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Minireview

Current thoughts on the phosphatidylinositol transfer protein family

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Abstract Monomeric transport of lipids is carried out by a class of proteins that can shield a lipid from the aqueous environment by binding the lipid in a hydrophobic cavity. One such group of proteins is the phosphatidylinositol transfer proteins (PITP) that can bind phosphatidylinositol and phosphatidylcholine and transfer them from one membrane compartment to another. PITPs are found in both unicellular and multicellular organisms but not bacteria. In mice and humans, the PITP domain responsible for lipid transfer is found in five proteins, which can be classified into two classes based on sequence. Class I PITPs comprises two family members, α and β, small 35 kDa proteins with a single PITP domain which are ubiquitously expressed. Class IIA PITPs (RdgBαI and II) are larger proteins possessing additional domains that target the protein to membranes and are only able to bind lipids but not mediate transfer. Finally, Class IIB PITP (RdgBβ) is similar to Class I in size (38 kDa) and is also ubiquitously expressed. Class III PITPs, exemplified by the Sec14p family, are found in yeast and plants but are unrelated in sequence and structure to Class I and Class II PITPs. In this review we discuss whether PITP proteins are passive transporters or are regulated proteins that are able to couple their transport and binding properties to specific biological functions including inositol lipid signalling and membrane turnover.

Key words: Phospholipase C; Phosphatidylinositol 3-kinase; Phosphoinositide; Membrane traffic; Growth factor; Lipid signaling

1. Introduction

Eukaryotic phosphatidylinositol transfer proteins (PITPs) are ubiquitous proteins that transport phosphatidylinositol (PI) or phosphatidylcholine (PC) between membranes and participate in cellular phosphoinositide metabolism during signal transduction and vesicular trafficking [1–3]. PITPs were first purified on the basis of their transfer activity towards PI [4] and subsequent studies have implicated PITP in delivering PI to specific cellular compartments, where PI can be further phosphorylated by specific lipid kinases [5–12]. This function of PITP depends on its ability to bind a single PI or PC molecule. PITPα has an internal cavity that can sequester the entire lipid within the body of the protein and therefore facilitate transport through the hostile aqueous environment [13]. The ability to transport hydrophobic ligands is not unique to PITPs and is shared by many other proteins. PI/PC transfer can also be mediated by Sec14p (also known as yeast PITP), a protein first identified in *Saccharomyces cerevisiae* [14]. However, Sec14p is unrelated to PITP proteins in both sequence and structure [13,15]. PC transfer can also be mediated by the START domain found in PC transfer protein (PC-TP), a 28 kDa protein [16–18]. PITP, PC-TP and Sec14p are examples of proteins that mainly comprise a single domain that can sequester a hydrophobic ligand in a cavity and so is able to mediate the movement of the ligand through the aqueous phase. With the completion of the sequencing of genomes of several organisms, it is increasingly clear that these domains are not only found in proteins, comprising a single domain, but can be present in larger proteins with several other domains.

2. Proteins containing the PITP domain

Table 1 provides a summary of PITP proteins identified in various organisms (by sequence only in some cases) and the proteins have been classified by sequence similarity. The best studied PITPs are the mammalian Class I PITPs, comprising PITPα and PITPβ which are 77% identical and 94% homologous. Class I proteins are widespread and are found in both unicellular and multicellular eukaryotes but not bacteria. Examples of such multicellular eukaryotes include mammals (humans, mice, rat, rabbit), flies (*Drosophila melanogaster*, *Anopheles gambiae*), worms (*Caenorhabditis elegans*) and fish (*Danio rerio* (zebrafish) and *Fugu rubripes* (Japanese puffer fish)). Examples of simple unicellular eukaryotes which contain proteins which are similar in size and sequence to PITPα/β and contain a single PITP domain include parasites (*Plasmodium falciparum*, *Encephalitozoon cuniculi*) and soil amoebae (*Dictyostelium discoideum*). *E. cuniculi* is an interesting parasite as its genome is compact meaning that many of the proteins are shortened compared to their counterparts in other organisms [19]. Thus the PITP protein is only 251 amino acids long compared to 270 in humans and has lost residues at the C-terminus. In PITPα, the C-terminus is important for maintaining the structure in a compact form (see later). How-

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Abbreviations: PITP, phosphatidylinositol transfer protein; PI, phosphatidylinositol; PC, phosphatidylcholine; PC-TP, PC transfer protein; PI(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PI(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate; SM, sphingomyelin; SMase, sphingomyelinase; DAG, diacylglycerol; PLC, phospholipase C; FRET, fluorescence resonance energy transfer; PYK2, a tyrosine kinase

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Table 1
Classification of the proteins containing the PITP domain from various organisms

Organism	Proteins with PITP domains			Comments
	Class I	Class IIA	Class IIB	
Homo sapiens/*Mus musculus*	PITPα (*vibrator*)	RdgBαI/Nir2/PITPnm	RdgBβ	PITPα – *vibrator* phenotype
	PITPβ	RdgBαII/Nir3		PITPβ – embryonic lethal RdgBαI – embryonic lethal RdgBαII – no obvious phenotype
Caenorhabditis elegans	PITP1 PITP2	RdgBα	Not present	RNAi knockout of PITP1 and RdgBα No obvious phenotype
Drosophila	PITP (*vib*)	RdgBα	RdgBβ	RdgBα – retinal degeneration
Dictyostelium	PITP I PITP II	Not identified	Not identified	Dd-Sec14p is also found in this organism which is a bona fide PITP
Plasmodium falciparum	PITP	Not identified	Not identified	Malarial parasite
Encephalitozoon cuniculi	PITP	Not present	Not present	Eukaryotic unicellular parasite containing 1997 potential protein coding genes only
Saccharomyces cerevisiae	Not present	Not present	Not present	Yeast PITP (Sec14p)
Schizosaccharomyces pombe	Not present	Not present	Not present	Yeast PITP (Sec14p)

The sequence-unrelated yeast PITPs (Sec14p family) are not classified in the table. Sec14p and Sec14p-related proteins are widespread in mammals, plants and yeasts (designated Class III) (see text).

ever, since the C-terminal region is the most divergent between PITPα and PITPβ, the protein may have a different strategy to maintain its compact structure.

Class II PITPs are the RdgB proteins, so named because the first isoform was identified in *Drosophila* as the gene responsible for degeneration of the retina (retinal degeneration mutant phenotype type B). RdgB proteins are classified into Class IIA (RdgBα) and IIB (RdgBβ) which is based on their size. RdgBα proteins are 160 kDa and possess an N-terminal PITP domain followed by an acidic calcium binding domain, six short hydrophobic regions, a putative metal ion binding domain and a carboxy-terminal PYK2 binding domain. Whilst *Drosophila* and *C. elegans* have a single RdgBα gene, mice/humans have two genes (m-RdgBαI and m-RdgBαII). In *Drosophila* RdgBα is required for both photoreceptor cell viability and the light response. Mammalian RdgBαI has widespread tissue distribution and can functionally rescue the *Drosophila* RdgB mutant phenotypes. RdgBαII possesses a neuronal-specific expression pattern, with high levels in the retina and the dentate gyrus of the hippocampus. RdgBαII cannot fully restore the light response in *Drosophila*, unlike RdgBαI, indicating that functional differences are likely to exist between the two mammalian RdgB homologues [20]. More recently, a 38 kDa soluble form of PITP was cloned which was more closely related in sequence to the PITP domain of RdgBα, and termed RdgBβ which forms Class IIB [21] (Table 1). RdgBβ is widely expressed in most tissues including heart, muscle, kidney, liver and peripheral blood leukocytes.

In the yeast *S. cerevisiae*, a 35 kDa protein has been characterised as a PITP (Sec14p) but bears no sequence or structural homology to Class I and Class II PITPs [15]. To distinguish between Class I and II PITPs and yeast PITPs (Sec14p), we will refer to this family of proteins as Class III proteins which includes Sec14p and other sequence-related proteins. Sec14p is localised to the Golgi and is essential for protein transport from the Golgi complex [22]. Most relevant to the discussion here is that the temperature-sensitive mutants (but not null) can be rescued by over-expression of either PITPα or PITPβ [23,24]. Sec14p-related proteins are widespread and are found in humans, mice, plants and many forms of yeast. Not all Sec14p-related proteins are PITPs, however; some bind and transfer other hydrophobic ligands such as α-tocopherol, 11-*cis*-retinal and squalene [25,26]. Also the biological function of Sec14p is different in various yeasts. In *Yarrowia lipolytica*, Sec14p is not required for viability nor for the secretory pathway but is required for dimorphic transition from the yeast to the mycelial form that typifies this species [27]. In *Schizosaccharomyces pombe*, Sec14p is required for both secretory function and forespore membrane formation [28]. Despite the different biological functions ascribed to Sec14p, it is probable that the underlying core biochemistry is the same. Thus lessons can be learnt from Sec14p studies in yeast. The Sec14p from *S. cerevisiae* has been subject of much investigation and the available genetic and biochemical data indicate that Sec14p modulates both PI and PC metabolism. Sec14p regulates the levels of PI 4-phosphate at the Golgi [29,30] and also levels of diacylglycerol (DAG) by inhibiting its utilisation for PC synthesis [31]. In this brief review, the Sec14p family of PITPs will be mentioned where pertinent in understanding the Class I PITPs. In particular this is most relevant for PITPβ, which, like Sec14p, is also Golgi-localised [32,33].

3. What gene knockout studies tell us about biological function

In mice, the essential nature of PITP is indicated by the *vibrator* mutation, which causes neuronal degeneration due to a fivefold reduction in PITPα levels [34]. The *vibrator* mutation causes an early-onset progressive action tremor, degeneration of brainstem and spinal cord neurones, followed by juvenile death. Further support for an in vivo role in neuronal function comes from the characterisation of *Drosophila rdgB* mutations where the null mutants undergo light-induced retinal degeneration. The *Drosophila* RdgB protein is a membrane-associated protein containing a PITP domain. In this organism, RdgBα functions together with rhodopsin, Gq-related G protein and a phospholipase Cβ isozyme in the visual signal transduction cascade [35,36]. In contrast, deletion of the gene encoding mouse PITPβ or RdgBαI is embryonic lethal [37,38]. In *C. elegans*, RNAi has been used to knock out two of the three PITP genes individually but no phenotype was recorded. The presence of five proteins with a PITP domain in

mammalian cells and their widespread expression supports the emerging concept that different PITP proteins have distinct functions in vivo. Whilst there is much evidence supporting an important role of PITPs in neuronal function in both flies and mammals, the presence of PITP genes in unicellular organisms, including parasites, indicates that PITP proteins have been put to use in multiple ways. The underlying core biochemical mechanism is, however, likely to be the same – its ability to regulate lipid metabolism. Knockout studies in these different organisms will be required to reveal the multitude of biological functions of this family of proteins.

4. Coupling the ability to transport lipids to their subsequent metabolism

The emergent view is that PITP proteins are not just passive mediators of lipid transport but function in complex ways by modulating phospholipid metabolic pathways and impacting on many cellular processes including lipid-mediated signalling pathways and membrane traffic. Studies from permeabilised cells indicate that PITP is an integral part of the machinery which is required for the receptor-regulated production of the two second messengers, inositol 1,4,5-trisphosphate and DAG by phospholipase Cs (PLCs) [5,6,39] and for phosphoinositide 3-kinase-mediated phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$) production [9]. The PLC signalling pathway is a major consumer of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and the plasma membrane pool of inositol lipids is replenished with PI from intracellular compartments by PITP, by virtue of its ability to bind and deliver PI [39,40]. PITP also participates in the subsequent phosphorylation of PI [6,8]. Thus PITP couples PI delivery and $PI(4,5)P_2$ synthesis in cell signalling. In membrane transport, PITP plays a role in the provision of PI for further phosphorylation. In particular, PITP can influence the production of 3-phosphorylated phosphoinositides including phosphatidylinositol 3-phosphate [7,10,41]. The intact phosphorylated inositol lipid then provides a binding site for cytosolic proteins to be recruited to membrane surfaces where they can mediate their biological functions [42].

PITPβ, in addition to transferring PI and PC, has been reported to transfer sphingomyelin (SM) [32,43]. In these studies PITPβ was reported to exhibit a higher transfer activity towards fluorescent SM than even PI, suggesting that PITPβ bound SM with high affinity. However, in two recent studies, transfer was examined using natural cellular SM, and it was concluded that both PITPα and PITPβ were capable of transferring SM with a low but comparable efficiency, and that PITP transfer activity towards SM was much less compared to that towards PI and PC. PITPβ was found to be marginally better than PITPα in transferring SM in both these studies [33,44]. Previous studies where fluorescent SM was used may be misleading due to the presence of the bulky fluorophore, which could affect both packing of the donor membrane and the binding of the lipid to the PITP. Thus the SM transfer function of PITPβ in SM signalling and traffic remains unclear.

Studies from *S. cerevisiae* have indicated that Sec14p is important for regulating PC metabolism and ensuring that DAG is available at the Golgi for vesicle formation [45]. These studies have prompted the examination of the possible role of PITPβ and PITPα in modulating PC metabolism. In addition, because PITPβ was reported to transport SM (as discussed above), the possible role of PITPβ in SM metabolism has been also examined. In mammalian cells, the synthesis of PC and SM are localised in the endoplasmic reticulum (ER) and Golgi respectively (Fig. 1). In three separate studies, the role of PITP in the metabolism of PI and PC has been examined. In the first study, antisense RNA was used to reduce the amount of PITPα by 25% in WRK-1 rat mammary tumour cells. This resulted in a 30–40% decrease in choline metabolites including PC, SM, lyso-PC, glycerophosphocholine and choline whilst cholineP and CDP-choline were increased. The results were interpreted to mean that the rate-limiting enzyme, phosphorylcholine cytidyl transferase, was inhibited (see Fig. 1). Thus the decrease in PC de novo synthesis observed here [46] is in contrast to the situation in yeast where suppression of Sec14p leads to enhanced PC synthesis [47]. No effects were observed on inositol-derived lipids before and subsequent to stimulation with vasopressin. However, a 25% decrease in PITPα may not be sufficient to show any effects as both PITPα and PITPβ are able to replenish PI to the plasma membrane (see later).

In a second study, PITPβ over-expression (over 10-fold) in NIH3T3 cells was shown to enhance re-synthesis of SM upon degradation by exogenous sphingomyelinase (SMase). The results were interpreted to mean that PITPβ regulated SM synthase activity and/or SM transport from the Golgi to the plasma membrane [48] (see Fig. 1). SM synthesis mainly occurs in the lumen of the Golgi from which it is transported to the outer leaflet of the plasma membrane by vesicular traffic. SM synthase transfers the phosphocholine moiety of PC to ceramide in order to generate SM and DAG [49]. Therefore, SM synthase may contribute to the reciprocal regulation of the levels of PC and DAG in Golgi membranes (see Fig. 1).

In the third study, we have examined whether over-expression of PITPα or PITPβ modulates sphingolipid and PC metabolism [50]. Using COS-7 cells, PITPβ over-expression did not stimulate SM re-synthesis upon degradation by exogenous SMase, suggesting that the ability of PITPβ to interfere with SM metabolism might be cell type-specific. On the other hand, the amount of endogenous PITPβ in COS-7 cells might be sufficient for stimulating SM synthase activity. Nevertheless, in purified Golgi membranes that are depleted in endogenous PITPβ, the addition of recombinant PITPβ does not increase SM production (but increases phosphatidylinositol 4-phosphate production (D. Jones and S. Cockcroft, unpublished)). Additionally, over-expression of either PITP did not affect de novo PC synthesis [50].

The conclusions drawn from studies where PITPs have been over-expressed or suppressed are inconsistent with each other and further work needs to be done.

5. Mechanism of lipid exchange

How do lipid transfer proteins exchange their cargo lipid? This is a common problem shared by all lipid transport proteins as the proteins have to be soluble but must transiently associate with the membrane in order to pick up their hydrophobic ligand. Another common property shared by PITP and other lipid transporters is that the ligand is in a binding pocket and the protein has to undergo a conformational change to open this cavity in order to allow the lipid to leave. What determines this process is critical in our understanding

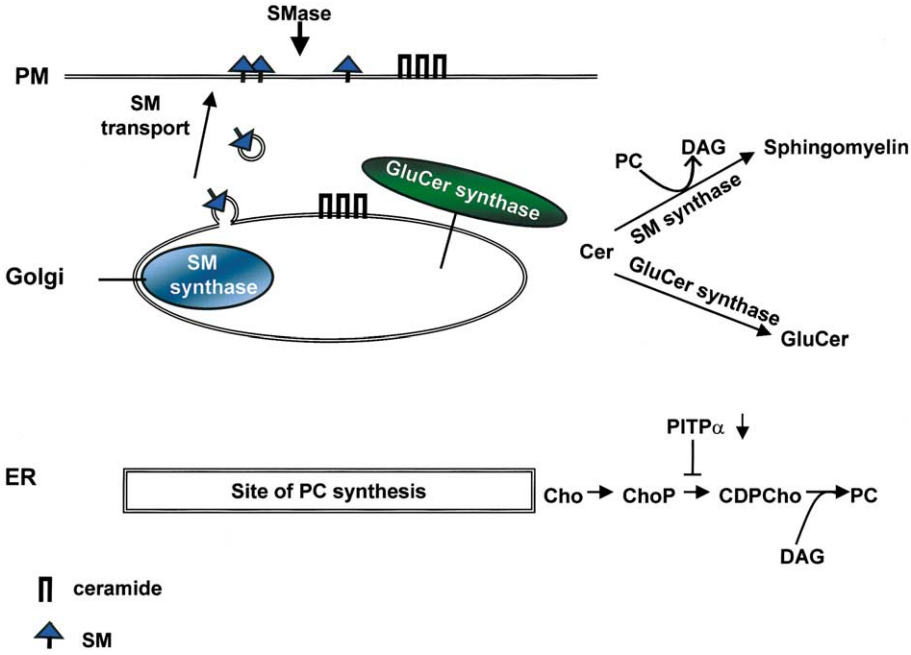

Fig. 1. An outline of the metabolic pathways to PC, SM and GluCer synthesis that have been examined following over-expression and suppression of PITPα and PITβ (see text for further details). Based on the study by Monaco et al. [46], a decrease in PITPα leads to partial inhibition of PC synthesis and the site for its action is shown. Two separate studies have examined the effects of over-expression of PITPβ (which localises at the Golgi) on sphingolipid metabolism. In a study using NIH 3T3 fibroblasts, hydrolysis of SM at the external leaflet of the plasma membrane by exogenous SMase, led to enhanced replenishment of SM at the plasma membrane [48]. In a second study using COS-7 cells, no changes in replenishment was observed [33].

of how these proteins can fulfil their functions. The structures of PITPα complexed with PC and the apo-PITPα have been solved by X-ray crystallography [13,51] and provide a mechanistic insight into this process. PITPα is characterised by an eight-stranded, concave β-sheet flanked by two long α-helices, which define an enclosed internal cavity in which a single molecule of PC is accommodated [13]. The polar headgroup lies in the centre of the protein with the fatty acyl chains projecting towards the surface (see Fig. 2A).

The structure of the apo-form, together with extensive biochemical analysis of C-terminal deletion mutants, provides the possibility of constructing events that could allow the protein to open its cavity thus allowing the lipid to depart (see Fig. 2B). At its C-terminus, PITPα has an α-helix (G-helix) followed by an extension of 11 amino acids [13]. Prior to any knowledge of the structure, limited proteolysis was used to obtain structural insights. The protease subtilisin removed 24 amino acids from the C-terminus (see Fig. 2A) and this led to the dimerisation of the truncated PITP molecule accompanied by loss of transfer function, as well as the ability to reconstitute PLC signalling [52] (PLC signalling provides a measure of both PI transfer and phosphoinositide synthesis

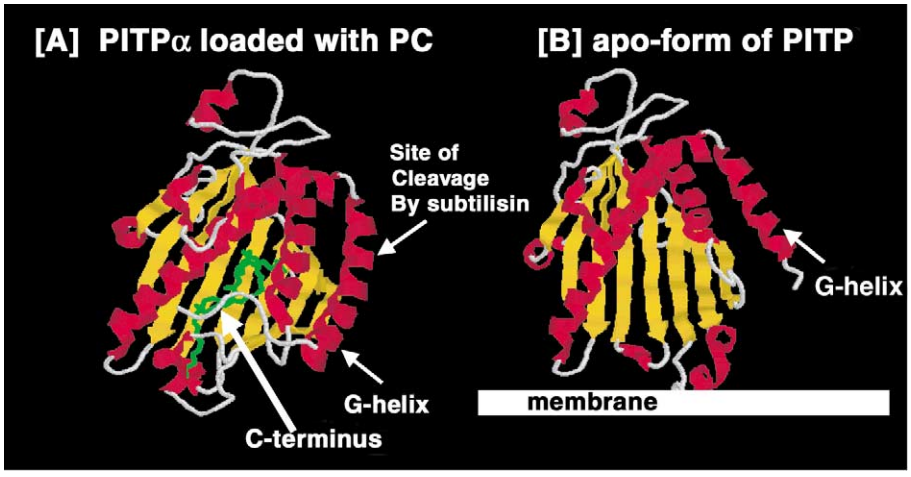

Fig. 2. Comparison of the PITP structure loaded with PC and without the lipid. The structures were made in Rasmol from the following PDB codes, (A) 1FVZ and (B) 1KCM. The β-sheets are in yellow and α-helices are in red. The PC molecule in the structure is in green. The C-terminus indicated in A wraps in the lipid whilst in the structure in B the G-helix has swung out exposing the lipid fatty acyl chains towards the membrane. The site of cleavage by subtilisin at Met^{246}, which removes 24 amino acids, is indicated. The position of the membrane in relationship to the PITP molecule is indicated for the apo-structure (B).

examined in permeabilised cells [5,39]). A systematic deletion of the C-terminus of five, 10 and 20 amino acids indicated that deletion of only five amino acids is sufficient to impair the ability of PITPα to function in the reconstitution of PLC signalling [53]. Nonetheless, the protein retained its ability to bind its lipid although its transfer activity was reduced. Limited protease digestion with trypsin only occurs in the presence of lipid vesicles and, in this case, the PITPα is cleaved to give two products, one lacking 12 amino acids and the other lacking 18 amino acids [54]. These C-terminal truncated proteins have a more relaxed conformation and show a higher affinity for membranes compared to the full length protein, which could explain the decrease in transfer activity [54–56]. Although membrane binding is a necessary condition of lipid transfer, if the association is too strong the protein will be immobilised and therefore unable to participate in multiple rounds of transfer. Thus, the C-terminus is critical in ensuring that the PITPα molecule has a compact structure and remains soluble. Movement of the C-terminus appears to be required to expose a hydrophobic surface (hence dimerisation of both the apo-structure and the deletion mutants) that would allow the protein to interact with the membrane. The apo-structure provides a snapshot of this process where the G α-helix and the C-terminal 11 amino acids have moved, exposing the lipid binding cavity [51] (see Fig. 2B). In this open state, a channel is created for the lipid to depart and key hydrophobic residues are exposed which can interact with the membrane, possibly including insertion into the membrane. Mutational analysis of the hydrophobic amino acids are now essential to confirm this model.

6. Is lipid transfer a regulated process?

Living cells maintain the identity of their organelles with respect to their lipid and protein composition in the face of ongoing traffic between these compartments. Despite the presence of uniformly high concentrations of PITPα or PITPβ (dependent on cell type) in the cytosol, the concentration of PI is not identical in the different organelles. There is a gradient of PI as one moves through the secretory pathway, the highest concentration at the ER followed by the Golgi and finally the plasma membrane.

So what can regulate the lipid transfer process? Since the lipid transport proteins have to dock to the membrane to exchange their cargo ligand, the key must lie either in the structure of the membrane or in a signal-induced change in the conformation of PITPα. A change in the membrane could occur when cells are actively consuming $PI(4,5)P_2$ by PLC hydrolysis or phosphorylation to $PI(3,4,5)P_3$, and this may cause local deformations in membrane structure. Thus, a possible scenario is that these transfer proteins constantly bump into a membrane, but do not open their hydrophobic pocket unless the membrane is perturbed in some way such that the local packing density of the lipids is disrupted. Under these conditions, the PITP molecules may accumulate at sites of perturbation, permitting the opening of the cavity and exit of the lipid. Binding of a lipid to the apo-PITP may then drive the cavity to close and the protein to move off the membrane.

The presence of a PITP domain in proteins, which are already membrane-associated, raises the question of other functions of these domains, in addition to lipid transfer. One common role for lipid binding motifs such as PH domains, FYVE domains, C1 domains, C2 domains and PX domains, which are widely distributed domains, appears to be in the targeting of proteins to specific subcellular compartments [57]. In these cases the lipid headgroups bind specifically to a pocket or groove on the protein surface, tethering the protein to the membrane. These structures are designed to recognise specific lipids embedded in lipid bilayers only. Since the PITP domain actually binds the monomeric lipid, the potential function of these proteins must be rather different. In principle, the PITP domain can switch between the PC- or PI-bound form and conceivably this may function as a molecular switch, similar to G proteins, which switch between the active GTP-bound form and the inactive GDP-bound form. This requires that the structures of the PC-bound and PI-bound forms of PITP are distinct if they are to function as molecular switches. Recent studies have shown that the PC-bound form of PITPα is a better substrate for protein kinase C-mediated phosphorylation at serine 166 compared with the PI-bound form of PITPα [58]. This suggests that the two forms of PITPα are structurally different. The availability of the structure of the PI-bound form of PITP is eagerly awaited, as this will provide substance to this idea.

In vitro, PITP proteins have constitutive transfer activity, not dissimilar to the case of PLCs when they are assayed in vitro. In intact cells, the transfer activity of PITPs has not been assessed so far. We have begun to examine whether PITP proteins can be regulated by cell surface receptors. One way to examine interactions of PITP when it goes to a membrane to pick a lipid is to use fluorescence resonance energy transfer (FRET). FRET is only observed when molecules are in close proximity (<10 nm), and therefore are able to transfer energy from the donor to the acceptor. We have monitored FRET between compatible fluorophores, green fluorescent protein (GFP) and BODIPY, attached to PITP and lipids respectively, in the presence and absence of epidermal growth factor [59]. In unstimulated cells, BODIPY PI or PC were not in close enough proximity to GFP-PITPα, and no FRET was observed. However, upon stimulation, FRET is observed at the plasma membrane indicating an accumulation of PITPα (or PITPβ) molecules bound with BODIPY-labelled PI or PC. FRET is not observed when BODIPY-PE is used indicating that this interaction is specific for PITP ligands, PI and PC, and one is not simply monitoring the association of PITP with a membrane. From these studies, it becomes apparent that the lipid transfer activity of the protein is regulated in vivo. We can now address the question of what promotes the accumulation of PITP proteins to the plasma membrane following stimulation.

7. The way forward

PITP proteins have come a long way since their discovery in the early 1970s as lipid transporters. Whilst much of the early thinking has been driven by this biochemical activity, it is now clear that these proteins play more complex roles, of which the transport function is only one. From studies where cytosolic proteins have been removed from cells by permeabilisation, PITP proteins have been found to reconstitute many aspects of signal transduction and vesicular traffic including exocytosis [1,3]. The major limitation of these studies is the insult that the cells undergo in such reconstitution studies. To

examine PITP function in intact cells, the available options have been to over-express PITP, to decrease the PITP concentrations using antisense technology, or to generate dominant negative mutants. We and others have applied these methods with only limited success [33,46,48,53]. We have attempted to generate dominant negative mutants to study PITP function in intact cells, but have not succeeded so far and this is most likely because PITP proteins may not directly associate with other proteins but with membrane lipids only. Over-expression does not result in any obvious phenotype and suppression of endogenous protein requires cell selection over a long period, which makes comparisons between two cell populations difficult.

There is light at the end of the tunnel with recent advances in RNAi technology that can be applied to mammalian cells in culture. We are currently engaged in using this approach to examine whether these proteins really play roles in membrane turnover and in signal transduction. Structural studies are in progress; we shall soon have the structure of the PI-liganded PITP and with this information, it will be possible to design PITPs that can only bind and transfer PI or PC. The use of RNAi coupled with PITP mutants selective for PI or PC transfer will provide insights into PITP function in intact living cells. Exciting times lie ahead for unravelling the biological functions of this family of enigmatic proteins and this will require the further development of new technologies to study membrane lipid/protein dynamics in living cells.

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