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Minireview

# Phosphatidylinositol transfer proteins: requirements in phospholipase C signaling and in regulated exocytosis

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**Abstract Phosphatidylinositol transfer proteins (PITP) are abundant cytosolic proteins originally identified because of their ability to act in vitro as specific transporters of phosphatidylinositol or phosphatidylcholine between membranes. However, the cellular function of mammalian PITP has remained enigmatic till recently. Due to the development of reconstitution assays in cytosol-depleted cells, PITP was found to be an essential component for phospholipase C-mediated hydrolysis of $PIP_2$ and for regulated exocytosis. The exact mechanism how PITP exerts its effects is not known but the PI binding/transfer activity of PITP can partly explain its cellular function. PITP would enable the local synthesis of $PIP_2$ by delivering PI to specialized signaling sites.**



*Key words:* Phosphatidylinositol bisphosphate; Phospholipase C; Exocytosis; Phosphatidylinositol transfer protein; SEC14

## 1. Introduction

The development of powerful reconstitution assay systems is beginning to reveal important function(s) of phosphatidylinositol transfer protein (PITP) in transmembrane signaling and in regulated exocytosis in mammalian cells. Using cell permeabilization to deplete cytosolic proteins followed by reconstitution assays, PITP was purified as a major factor that could restore G-protein-mediated phospholipase C (PLC) activation in HL60 cells [1]. Application of a similar strategy in PC12 cells simultaneously revealed that PITP could restore $Ca^{2+}$-dependent exocytosis [2]. The cellular functions of PITP are just beginning to be identified although the existence of PITP has been known for nearly 30 years [3].

This review will focus mainly on these recent developments and discuss the possible mechanisms of how PITP accomplishes its function in PLC signaling and regulated exocytosis in mammalian cells. PITPs SEC 14p have also been identified in the yeast *Saccharomyces cerevisiae* where it regulates the exit of vesicles from the Golgi destined for the plasma membrane [4]. The way the yeast PITP homologue executes this function has been discussed recently and differs with what is thought to take place in mammalian cells [5]. Finally, PITP has also been identified in Drosophila from a retinal degeneration mutant [6] in the visual system which consists of a $G_q$-protein-regulated PLCβ isozyme (referred to as NorpA).

PITP was originally identified as a protein that could mediate the transfer of PI or PC between membrane compartments, and hence its name [3]. PITP has a single phospholipid binding site which is occupied by either PI or PC with the affinity for PI being 16-fold higher than for PC. PITP has 270 amino acid residues and is a 35–36 kDa protein as estimated by SDS-PAGE. Two distinct PITPs have now been identified: the originally identified PITP (now named α) and the newly characterised PITPβ isoform. PITPβ contains 271 amino residues and is 77% identical (94% homologous) at the primary sequence level to PITPα and represents the product of a separate gene [7,8]. PITPβ was identified because of its ability to complement SEC14 mutants in yeast and can thus be regarded as the functional equivalent of SEC14. In this regard, PITPβ isoforms, like SEC14p, are found associated with Golgi membranes [9–11].

The PI and PC binding and transfer activity of mammalian PITP (α and β) is also shared with SEC14p. The product of the *SEC14*$^{sc}$ gene from *S. ceresvisiae* is required for export of yeast secretory proteins including invertase from the Golgi complex [4]. Phenotypically, SEC14 mutants show an expansion of the Golgi complex and the accumulation of secretory granules within the cytoplasm [12,13]. Release of invertase to the external medium is blocked in SEC14 mutants [14]. In contrast to *S. ceresvisiae*, in the dimorphic yeast *Yarrowia lipolytica*, SEC14 mutants do not have a defect in the secretory pathway. Instead, SEC14$^{yp}$ mutants cannot undergo the characteristic dimorphic transition from the yeast to the mycelial form that typifies this species [15].

The PITP from *S. cerevisiae* is a 35 kDa protein but shares no primary sequence homology with mammalian PITPs. SEC14p$^{SC}$ shares significant homology to other yeast PITPs, including that from *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Yarrowia lipolytica*. Table 1 summarises the known family members that belong to the PITP family in mammalian cells, yeast, and other organisms. Despite the lack of homology at the amino acid sequence level, all these proteins share the ability of transferring PI or PC between membrane compartments and bind PI or PC. Recent studies in Drosophila have revealed the existence of a membrane-anchored form of PITP. This protein is an integral membrane protein of 1054 amino acid residues (160 kDa); the 281 amino acids of the amino-terminus show strong homology to mammalian PITPs and a truncated rdgB protein, which only contains this amino-terminal domain, possesses PI transfer activity in vitro.

Fig. 1 summarizes the known functions of PITP in mammalian cells. How PITP can account for supporting such a diverse range of biological function from signaling events to membrane traffic is not clear but some common themes occur

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in mammalian cells and this relates to the ability of PITP to participate in the compartmentalized delivery of PI for lipid phosphorylation. Whether this explanation can be extended to yeast is not clear and other mechanisms have been proposed ([5] for discussion).

## 2. Requirement of PITP in phospholipase C-mediated $PIP_2$ hydrolysis

The identification of an essential requirement for PITP was made when G-protein-mediated PLC hydrolysis of phosphatidylinositol bisphosphate ($PIP_2$) was examined in permeabilized HL60 cells. It was found that hydrolysis of $PIP_2$ when stimulated by GTPγS or the receptor-directed agonist, fMet-LeuPhe was impaired when cytosolic proteins were depleted from the permeabilized cells. The cytosol-depleted cells, however, retained all the components that were known to be required for $IP_3$ formation: the receptor, the G-protein and the phospholipase Cβ. Re-addition of exogenous cytosol was found to restore the ability of the fMetLeuPhe receptor-mediated $IP_3$ production. PITP was identified as the major reconstituting factor in the cytosol [1,16]. A second activity was also found and this was identified as phospholipase Cβ. PITP and PLCβ both participate in the production of $IP_3$.

PLC enzymes are classified into three families (β, γ and δ) which are differentially regulated. PLCβ isozymes are regulated by G-proteins and PLCγ forms are regulated by tyrosine phosphorylation. A requirement for PITP in PLC-γ1-mediated hydrolysis of $PIP_2$ stimulated by receptor tyrosine kinases was subsequently identified [17,18]. In A431 cells, the EGF receptor autophosphorylates tyrosine residues and allows the recruitment of PLC-γ1 through interactions with the SH2 domains present on PLCγ1. In RBL cells, crosslinking of the IgE receptor by antigen stimulates inositol lipid hydrolysis and is mediated by PLCγ1. In both cell-types, extensive permeabilization led to loss of $IP_3$ production stimulated by the appropriate agonists and was accompanied by the loss of cytosolic proteins including PLCγ1 and PITP. Re-addition of PLCγ1 was not sufficient to restore receptor-mediated inositol lipid hydrolysis in these cytosol-depleted cells. However, the combined presence of PLCγ1 and PITP was fully capable of restoring $IP_3$ production.

The requirement in PITP for PLCβ- and PLCγ-catalysed hydrolysis of $PIP_2$ to produce $IP_3$ in permeabilized cells clearly suggests that PITP functions at a common point in these differentially-regulated signaling pathways and that could be at the level of substrate availability. Although the mechanism of PLCδ regulation is currently not known, it can be predicted that PITP will also be required.

## 3. PITP is required for substrate provision for the phospholipases

The execution point for PITP in PLC signaling was identified to lie upstream of PLCs. Reconstitution of PITP for G-protein-PLCβ is strictly dependent on the presence of MgATP [1]. The substrate for the PLCs is $PIP_2$ and its concentration is a limiting factor in vivo. $PIP_2$ is synthesized from PI by sequential phosphorylation of PI by PI 4-kinase and PIP 5-kinase. In stimulated cells, the amount of $IP_3$ generated far exceeds the resting levels of $PIP_2$ [16]. In addition, only very transient decreases in $PIP_2$ concentrations occur during stimulation. All this points to PITP participating in the synthesis of $PIP_2$. Evidence that PITP increases the phosphorylation of PI to PIP and $PIP_2$ has been presented [16,17,19].

The lipid kinases, PI 4-kinase and PIP 5-kinase sequentially phosphorylate PI to $PIP_2$ at the plasma membrane where PLCs are activated by cell-surface receptors. In contrast, synthesis of PI occurs at the endoplasmic reticulum [20]. Thus the transfer of PI by PITP from this intracellular site to the plasma membrane would be required during cell surface stimulation. The property of PITP would be well-suited for this function.

PITPα, PITPβ and the yeast PITP, $SEC14^{sc}$ are equally effective in supporting PLC activation [18] and the common feature shared by all three transfer proteins is the ability to

Table 1
PITP proteins in mammalian cells, yeast, fungi and Drosophila

| | | | Size characteristics | Comments |
|---|---|---|---|---|
| Mammalian PITP | | | | |
| PITPα | (human, rat, mouse, rabbit) | [25,26] | 270–271 amino acid residues; 35 kDa | can transfer PI or PC between membranes |
| PITPβ | (rat) | [7,8] | 271 amino acid residues; 36 kDa | as PITPα but can also transfer sphingomyelin |
| Drosophila PITP | | | | |
| rdgB | (*Drosophila*) | [6] | 1054 amino acid residues | the amino terminal 281 residues are homologous to mammalian PITPα; a mammalian homologue has also been identified |
| Yeast PITP | | | | |
| $SEC14p^{SC}$ | (*Saccharomyces cerevisiae*) | [4,14] | 303 amino acid residues; 36 kDa | can transfer PI or PC between membranes |
| $SEC14p^{KL}$ | (*Kluyveromyces lactis*) | [14] | 301 amino acid residues | sequence homology to $SEC14p^{SC}$ |
| $SEC14p^{YL}$ | (*Yarrowia lipolytica*) | [15] | 497 amino acid residues | sequence homology to $SEC14p^{SC}$ |
| $SEC14p^{CA}$ | (*Candida albicans*) | [27] | 301 amino acid residues | sequence homology to $SEC14p^{SC}$ |
| Fungi PITP | | | | |
| *Mucor mucedo* | (Zygomycetes) | [28] | not cloned, 24 kDa | can transfer PG > PI. > PC; N terminal sequence determined and shares homology to other fungi (Aspergillus oryzae) |
| *Neurospora crassa* | (Ascomycetes) | [29] | not cloned; 19 kDa | can transfer PG > PI. > PC; |
| *Asperpergillus oryzae* | (Deuteromycetes) | [30] | not cloned; 19 kDa | can transfer PG > PI. > PC; |

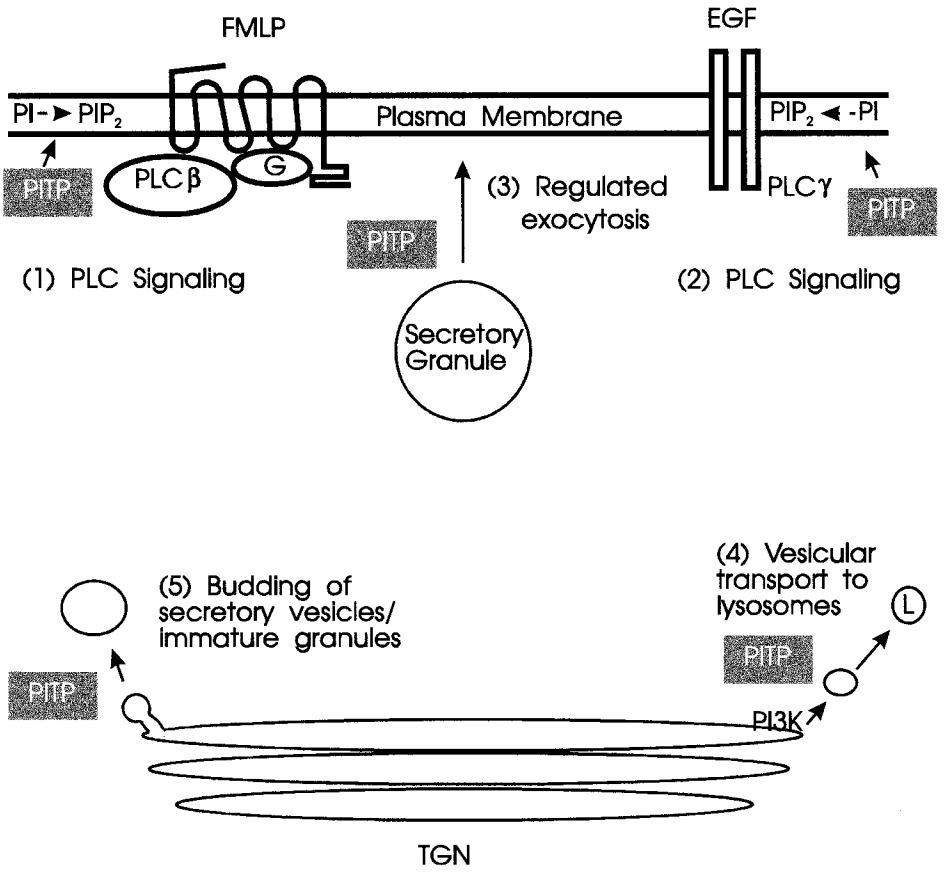



Fig. 1. PITP function identified in mammalian cells. Several different functions of PITP have been recently identified in mammalian cells. Biochemical data indicate a role for PITP in (1 and 2) the compartmentalized synthesis of $PIP_2$ for PLCβ-mediated $PIP_2$ hydrolysis [1,16] and for PLCγ-mediated $PIP_2$ hydrolysis. [31]. (3) PITP is required for regulated exocytosis in PC12 and HL60 cells [2,23]; in PC12 cells PITP functions together with PIP 5-kinase and $PIP_2$ is important here [19] (4) PI 3-kinase (PI ⇒ PI3P) is required for vesicle-mediated transfer to lysosomal compartments and PITP is also implicated here [21]; (5) PITP can cause budding of secretory vesicles and immature granules in PC12 cells [24].

bind and transfer PI. Thus the known properties of PITP in vitro strongly suggest that the requirement for PITP in PLC signalling can be partly due to transfer of PI from sites of synthesis in the endoplasmic reticulum to sites of lipid phosphorylation and hydrolysis at the plasma membrane.

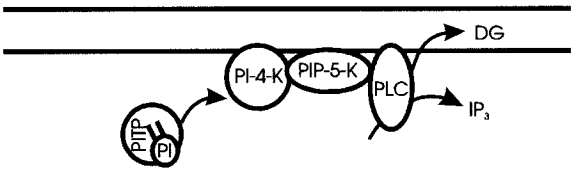
We have hypothesized that in addition to the requirement of the transfer activity of PITP, it also acts as a cofactor in the synthesis of $PIP_2$. The cofactor function proposes that the lipid carried by PITP is preferentially used by the lipid kinases (see Fig. 2). Thus it can be envisaged that the PI molecule bound to PITP is channeled through a complex of enzymes through phosphorylation and hydrolysis. Channeling is defined as a concept where metabolic intermediates are transferred between sequential enzymes in a pathway without equilibration of the free intermediates with the bulk environment.

PITP can essentially provide a source of PI to appropriate membrane compartments on demand where it can be used by the appropriate lipid kinases (PI 4-kinases and PI 3-kinase) for synthesis of phosphorylated derivatives of PI. PITP has been found to associate with PI 4-kinase and PLCγ1 in A431 cells subsequent to stimulation by EGF [17]. PITP has also been found to interact with a 3-kinase that specifically phosphorylates phosphatidylinositol to PI3P [21]. In vitro, PITPα, PITPβ and SEC14p also increase the rate of PI3P formation when added to PI vesicles and PI 3-kinase. All this supports the notion that PITP plays a cofactor role in lipid substrate presentation to the lipid kinases [21]. The substrate presentation model to the lipid kinases is analogous to the GM2 activator protein where this protein is thought to lift the glycolipid from the membrane to enable hydrolysis by β-hexosaminidase to occur [22].

## 4. PITP in regulated exocytosis in mammalian cells

The use of permeabilized cells revealed an unexpected role for PITP in regulated exocytosis. In this case, examination of $Ca^{2+}$-regulated release of noradrenaline from dense core granules in PC12 cells was under investigation. MgATP and cytosol are required for 'priming' prior to $Ca^{2+}$-triggered exocytosis. A reconstitution assay was established to identify these 'priming' factors in the cytosol. Three separate priming factors were detected and two of them have been identified: one is PITP and the other is PIP 5-kinase [2,19]. These two proteins function together with PI 4-kinase that is membrane-localized in the conversion of PI to $PIP_2$. The site of this conversion has not yet been elucidated.

The requirement for PITP in exocytosis has also been shown for HL60 cells and RBL cells. Permeabilized HL60 cells (a cell-line related to the neutrophil) and RBL cells (a cell-line related to mast cells) can be induced to secrete speci-




PITP "channels" PI directly to PI-4-K

Fig. 2. Interactions of PITP with lipid kinases. A possible model illustrating how PITP presents PI to the lipid kinases. The PI molecule bound to PITP is channeled through a complex of enzymes through phosphorylation and hydrolysis. Channeling is defined as a concept where metabolic intermediates are transferred between sequential enzymes in a pathway without equilibration of the free intermediates with the bulk environment. This model takes into account the observation that Sec14p, PITPα and PITPβ are all equally effective in supporting PLC signaling [21].

alized lysosomal granules by micromolar $Ca^{2+}$ in the presence of GTPγS. Exocytosis becomes refractory to stimulus in cytosol-depleted cells and this can be restored upon re-addition of PITP [23]. Secretion is MgATP dependent indicating that phosphorylation events need to take place, most likely PI phosphorylation to $PIP_2$.

As in PLC signaling, PITP (α and β) and SEC14p can be used interchangeably in mammalian cells to support regulated exocytosis. Current evidence points to intact $PIP_2$ as a requirement for exocytosis of secretory granules. How $PIP_2$ participates in exocytosis is not clear but numerous $PIP_2$ binding proteins have been identified and these are potential targets. Examples might include synaptotagmin, cytoskeletal and actin-binding proteins. In addition to exocytosis, these three PITP proteins have also been found to support budding of secretory vesicles from the Golgi membranes in PC12 cells [24], a function analogous to SEC14p in the yeast, *S. cerevisiase*. No data have been presented to address whether budding is dependent on phosphorylated PIs by PI 3-kinases or PI 4-kinases in mammalian cells.

## 5. Conclusions

PITP has been identified as a major player in PLC signaling and in regulated exocytosis in mammalian cells and, an underlying theme has emerged. The cellular function of PITP is the participation in the synthesis of phosphorylated derivatives of PI including $PIP_2$. $PIP_2$ has recently been shown to be a key phospholipid that is not only a substrate for the phospholipases and for PI 3-kinases, but also has as a second messenger function as an intact lipid. The concentration of $PIP_2$ in cells is tightly regulated and arises from the activities of several PI 4-kinases PIP 5-kinases, some of which are subject to regulation. The individual lipid kinases responsible for the provision of $PIP_2$ as substrate for PLC signaling and PI 3-kinase activities, and as a second messenger in exocytosis remain to be identified.

The requirement for PITP in other aspects of inositol lipid signaling is likely to emerge, such as in PI 3-kinase signaling pathways. PITP, a cytosolic protein is well suited to provide PI for the spatially segregated events in any part of the cell including the Golgi membranes, secretory vesicles, the plasma membrane and even the nucleus. The highly conserved nature of mammalian PITP in evolution, presumably underlines the importance of this molecule in different aspects of biology. Whether this function of PITP can be extended to yeast remains unproven.

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