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INOSITOL LIPID REGULATION OF LIPID TRANSFER IN SPECIALIZED MEMBRANE DOMAINS

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SUMMARY

The highly dynamic membranous network of eukaryotic cells allows spatial organization of biochemical reactions to suit the complex metabolic needs of the cell. The unique lipid composition of organelle membranes in the face of dynamic membrane activities assumes that lipid gradients are constantly generated and maintained. Important advances have been made in identifying specialized membrane compartments and lipid transfer mechanisms that are critical for generating and maintaining lipid gradients. Remarkably, one class of minor phospholipids -- the phosphoinositides -- is emerging as important regulators of these processes. Here, we summarize several lines of research that led to our current understanding of the connection between phosphoinositides and the transport of structural lipids and offer some thoughts on general principles possibly governing these processes.

Inositol lipids are important for lipid composition and homeostasis

The central role of inositol lipids in transmembrane signaling, ion channel regulation and cellular trafficking has been well established ¹. However, in addition to these well-recognized roles, inositol lipids are repeatedly linked to processes affecting general lipid composition and homeostasis. In early studies identifying *myo*-inositol as a “vitamin”, it was observed that some rodents kept on inositol-free diet developed fatty liver ². A long time has passed since these early observations for which we still do not have an explanation, but inositol lipids have since been often recognized as regulators of composition of structural lipids. The Stt4 phosphatidylinositol 4-kinase (PI4K) ^{3, 4}, the PtdIns4P phosphatase, Sac1 ^{5, 6} and phosphatidylinositol (PtdIns) itself ⁶, have all been tightly connected to sphingolipid synthesis and the ergosterol cell wall integrity pathway ^{7, 8} in yeast. The Stt4 PI4K was also found in a screen seeking genes important for phosphatidylserine (PS) decarboxylation at the ER-Golgi interface ⁹. The yeast Sec14 protein, a phosphatidylinositol (PtdIns or PI) and phosphatidylcholine (PC) transfer protein has a major role in protein secretion out of the Golgi but how this happens is still being worked out ^{10, 11}. As seen from this list, yeast studies have been instrumental in furthering our understanding of these processes. Much less is known about the same topics in mammalian cells. Regardless of the species, two main lines of research have shaped this field, and their merger happening in recent years forced us to rethink old paradigms and

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entertain new ideas concerning the connection between inositol lipids and overall lipid homeostasis. The first topic is concerned with PtdIns transfer proteins (PITPs) and the notion that despite their name, their PI transfer function has been really hard to prove and their real functions remain obscure. The other line was born out of findings that PtdIns4P binding protein modules are frequently found in proteins that transfer various forms of lipids between membranes. We will review these developments in more detail and in the end will try to draw some conclusions that transpire from this still evolving and often controversial research field.

Inositol lipid transfer proteins support phosphoinositide function

Proteins capable of PtdIns transfer between membranes have been identified in brain extracts in the early 70s¹². However, questions on their possible functions and significance were only resurfaced when it was discovered that PITPs are required for PLC-mediated hydrolysis of PtdIns(4,5) P_2 in the plasma membrane (PM)¹³. Curiously, the same proteins were also found as important factors required for ATP-dependent priming of exocytic vesicles¹⁴ presumably also supporting PtdIns(4,5) P_2 synthesis. These findings together with the fact that PtdIns synthesis occurs in the ER, and PtdIns(4,5) P_2 is formed in the PM, logically led to the assumption that PITPs transfer PtdIns from the ER to the PM for further phosphorylation by PI4K and PIP 5-kinase enzymes. In parallel studies it was discovered that the RdgB mutation, causing retinal degeneration in *Drosophila*, affects a protein that contains a PITP domain. This, together with the localization of the RdgB protein to the subplasmalemmal ER of photoreceptor cells led to the suggestion that the function of RdgB is to transfer PtdIns from the ER to the PM for PtdIns(4,5) P_2 synthesis in photoreceptor cells where PLC-mediated PtdIns(4,5) P_2 hydrolysis is the major signaling pathway in photo-signal transduction. It is noteworthy though that the retinal degeneration of RdgB flies is more indicative of a signal termination defect than a signal exhaustion one, which is hard to explain if the role of the protein is to maintain PtdIns(4,5) P_2 (reviewed in¹⁵). In fact, the idea that PITPs transfer PtdIns from the ER to the PM is still awaiting formal experimental confirmation.

At the same time, several observations suggested that the most important function of PITPs could be different from simple PI transfer. The essential yeast PITP protein, Sec14, was found to be required for protein secretion out of the Golgi¹⁶. However, *Sec14* mutants can be rescued when PC synthesis is compromised, or when the Sac1 PtdIns4P phosphatase is impaired¹⁷. These findings together with many others that followed led to the idea that Sec14 is important for PtdIns4P generation and at the same time keeps PC synthesis (a major DG consuming pathway) under control thereby helping maintain the DG content of Golgi membranes¹¹. A similar role for the mammalian RdgB homologue, Nir2 (also called PITPnm1) in controlling Golgi DG content has been proposed¹⁸. How Sec14 or Nir2 achieves such a control is still not clear but in the case of Sec14, structural and functional evidence suggest that the protein acts as a lipid chaperone presenting PtdIns to PI 4-kinase enzymes and the heterotypic PC-PI exchange is integral to this function¹⁹. Similar roles for mammalian PITPs have been shown for PI3Ks^{20, 21} or the Golgi-localized PI4Ks^{22, 23}. These findings led to the concept that Sec14 and PITPs are critical components of signaling platforms that control the lipid composition of membranes, hence interjecting a role of PtdIns (or a derivative thereof) in all of these processes¹⁰. It should be noted that Sec14 proteins are not the yeast homologues of mammalian PITPs, even though the mammalian PITPs can compensate to some extent for the yeast *Sec14* defect²⁴ (Fig. 1). However, there are five Sec14 homologues in yeast (called Sfh1-5), all of which are functionally related to lipid metabolism. All Sfh proteins (with the possible exception of Sfh3) are also linked to Stt4-mediated PtdIns4P and PtdIns(4,5) P_2 synthesis^{25, 26}. Many proteins containing Sec14 domains are found in higher eukaryotes and a number of stand-alone Sec14 domain-like

proteins, such as the retinaldehyde binding protein (CRALBP), cactaxin, α -tocopherol and TAP/SPF, all function as lipid transfer proteins (see ¹¹ for original references). It will be important to see if the functions of any of these proteins are under phosphoinositide control.

As we see from this short summary, it is certain that PITPs do have a role in phosphoinositide signaling even if the way they do it is not all that clear. The flip side of the coin, however, is that phosphoinositides also have a role in lipid transfer and this will be the topic of the next section.

Inositol lipids regulate lipid transfer proteins

Mouse studies on mammalian PITPs have also pointed to their link to lipid metabolism. While PITP β knockout mice are early embryonic lethal at the implantation stage, PITP α $-/-$ mice develop normally but die quickly after they start suckling. Their pathology shows intestinal and liver steatosis indicating a severe defect in lipid handling by the gut and the liver and spinocerebellar degeneration apparently unrelated to the gut and liver problems ^{27, 28}. Progressive spinocerebellar degeneration is also characteristic of the *vibrator* mouse that expresses a very low level of PITP α ²⁹. The mechanism of how PITP α deficiency affects neurons is not yet understood, but one study identified PITP α as an interacting partner of netrin-1 receptors and being important for neurite growth and guidance ³⁰. It is yet to be determined whether the neuronal degeneration is also related to alterations of lipid metabolism in neurons.

In separate studies looking for protein domains that interact with phosphoinositides, namely with PtdIns4P, it was found that a selected group of pleckstrin homology (PH) domains that showed preferential binding to PtdIns4P, and to a lesser degree, PtdIns(4,5)P₂, are found in various lipid transfer proteins. These included the oxysterol binding protein, OSBP ³¹, some of its yeast homologues (OSH₁₋₄) ³² and several mammalian OSBP related proteins, the ORPs ³³. It also included the ceramide transfer protein, CERT ³⁴ and the FAPP2 protein that transports glucosyl-ceramide ³⁵. Many of these proteins contain a PH domain at their N-termini, a lipid binding domain at their C-termini and a FFAT domain (a double phenylalanine in an acidic track ^{36, 37}), which provides anchoring via the ER-localized VAP proteins (Fig. 2). That PtdIns4P is critical for the transport function of these proteins was indicated by the finding that a mutation within the PH domain of CERT that eliminates its PtdIns4P binding renders the protein functionally incompetent ³⁴. Later studies showed that PtdIns4P and PI 4-kinases were required for the functions of both CERT ³⁸, OSBP ³⁹ and the FAPP2 proteins ³⁵. It is now generally believed that the PH domain serves as a localization motif that allows the lipid transfer protein to find its intended target membrane, mainly in the PtdIns4P-rich Golgi compartment, and PtdIns4P binding presumably facilitates the release of the lipid cargo from the lipid transfer protein to the target membrane ⁴⁰ (Figure 2).

Lipid transfer proteins meet PITPs

The PITPs and the lipid transfer proteins crossed paths when the curious connection between the yeast Sec14 protein and the oxysterol binding protein, Kes1 (also called Osh4) was discovered ⁴¹. The ensuing research on this topic has been extremely instructive in moving the field forward, although the picture emerging is still fuzzy and not without controversies. The key observation was that Sec14 mutants are rescued by deletion of the Kes1 protein ⁴¹. Why this happens is still debated but a number of observations suggested that Kes1 function was related to antagonizing Pik1-generated PtdIns4P in the Golgi (e.g. ⁴²). In this context, it was an important discovery that Kes1 is able to bind PtdIns(4,5)P₂ in addition to sterol, and inositol binding as well as PtdIns4P production by Pik1 were both important for Kes1 Golgi localization and functions ⁴³. The inositol recognizing residues of Kes1 reported

in ⁴³ were later mapped to the outer surface of the sterol-binding domain ⁴³, once the Kes1 structure became available ⁴⁴. Subsequent structural studies identified another PtdIns4P binding site in Kes1, which partially overlapped with the sterol binding pocket thereby the two ligands were found to compete for the same site ⁴⁵.

Different models have been introduced to explain Kes1 function and its relationship to Sec14 and PtdIns4P. One study showed that anionic phospholipids, such as PtdIns(4,5)P₂ facilitate sterol exchange by Kes1p between membranes *in vitro* and between the ER and the PM in yeast cells ⁴⁶. The authors suggested that inositol lipid binding (probably at the acceptor membrane) facilitates the release of the sterol from Kes1 to the membrane (Fig. 3C). Other reports, however, did not substantiate the role of Kes1 (or other OSH proteins) in non-vesicular transport of sterols between the PM and other membranes ⁴⁷. In fact, sterol binding defects were shown to enhance rather than impair Kes1 activity, including its PtdIns4P “antagonism” ^{48, 49}. Kes1 activities included transcriptional activation of general amino acid control, modulation of amino acid activation of mTOR and the control of sphingolipid signaling, all of which required Golgi recruitment by PtdIns4P. PtdIns4P binding by Kes1 in the Golgi also competes with other PtdIns4P effectors that regulate the secretion process in the late Golgi ⁴⁸ explaining why Kes1 antagonizes Sec14 (Fig. 3D). A third model was proposed by another group that found that Osh4/Kes1 not only bound sterols but also bound PtdIns4P and the latter binding competes with the ability of the protein to bind the sterol. The solved structure of PtdIns4P-bound Kes1 also provided structural insights for this behavior of the protein ⁴⁵. These authors proposed that the ER to Golgi transport of sterol is linked to a Golgi to ER transport of PtdIns4P. Here, the PtdIns4P gradient is maintained by the action of the Golgilocalized PI 4-kinase, Pik1 and the ER-localized PtdIns4P phosphatase, Sac1. This model also invokes Sec14 as a protein that delivers and presents PtdIns to the Pik1 enzyme as it exchanges PtdIns for PtdCho in the Golgi membrane (Fig. 3E). It has yet to be seen whether this latter model is compatible with all of the findings reported in ^{48, 49} and whether there are any parallels to it in mammalian cells.

In fact, some evidence also suggests a connection between sterols and PtdIns4P in mammalian cells. Here the lipid composition of the membranes was shown to have an impact on the activity of type II PI4Ks. These enzymes were originally found enriched in sphingolipid- and cholesterol-rich membrane domains ⁵⁰ and later shown to be present in the TGN and various endosomal compartments, where they are kept in the membrane by palmitoylation ⁵¹. Cell fractionation studies revealed that these enzymes displayed their highest activity in membranes that contained high level of cholesterol ⁵². Moreover, changing cholesterol content of the membrane affected both their activity ⁵³ and the extent of their palmitoylation ⁵⁴. These data also suggest a strong connection between the sterol status of the cell and PtdIns4P generation.

Special compartments formed by adjacent organelles are sites of lipid transfer

Theoretically, non-vesicular lipid transfer could take place between distant membranes given the diffusion speed of the lipid transfer proteins in the cytosol. However, it has become clear by now that most non-vesicular lipid transfer processes take place between membranes of different organelles at contact sites that can be functionally and anatomically defined ⁵⁵. Such interorganelle contact zones have long been known to exist between the ER and the mitochondria (called MEMs for mito-ER-membranes), and these sites are critical for mitochondrial lipid synthesis ^{56, 57} as well as for efficient transfer of Ca²⁺ from the ER to the mitochondria ^{58, 59}. More recently, similar contacts are recognized between the Golgi and the ER where the exchange of lipids by the aforementioned mechanisms takes place ⁶⁰.

The functional importance of the contact sites between the ER and the PM have been highlighted by recent studies in yeast ^{61, 62} as well as in mammalian cells ⁶³.

The yeast Stt4 PI 4-kinase controls the production of PtdIns4P in the PM and as such, it is essential for the maintenance of PM PtdIns(4,5)P₂ pools ³. Curiously, early studies showed that Stt4 controls a PtdIns4P pool that is degraded by the Sac1 phosphatase enzyme ⁶⁴. These two sets of results, however, were difficult to reconcile since Stt4p is located in the PM while Sac1 is mainly an ER-localized enzyme ⁶⁵. As it turns out, Stt4 is not evenly distributed in the PM but is organized into discrete signaling domains with the help of the Ypp1 and Efr3 proteins ^{66, 67}. It has also been shown that the ER forms close appositions to the PM in yeast and Sac1 can be found in these locations. Recent studies suggested that ER-anchored Sac1 is capable acting in *trans* on the PtdIns4P pool of the PM but Sac1 requires the Osh3 protein for activation in these contact sites. The PH-domain containing Osh3 protein binds PtdIns4P in the PM and the VAP homologue Scs2/Scs22 proteins in the ER, and hence, it acts as a dynamic bridge between PtdIns4P produced by the Stt4 kinase and the ER-bound Sac1 phosphatase enzyme ⁶¹ (Fig. 4A). Disruption of PM-ER contact sites by deletion of several proteins that contribute to this anatomical arrangement ⁶² significantly increased PtdIns4P levels confirming that this junctional compartment is essential for the ability of the Sac1 phosphatase to access PM-bound PtdIns4P. It should be noted, though, that Sac1 is not confined to the ER and shuttles between the ER ⁶⁸ and the Golgi and can also be found in endosomes ⁶⁹.

In mammalian cells the importance of the ER-PM junctional compartment has been highlighted by studies on the Ca²⁺ entry pathways activated by the depletion of ER luminal Ca²⁺ pools. It has long been known that depletion of ER Ca²⁺ stores activates a Ca²⁺ entry pathway named SOCE (store-operated Ca²⁺ entry) ⁷⁰. The molecular entities underlying this phenomenon have been identified in the last 6 years or so. These include the ER-localized STIM1/2 proteins and the PM-localized Orai1 channels that form physical contacts upon ER luminal Ca²⁺-depletion ^{71, 72}. It has been widely speculated that the C-terminal polybasic domain of STIM1 interacts with PM phosphoinositides to aid contacts between the ER and the PM ⁷³, although our studies did not substantiate such a role of these lipids in this process ⁷⁴. This compartment, however, also has potential importance in the synthesis or delivery of PtdIns4P to the PM. The enzyme that generates the PM pool of PtdIns4P is PI4KA ⁷⁵, a homologue of the yeast Stt4 protein ⁷⁶. For a long time PI4KA was believed to be exclusively ER localized ^{76–78}, with no traces detectable in the PM or in PM-ER junctions. This apparent discrepancy has been solved by a recent study from the De Camilli group showing that the mammalian EFR3 and TTC7 proteins can bring PI4KA to the PM ⁷⁹ analogously to the Ypp1/EFR3/Stt4 complex described earlier in yeast ⁶⁶.

PtdIns-loaded membranes on the move

While research in the last 30 years have firmly established the pivotal role of phosphoinositides in signaling and vesicular trafficking, we still have a very poor understanding of why these lipids have special importance in controlling the movements of other lipids in the cell. This problem is especially hard to untangle as phosphoinositides are lipids themselves and their synthesis and distribution also needs better understanding. In light of the importance of PIP proteins in lipid homeostasis and phosphoinositide formation, it is surprising how little we know about the localization of PtdIns in the cell and the processes by which this lipid reaches other membranes. Our recent studies trying to address this question have yielded some unexpected findings ⁸⁰. The synthesis of PtdIns requires two enzymes, the CDP-DG synthase (CDS) that converts PtdOH to CDP-DG and the PI synthase (PIS) that combines CDP-DG with inositol to make PtdIns. Both of these enzyme activities were detected in microsomal fractions in early studies, and presumed to be

located in the ER (see ⁸⁰ for original citations). However, while the CDS enzymes (there are two isoforms, CDS1 and CDS2) are, indeed, located in the ER, the PIS enzyme is not only found in the tubular ER but is also associated with highly mobile structures. (Both of these proteins have several membrane-spanning domains so they must be part of membranes). These moving objects originate from the ER and seem to cycle back to the ER making ample contacts with several other organelles such as the Golgi, endosomes and the PM during their lifetimes. However, importantly, they do not appear to fuse with those other membranes. Recent studies suggested that the Rab10 protein has an important role in forming the ER tubules and that the PIS enzyme and the PC-synthesizing CEPT1 enzyme were both found on the tip of these ER structures perhaps synthesizing the lipid for the growing ER tubules ⁸¹. The stochastic movements and lack of full membrane fusion of the PIS organelle with the PM raises many questions and opens new possibilities concerning how these mobile platforms can facilitate PtdIns distribution within the cell. Is there lipid exchange during the contacts and if so is it due to hemifusion or is it mediated by lipid transfer proteins? Since these new “organelles” do not co-localize with any organelle markers and do not contain PI kinases or phosphorylated PIs in detectable amounts, we assume that they serve as PtdIns distributors to other membranes where PtdIns phosphorylations take place. Essentially, these moving structures can be considered as an extended ER compartment that forms dynamic contacts and multiplies the probability of lipid exchange that has previously been assigned to the less dynamic contact zones between the ER and other membranes. Clearly, more studies are needed to understand the genesis, lipid- and protein composition, regulation and functional relevance of these PtdIns synthesizing platforms.

Concluding remarks

Our perception of phosphoinositides has gone through several iterations. These lipids were first recognized as precursors for messenger molecules when acted upon by PLC enzymes ⁸² and as later discovered, by PI3K enzymes ⁸³. However, their roles as membrane-bound docking sites during the recruitment and regulation of soluble signaling proteins has also gained equal importance, as did their roles in the control of ion channel and transporter activities. PI4Ks and PtdIns4P have proven to have roles distinct from just supplying precursors for PtdIns(4,5) P_2 ⁸⁴. Their importance in controlling vesicular trafficking via recruitment of clathrin adaptors at the Golgi and TGN were recognized in the last decade ^{85, 86}. This was followed by the realization that PI4Ks have strong connections with sterol and sphingolipid synthesis and distribution related to non-vesicular lipid transfer. With all of these roles, it is fitting that the most ancient PI kinases are the PI 4-kinases with three distinct isoforms already appearing in yeast. It is notable that both type III PI4Ks in yeast work at membrane contact areas: Pik1 at the ERGolgi interface, whereas Stt4 at the ER-PM contact zones ⁸⁷ (Fig. 4A).

It is hard not to see parallels between the already detailed mechanism for PtdIns4P control and sterol binding and/or transport between the ER and the Golgi with the participation of Pik1, Kes1 and Sac1 ⁴⁵ (see Fig. 3E) and the process that takes place between the ER and the PM, where the Stt4 kinase generates PtdIns4P in the PM and the ER-bound Sac1 enzyme using the help of Osh3 degrades PtdIns4P. Although the model proposed in ⁶¹ posits that the ER-bound Sac1 acts in trans at the PM with the help of the Osh3 protein, the data may also be compatible with a model in which the PtdIns4P made by Stt4 in the PM is presented by the Osh3 protein to the Sac1 phosphatase in the ER. The only difference is where the PtdIns is liberated: in the PM or in the ER membrane (Fig. 4A). One can further speculate whether the PH domain of the Osh3 and other lipid-transfer proteins function as PtdIns4P binding sites holding the head-group while the acyl chains are buried in the lipid transfer domain of the molecule (Fig. 4B). Could this be a general feature of lipid transfer proteins that have

PtdIns4*P*-recognizing PH domains? The larger question is whether PtdIns 4-phosphorylation is part of the energy conversion process that drives the non-vesicular transfer of specific lipids between the ER and other membranes (Fig. 4C), or is it the opposite, whereby sterols and other lipids control the levels of PtdIns4*P*? There are many examples where the driving force for the conformational change of a transporter is provided by the buildup of chemical (mostly ionic) gradients. It may be possible that PtdIns4*P* gradients formed between adjacent membranes drive the non-vesicular counter-transport of a variety of lipids mediated by a variety of lipid transfer proteins. These questions are born out of pure speculation at the moment, and are not necessarily compatible with all experimental findings. They are presented only to facilitate further thinking and discussions and to raise the possibility that PtdIns 4-phosphorylation serves as a general means of driving lipid transport between membranes.

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Fig. 1. PITPs and Sec14 proteins

(A) The prototypical and first-described mammalian PITPs are the Class I PITP α and PITP β , the latter having two splice forms (not shown). Another family of PITPs includes the *Drosophila* RdgB and its mammalian homologues, Nir2 and Nir3 forming the Class IIA group (Nir1 is a homologue lacking the PITP domain). RdgB/Nir2 are multi-domain proteins associated with the ER membrane via an FFAT domain⁶⁰. A stand-alone PITP most homologous to the PITP domain of the Class IIA group is named RdgB β and it constitutes to Class IIB. It also has two splice forms (not distinguished here). RdgB β was recently found to be a PtdOH transfer protein⁸⁸. (B) The yeast Sec14 protein has a stand-

alone PI/PC binding domain. Many mammalian proteins contain Sec14 homology domains (not shown here) but there are also stand alone mammalian Sec14 homologues. The Spf protein also contains a GOLD (Golgi localization) domain. (C) The structure of PITP α with or without the ligand PtdIns in the binding pocket (pdb: 1KCM and 1UW5, respectively). Beta-strands are colored brown. Notable differences between the two structures are the position of the C-terminal helix (without the C-terminal tail) (colored cyan) leaving the lipid binding tunnel open and a small helical domain (colored purple) that backs the C-terminal 6 amino acid tail as it closes the binding cavity. Note that the PtdIns molecule is positioned its head deep inside the pocket. (C) The structure of the yeast Sec14 protein void of lipid and the PtdIns-loaded form of one its homologues, Sfh1 (pdb: 1AUA and 3B7N). The structural difference is clear compared to the mammalian PITPs. Notable difference between the two structures is the position of the helices serving as “lids” (colored cyan) covering the exit from the binding pocket. Also note that the position of the head of the PtdIns molecule is the opposite compared to that in mammalian PITPs: here the fatty acid chains are positioned inside the cavity and the head is facing outward. The structural similarity between yeast Sec14 and the mammalian Sec14 proteins is remarkable (here shown is the structure of Spf bound to palmitic acid, pdb: 1O6U). The additional GOLD-domain⁸⁹ is an extra feature not found in the yeast Sec14 proteins. The structure pictures were generated with PyMOL.

Fig. 2. Lipid transfer proteins containing PtdIns4P recognizing PH domains

(A) A unique group of pleckstrin homology (PH) domains is found in selected lipid transfer proteins. To this group belong the mammalian oxysterol binding protein (OSBP) and several of its yeast OSH and mammalian ORP homologues. These proteins all contain an N-terminal PH domain, a FFAT domain, for interaction with ER-localized VAP proteins, and a lipid transfer domain that belongs to the StAR protein family (START). The mammalian ceramide-transfer (CERT) protein also shares this domain structure. The mammalian FAPP2 protein also has a PH domain and a glycolipid transfer domain, while the FAPP1 protein lacks a lipid transfer domain and only possesses a highly homologous PH domain. (B) The

START domain of CERT with or without its ceramide ligand (pdb: 2E3M and 2E3Q, respectively) shows very minor differences and is more similar to the PITPs than to the Sec14 domains. The structures of the CERT and FAPP1 PH domains share the classical PH domain fold with the C-terminal helix (red) and the 7 betastrands arranged in two beta-sheets (brown). Unique is the loop (colored yellow) that penetrates into the membrane explaining the curvature and tubulating activity of the domain. Some of the basic residues that contribute to PtdIns4P binding are labeled blue. (C) Current model of how these lipid transfer proteins work between juxtaposed ER and the Golgi membranes. The PH domain binds PtdIns4P made by PI4KB in the Golgi membrane, while the FFAT domain binds the VAP proteins in the ER. The START domain is then capable of transferring ceramide from the ER to the Golgi, where ceramide flips to the luminal side and is converted to sphingomyelin⁹⁰. The structure pictures were generated with PyMOL.

Fig. 3. Sterol transport and PtdIns4P levels in the yeast ER and Golgi membranes

(A) The structure of Osh4/Kes1 liganded with cholesterol (pdb: 1ZHY). In addition to the sterol ligand found in the lipid binding pocket, also shown are in blue the basic residues that were found to be important for PtdIns(4,5)P₂ binding in ⁴³. (B) The structure of Osh4/Kes1 liganded with PtdIns4P (pdb: 3SPW). Note that the PtdIns4P binding site is different from the PtdIns(4,5)P₂ site described earlier. Here the fatty acid chains of PtdIns4P partially occupy the lipid binding space that houses cholesterol and the Ins4P headgroup rearranges the helices that form the lid over the lipid binding pocket (colored in cyan). Binding of the two ligands is mutually exclusive. (C-E) Suggested models for the interplay between the

Kes1 protein and Sec14/PtdIns4P signaling. (C) The sterol transport between the ER and other membranes mediated by Kes1 is facilitated by phosphoinositide binding, presumably by promoting the release of the sterol cargo in the acceptor membranes⁴⁶. This model does not deal with the Kes1-Sec14 antagonism. (D) The Sec14 protein delivers PtdIns to the Pik1 enzyme by a mechanism that involves heterotypic exchange of PtdCho and PtdIns. Kes1 is not a sterol transfer protein but its sterol binding antagonizes the interaction of Kes1 with PtdIns4P in the Golgi. Only Golgi-bound Kes1 is competent to control several processes (see text for details) but its PtdIns4P binding competes with other PtdIns4P binding effectors that are important for vesicular budding⁴⁸. (E) Transport of sterol from the ER to the Golgi is coupled with the PtdIns4P gradient maintained by Pik1 and Sac1 actions in the respective compartments⁴⁵. The complete cycle also involves the Sec14 protein that transfers PtdIns to the Golgi and trades it for PtdCho. This arrangement essentially links sterol and PtdCho transports in opposite directions using PtdIns phosphorylation to drive the process. The structure pictures were generated with PyMOL.

Fig. 4. Possible connections between PtdIns-PtdIns4P cycles and non-vesicular lipid transfer between adjacent membranes of distinct organelles

(A) Model describing how the ER-bound Sac1 can access the Stt4-made PtdIns4P when Osh3 is present ⁶¹. (B) Speculative alternative model for the same process drawing parallels between the PM-ER PtdIns4P cycle and the cycle described in the Golgi ⁴⁵ as shown in Fig. 3, panel E. (C) Hypothetical model raising the question whether similar principles apply to lipid transfer by CERT or OSBP. Could the PH domains help scoop up PtdIns4P to be transferred in the retrograde direction with the help of the START domains? (D) Highly hypothetical model where vesicular lipid transfer is energized by PtdIns phosphorylation. LPT1 is the PtdIns transfer protein and LPT2 is the transport step taking lipids from

membrane B to membrane A while transferring PtdIns4P in the reverse direction. It is important to note that these models are highly speculative and not proven yet by experimental data.