–12).

PITPa was identified as the major reconstituting factor that allowed restoration of PLCβ signaling in HL-60 cells (7). A requirement for PITP has also been identified for inositol 1,4,5-trisphosphate production by receptors that activate PLCγ1. When activated by the appropriate agonist, both the epidermal growth factor and IgE receptors are dependent on PITP for PLCγ1 signaling in A431 and RBL-2H3 cells, respectively (8, 9). The mechanism of PITP function in lipid signaling has been attributed to the lipid binding/transfer properties of PITP, and the delivery of PI to a signaling complex composed of PI 4-kinase, phosphatidylinositol-phosphate 5-kinase, and the receptor has been proposed as its physiological function (7–9, 13).

A separate role for PITP in exocytosis has also been identified (10, 12, 14). PITP was purified as a reconstituting factor together with phosphatidylinositol-phosphate 5-kinase for restoration of ATP-dependent priming of secretory vesicles for fusion with the plasma membrane in PC12 cells (10, 14). Cells of the myeloid lineage including neutrophils and HL-60 cells secrete lysosomal enzymes when activated with Ca2+ and guanine nucleotides. HL-60 cells depleted of cytosolic proteins become refractory to stimulation, and PITP was found to be required to restore secretory competence for Ca2+ and guanine nucleotide-mediated exocytosis (12). The addition of PITP led to increased synthesis of PI 4,5-bisphosphate, and this function of PITP provides the most likely explanation of how PITP participates both in exocytosis and in PLC-mediating signaling. In PLC signaling, PI 4,5-bisphosphate functions as a substrate, whereas its function in exocytosis is probably due to the recruitment of specific protein(s) required for the exocytotic machinery.

Secretory vesicle formation is also dependent on cytosolic proteins, and PITPa and PITPβ were identified as the active components (11). This function of PITP in mammalian cells is analogous to the requirement of yeast PITP (SEC14p) for export of secretory proteins from the Golgi complex (15). Evidence has been presented that suggests that it functions as a lipid sensor that controls the PC content of yeast Golgi membranes (16). Although yeast PITP (SEC14p) shares no primary sequence homology with mammalian PITP, SEC14p can restore the function of mammalian PITPs in PLC signaling, exocytosis, and vesicle formation (9–12). The function of yeast PITP is not conserved as mutations in SEC14p in the dimorphic yeast Yarrowia lipolytica do not lead to impaired secretion. Instead, it is required for differentiation from the yeast to the mycelial form (17).

In this report, we show that the carboxyl terminus of the...
PITP molecule plays a critical role in the restoration of PLC signaling. Deletion of 5 amino acids is sufficient to inactivate the protein with regard to PLC-mediated signaling, although lipid transfer is only reduced. In addition, the Δ5- and Δ10-PITP deletion mutants were found to inhibit restoration of PLC signaling by wild-type PITP, although they did not interfere with the lipid transfer function of PITP in vitro. We conclude that (a) the carboxyl terminus of PITP plays a critical role in PLC signaling; (b) the transfer activity is not the only determining factor that dictates the restorative function of PITP in inositol lipid signaling; and (c) the dominant inhibitory effects of Δ5- and Δ10-PITP on wild-type PITP in PLC signaling suggest the existence of a receptor for PITP.

**Materials and Methods**

Preparation of Recombinant PITP Proteins—Recombinant wild-type and mutant PITP proteins were cloned by the polymerase chain reaction using human PITPα cDNA as template and the following primers: 5′-GAACCTTCAATCCATAGTGGGCTGCTCA-3′ and 5′-CTCCGGGATCTTCAATCTGTCGTCACT-3′ for wild-type PITP; 5′-CATCTGAGATCCTTCTTCTTCTTCTTCTTCTTCTTCT-3′ and 5′-GACHTTGGGATCTTCAATCTGTCGTCACT-3′ for Δ5-PITP; 5′-GGGTGATCCTTCTTCTTCTTCTTCTTCTTCTTCTTCT-3′ and 5′-GACHTTGGGATCTTCAATCTGTCGTCACT-3′ for Δ10-PITP; 5′-GGGTGATCCTTCTTCTTCTTCTTCTTCTTCT-3′ and 5′-GACHTTGGGATCTTCAATCTGTCGTCACT-3′ for Δ10-PITP. The polymerase chain reaction products were cloned into the NdeI-BamHI restriction site of the pET14b expression vector (Qiagen Inc.) and transformed into XL-1 Blue cells (Stratagene). Positive clones were sequenced, compared with published human PITPα cDNA sequence, and transformed into DE3 (pLysS) cells (Promega). Expression of recombinant PITPα was induced with isopropyl-β-D-thiogalactopyranoside (0.1 mM) for 4 h at room temperature, and bacterial cells were collected by centrifugation. The pellet was resuspended in buffer containing 50 mM sodium phosphate and 300 mM NaCl (pH 8.0). After freeze-thaw, the samples were centrifuged at 100,000 × g for 30 min at 4 °C. Recombinant proteins were purified from the supernatants using Ni²⁺-nitrilotriacetic acid-agarose resin (QIAGEN Inc.) and transformed into XLI-Blue cells (Stratagene). Positive clones were sequenced, compared with published human PITPα cDNA sequence, and transformed into DE3 (pLysS) cells (Promega). Expression of recombinant PITPα was induced with isopropyl-β-D-thiogalactopyranoside (0.1 mM) for 4 h at room temperature, and bacterial cells were collected by centrifugation. The pellet was resuspended in buffer containing 50 mM sodium phosphate and 300 mM NaCl (pH 8.0). After freeze-thaw, the samples were centrifuged at 100,000 × g for 30 min at 4 °C. Recombinant proteins were purified from the supernatants using Ni²⁺-nitrilotriacetic acid-agarose resin (QIAGEN Inc.) as described previously (9).

**Assay for PI and PC Transfer**—PI transfer activity was assayed as described previously (7). This assay measures the transfer of [H]PI from rat liver microsomes to unlabeled liposomes in the presence of transferrin receptor. Briefly, protein samples were added to the [H]PI-labeled microsomes prepared as described above. Lipids were extracted from postnuclear supernatants and separated by TLC (solvent: chloroform/methanol/acetone/aqueous solution 75:45:30:0.2, v/v/v). Unlabeled standards were added to the samples to aid recovery and for identification on the TLC plate. The TLC plate was stained with iodine to locate the lipids, and the radioactivity was measured after excision of the spots for cardiolipin, PG, and PE. The TLC plate was also analyzed by imaging the radioactivity on a PhosphorImager.

Exchange of the Endogenous Lipids with PI and PC—50 μg of purified PITPα proteins (45 μl) were incubated with 5 μl of [3H]PI/PC-phosphatidic acid (70:30 mol %; 120 μCi/μl and 0.1 μCi/μl final concentrations) or [3H]PI (120 μCi and 0.0135 μCi/μl final concentrations) in 20 mM Tris buffer containing 5 mM MgCl₂ and 60 mM NaCl (pH 7.4). After the solution was incubated at room temperature for 1 h, PITPα proteins were repurified with nitrilotriacetic acid spin (QIAGEN Inc.), and the radioactivity was counted.

For exchange of endogenous bacterial lipids for PI or PC prior to use in PLC assays, purified wild-type PITP and Δ5- and Δ10-PITP were incubated with the appropriate lipid vesicles at a ratio of 1 mg of protein to 2.4 mg of liposomes (100 nmol ratio) and incubated at 4 °C overnight. The lipid vesicles were removed by the addition of DE52 in 20 mM Tris buffer containing 5 mM MgCl₂ and 60 mM NaCl (pH 7.4) as described previously (7).

**Reconstitution of G-protein-regulated Phospholipase C Activity in Cytosol-depleted HL-60 Cells with PITP Proteins**—HL-60 cells were grown and labeled with [3H]inositol as described previously (13). Labeled HL-60 cells (5 × 10⁶) were permeabilized with 0.6 IU/ml streptolysin O in PIPES (supplemented with 1 mM MgCl₂-ATP at pCa 7) for 10 min to deplete the cells of cytosolic proteins including PITP. The permeabilized cells were washed and finally resuspended in PIPES supplemented with 4 mM MgCl₂-ATP, 20 mM LiCl, and 4 mM MgCl₂ at pCa 6. Permeabilized cells (20 μl) were incubated with recombinant proteins in the presence of 10 μg of receptor in a final volume of 45 μl at 37 °C for 20 min. The reaction was quenched by the addition of chloroform/methanol, and inositol phosphates were extracted, separated by passage through Dowex 1-X8 anion-exchange resin, and counted for radioactivity.

**Results**

C-terminal Truncation of PITP Reduces Its Ability to Transfer Phospholipids—We have constructed three C-terminally truncated forms of PITPα (Δ5, Δ10, and Δ20) in which 5, 10, and 20 amino acids residues are deleted, respectively (Fig. 1A). Proteins were expressed in E. coli and purified, and the in vitro transfer activities of these recombinant proteins were compared with those of wild-type PITP (Figs. 1B and 2A and B). In addition to these three mutants, we constructed Δ50-PITP, but this mutant could not be recovered from the soluble fraction of E. coli.

Transfer activity was monitored using a donor membrane that contained radiolabeled PI or PC and an acceptor membrane compartment. The acceptor compartment was reisolated and monitored for the transferred radiolabeled lipid. Deletion of 5 amino acids was sufficient to reduce the transfer activity for both PI and PC (Fig. 2, A and B). 1 μg/ml wild-type PITP was maximal in the PI transfer assay, and corresponding concentrations of the mutant proteins were inactive. The Δ5- and Δ10-PITP mutants displayed substantial PI transfer activity when concentrations as high as 200 μg/ml were tested (Fig. 2A, panel b). In comparison, near normal transfer of PC could be observed at high concentrations of the Δ5- and Δ10-PITP mutant proteins. Although Δ5- and Δ10-PITP retained some ability to transfer PI and PC, Δ20-PITP could not transfer PI or PC (Fig. 2, A and B).

To carry out phospholipid transfer, PITP has to interact with a phospholipid membrane surface and exchange the endogenous lipid for another. When PITP is purified from mammalian tissues, the two major lipids that are bound to the protein are PI and PC at a ratio of 65:35 (3, 4). The recombinant proteins were expressed in E. coli, an organism devoid of both PI and PC. When expressed in E. coli cells, PITPs are exposed to PE (84.4%), PG (12.5%), and cardiolipin (2.6%), the main phospholipids present in these cells. We first established which of the endogenous E. coli lipids were associated with purified PITPs.

E. coli cells were grown in the presence of [3H]acetate overnight prior to induction to label the endogenous E. coli lipids. PITP was purified, and the lipids associated with the protein were extracted and analyzed by TLC (Fig. 3A). The lipid incor-
Critical Role of PITP C Terminus in PLC Signaling

The presence of GTP

Incubated with wild-type PITP and were depleted of endogenous PITP, and the washed cells were

PLC

Used at high concentrations. We utilized the G-protein-driven

Retain some residual capacity to restore signaling in cells when

In PLC signaling, it would be expected that these proteins may

Reduced PI transfer activity, but near normal PC transfer ac-

Deletion Mutants Have No Ability to Restore Inositol Signaling in Cytosol-depleted HL-60 Cells—

Δ5- and Δ10-PITP mutant proteins had a higher proportion of PE, shifting the ratio to 6:4 PG/PE. This change in binding properties was more marked in Δ20-PITP, where the ratio was nearly 5:5. In addition, the amount of lipid bound to Δ20-PITP was decreased by >60%. The results in Fig. 3B indicate that Δ5- and Δ10-PITP have a similar occupancy compared with wild-type PITP, but only 35% of Δ20-PITP has a lipid bound to the protein.

The exchange of the endogenous lipids with radiolabeled PI and PC was also examined. The proteins were incubated with PC and PI lipid vesicles and reisolated using the His tag, and the radioactivity associated with the proteins was compared with that of wild-type PITP. The data in Fig. 3C illustrate that although Δ5-PITP can exchange the endogenous lipids with PI and PC as well as wild-type PITP, the abilities of the Δ10- and Δ20-PITP mutants to exchange the lipids are significantly decreased.

Deletion Mutants Have No Ability to Restore Inositol Signaling in Cytosol-depleted HL-60 Cells—Δ5- and Δ10-PITP had reduced PI transfer activity, but near normal PC transfer activity at high concentrations of protein. If lipid transfer was the sole determining factor that was responsible for PITP function in PLC signaling, it would be expected that those proteins may retain some residual capacity to restore signaling in cells when used at high concentrations. We utilized the G-protein-driven PLCβ signaling in HL-60 cells for this purpose. HL-60 cells were depleted of endogenous PITP, and the washed cells were incubated with wild-type PITP and Δ5-, Δ10-, and Δ20-PITP in the presence of GTPγS (Fig. 4). Near maximal restoration of PLC signaling occurred around 100 μg of PITP/ml. None of the truncated proteins were able to restore PLC-mediated signaling. The maximal concentration that was practical to test in this assay was 1.5 mg/ml, and despite the addition of such a huge concentration, no reconstitution was observed. Instead, at these high concentrations, a slight inhibition of the GTPγS response was evident.

To exclude the possibility that the bacterial lipids could in any way interfere with the restoration of PLC signaling when added to permeabilized cells, the bacterial lipids were exchanged for PI or PC. Wild-type PITP and Δ5-PITP were converted into the PC and PI forms and compared with the protein loaded with the bacterial lipid PG. Fig. 4B illustrates that the ability of PITP to function in permeabilized cells in PLC signaling is independent of the nature of the loaded lipid.

Site-directed Mutational Analysis—To identify which specific residues may be important for the lipid binding/transfer properties of PITP, we mutated 2 candidate residues. There are clusters of basic amino acids in the C-terminal region in both PITPa and PITPβ, and it was reported that basic amino acid residues are very important for PI 4,5-bisphosphate binding (18). PITPa contains a lysine residue at position 264, whereas PITPβ has a similar basic amino acid, arginine, in this position. In addition to basic amino acids, Alb et al. (19) have reported that replacement of threonine 267 with several different amino acids abolished the PI transfer activity of PITP without any effect on PC transfer activity. Threonine 267 in PITP is a serine in PITPβ, again a conservative change. Although we replaced lysine 264 with isoleucine and threonine 267 with valine or alanine, the site-directed mutants had normal lipid transfer activity (Fig. 2C) and exhibited normal activity in the PLC reconstitution assay (data not shown).

Δ5-PITP Inhibits the Function of Wild-type PITP in PLC

FIG. 1. A, linear display of the mutants used in this study; B, SDS gel electrophoresis illustrating recombinant PITPa proteins (10 μg of protein/lane) and staining with Coomassie Blue. Lane 1, molecular mass markers (205, 116, 97, 66, 45, 29, 20.1, and 18.4 kDa); lane 2, wild-type PITP; lane 3, Δ5-PITP; lane 4, Δ10-PITP; lane 5, Δ20-PITP. a.a, amino acids.

FIG. 2. Comparison of in vitro transfer of PI and PC of wild-type PITP with C-terminally truncated mutants and with site-directed mutants. Recombinant PITP (rPITP) proteins were examined in a PI (A) or PC (B) in vitro transfer assay. The results are expressed as % of maximal transfer observed with wild-type PITP. ●, wild-type PITP; ■, Δ5-PITP; ▲, Δ10-PITP; ▼, Δ20-PITP. Also shown is the PI transfer of two site-directed mutant PITP with wild-type PITP (C). ●, wild-type PITP; ■, T267V; ▲, K264I. Data are means of duplicate measurements that did not vary by >5% and are representative of three independent experiments.
Critical Role of PITP C Terminus in PLC Signaling

In vitro

PITP has diverse effects on several cell functions ranging from PLC signaling to membrane traffic. In all cases reported to date, mammalian PITPa and PITPb can be used interchangeably with SEC14p, the yeast form of PITP, despite the lack of sequence homology with mammalian PITP. Since the PI binding/transfer activity is the common feature shared by the two mammalian forms of PITP and SEC14p, it must be the relevant activity that determines their abilities to restore PLC signaling and membrane traffic. Rescue of SEC14 defects in yeast with mammalian PITP has also been observed (20). However, PITPa only rescues the temperature-sensitive mutations, but not the null mutation. This result clearly indicates that SEC14p and mammalian PITP have distinct as well as overlapping functions.

To identify the structural requirements of PITP, we have systematically deleted the carboxyl terminus of PITPα and have compared the lipid binding/transfer characteristics in vitro with the ability of the truncated proteins to restore inositol lipid signaling in HL-60 cells. Deletion of just 5 amino acid residues was sufficient to impair the function of PITP both in vitro with the ability of the truncated proteins to restore PLC signaling and membrane traffic. Rescue of SEC14 defects in yeast with mammalian PITP has also been observed (20). However, PITPa only rescues the temperature-sensitive mutations, but not the null mutation. This result clearly indicates that SEC14p and mammalian PITP have distinct as well as overlapping functions.

To identify the structural requirements of PITP, we have systematically deleted the carboxyl terminus of PITPα and have compared the lipid binding/transfer characteristics in vitro with the ability of the truncated proteins to restore inositol lipid signaling in HL-60 cells. Deletion of just 5 amino acid residues was sufficient to impair the function of PITP both in lipid transfer and in PLC signaling. In addition, the Δ5-truncated form of PITP was inhibitory to wild-type PITP and potentially functions as a dominant-negative mutant.

Deletion of 5 residues at the C terminus decreased the lipid transfer activity of both PI and PC. PI transfer activity was affected more than PC transfer activity. For example, Δ5-PITP retained 30% of PI transfer activity when examined at 10 times the concentration of wild-type PITP, whereas PC transfer activity was comparable to that of wild-type PITP except that...
Critical Role of PITP C Terminus in PLC Signaling

Three to four times more protein was required. Despite the residual transfer activity obtained with Δ5- and Δ10-PITP, both these deletion mutants had no activity when examined for restoration of G-protein-mediated PLC signaling in HL-60 cells even when 15-fold higher concentrations over wild-type protein were used. The uncoupling of transfer function with reconstitution supports the emerging view that the function of PITP is not just to passively transfer PI from intracellular membranes where it is synthesized to sites of PLC signaling, but to directly participate in the synthesis of PI 4,5-bisphosphate (8, 12, 13).

It is possible that the deletion of the 5 amino acids from the C terminus affects the lipid binding properties of PITP significantly and hence the loss in transfer function. This was not found to be the case. We first examined which lipids were found to be the case. We first examined which lipids were participate in the synthesis of PI 4,5-bisphosphate (8, 12, 13).

Deletion mutants of PITP (Δ5 and Δ10) were still capable of binding the endogenous E. coli lipid, but had slightly altered ratios of anionic lipids (PG) to zwitterionic lipids (PE). Deletion of 20 amino acids had a more dramatic effect that in the amount of lipid associated was decreased by 60%, and this implies that a substantial amount of PITP does not have a lipid bound to it. In all three deletion mutants, a detectable amount of label in cardiolipin was also observed, suggesting that PITP mutants are less discriminatory than wild-type PITP (Fig. 3B).

Truncation also affected the ability to exchange the endogenous lipids with PI or PC in vitro. But the effects were small, and Δ5-PITP could exchange PI or PC as well as wild-type PITP. However, their ability to transfer PI or PC was significantly impaired. Δ20-PITP was completely inactive when examined for PI or PC transfer. Loss of PI transfer with Δ5-PITP was reduced by 30%, whereas restoration of PLC signaling was completely disrupted taking into account the 10-fold higher concentrations of Δ5-PITP used for both assays. This uncoupling of transfer with PLC signaling indicates that in addition to transfer, the PITP molecule has additional properties that are required for PLC signaling.

Δ5-PITP inhibits PITP function in cells, but not in the in vitro PI transfer assay. Inhibition by Δ5-PITP was not of a competitive nature. This suggests that in cells Δ5-PITP interacts with the “putative” receptor for PITP more strongly and effectivlely inhibits full-length PITP from exerting its effect. In the in vitro transfer assay, lipid transfer is a passive event and is not dependent on interaction with another protein. The identification of the binding partners for PITP will provide additional insights into the mechanism of PITP function. It should be recognized that in the visual transduction system in Drosophila, PITP is part of a larger entity that is an integral membrane protein (22).

In summary, we have identified the carboxyl terminus of PITP as being critical in PLC-mediated signaling. Deletion of the carboxyl terminus does not lead to complete loss of transfer activity, but does lead to complete loss of signaling in cells. Thus, the transfer activity is not the only determining factor that dictates the restorative function of PITP in inositol lipid signaling. It has been recently reported that limited proteolysis of PITP by trypsin cleaves the carboxyl terminus at Arg259 and Arg260, and this leads to a decrease in PC transfer activity (23). These data are in accord with our results. In addition, our results suggest that in cells PITP interacts with a putative receptor. The identification of the binding partners for PITP will be greatly facilitated by taking advantage of Δ5-PITP. The ability of the mutant proteins to block signaling will also provide valuable tools in assessing the function of PITP in living cells.

REFERENCES
1. Wirtz, K. W. A. (1991) Annu. Rev. Biochem. 60, 73–99
2. Helmkamp, G. M., Jr. (1990) in Subcellular Biochemistry (Hilderson, H. J., ed) pp. 129–174, Plenum Publishing Corp., New York
3. van Paridon, P. A., Gadella, T. W. J., Jr., Somerharju, P., and Wirtz, K. W. A. (1987) Biochim. Biophys. Acta 903, 68–77
4. van Paridon, P. A., Visser, A. J. W. G., and Wirtz, K. W. A. (1987) Biochim. Biophys. Acta 898, 172–180
5. Somerharju, P., van Paridon, P., and Wirtz, K. W. A. (1983) Biochim. Biophys. Acta 731, 186–195
6. Westerman, J., de Vries, K.-J., Somerharju, P., Timmermans-Herejgers, J. L. P. M., Snoek, G. T., and Wirtz, K. W. A. (1995) J. Biol. Chem. 270, 14263–14266
7. Thomas, G. M. H., Cunningham, E., Fensome, A., Ball, A., Totty, N. F., Truong, O., Hauan, J., and Cockcroft, S. (1993) Cell 74, 919–928
8. Kauffmann-Zeh, A., Thomas, G. M. H., Ball, A., Prasser, S., Cunningham, E., Cockcroft, S., and Hauan, J. (1996) Science 270, 1188–1190
9. Cunningham, E., Ball, A., Tan, S. W., Swigart, P., Hauan, J., Bankaitis, V., and Cockcroft, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6589–6593
10. Hay, J. C., and Martin, T. F. J. (1993) Nature 366, 572–575
11. Ohashi, M., de Vries, K. J., Frank, R., Snoek, G., Bankaitis, V., Wirtz, K., and Huttner, W. B. (1995) Nature 377, 544–547
12. Fensome, A., Cunningham, E., Prasser, S., Tan, S. K., Swigart, P., Thomas, G., Hauan, J., and Cockcroft, S. (1995) Curr. Biol. 6, 780–784
13. Cunningham, E., Thomas, G. M. H., Ball, A., Hiles, I., and Cockcroft, S. (1995) Curr. Biol. 5, 775–783
14. Hay, J. C., Pisette, P. L., Jenkins, G. H., Fukushima, T., Takenawa, T., Anderson,
Critical Role of PITP C Terminus in PLC Signaling

15. Bankaitis, V. A., Aitken, J. R., Cleves, A. E., and Dowhan, W. (1990) *Nature* 347, 561–562
16. McGee, T. P., Skinner, H. B., Whitters, E. A., Henry, S. A., and Bankaitis, V. A. (1994) *J. Cell Biol.* 124, 273–287
17. Lopez, M. C., Nicaud, J. M., Skinner, H. B., Vergnolle, C., Bankaitis, V. A., Kader, J. C., and Gaillardin, C. (1994) *J. Cell Biol.* 124, 113–127
18. Fukami, K., Sawada, N., Endo, T., and Takenawa, T. (1996) *J. Biol. Chem.* 271, 2646–2650
19. Alb, J. G., Jr., Gedvilaitie, A., Cartee, R. T., Skinner, H. B., and Bankaitis, V. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 8826–8830
20. Skinner, H. B., Alb, J. G., Whitters, E. A., Helmkamp, G. M., and Bankaitis, V. A. (1993) *EMBO J.* 12, 4775–4784
21. Geijtenbeek, T. B. H., De Groot, E., Van Baal, J., Brunink, F., Westerman, J., Snoek, G. T., and Wirtz, K. W. A. (1994) *Biochim. Biophys. Acta* 1213, 309–318
22. Vihtelic, T. S., Goebl, M., Milligan, S., O'Tousa, S. E., and Hyde, D. R. (1993) *J. Cell Biol.* 122, 1013–1022
23. Tremblay, J. M., Helmkamp, G. M., Jr., and Yarbrough, L. R. (1996) *J. Biol. Chem.* 271, 21075–21080
The First 5 Amino Acids of the Carboxyl Terminus of Phosphatidylinositol Transfer Protein (PITP) α Play a Critical Role in Inositol Lipid Signaling: TRANSFER ACTIVITY OF PITP IS ESSENTIAL BUT NOT SUFFICIENT FOR RESTORATION OF PHOSPHOLIPASE C SIGNALING

Shuntaro Hara, Phil Swigart, David Jones and Shamshad Cockcroft

J. Biol. Chem. 1997, 272:14908-14913.
doi: 10.1074/jbc.272.23.14908

Access the most updated version of this article at http://www.jbc.org/content/272/23/14908

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 22 references, 8 of which can be accessed free at http://www.jbc.org/content/272/23/14908.full.html#ref-list-1