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Topical Review

Inositol Lipids as Spatial Regulators of Membrane Traffic

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Introduction

Phosphoinositides have been in the limelight for over two decades following their identification as sources of the second messengers, diacylglycerol, inositol(1,4,5) trisphosphate (IP_3) and phosphatidylinositol(3,4,5) trisphosphate (PtdIns(3,4,5)P_3). The last 10 years have witnessed an explosion of experimental results that demonstrate that inositol lipids themselves should be considered as second messengers in their own right. The intact lipid molecule functions as a reversible recruiting device for proteins to transiently bind to membranes and as an allosteric regulator of many proteins. The inositol headgroup of phosphatidylinositol (PtdIns) can be phosphorylated at a single or a combination of positions (3′, 4′ or 5′) to give rise to seven different phosphoinositides (Fig. 1). Phosphoinositides can control membrane trafficking via their structural role as membrane components and via their ability to engage specific protein domains (e.g., pleckstrin homology (PH) or FVYE) which bind with high affinity and specificity to the different phosphorylated species of phosphoinositides, and thus recruit molecular machinery driving specific membrane trafficking events and regulating actin assembly.

The first hint that phosphoinositides were important in membrane traffic came from studies showing that a bacterial phospholipase C inhibited regulated exocytosis in PC12 cells [20] and from genetic studies in *Saccharomyces cerevisiae* where the secretory mutant, *Sec14* showed defects in exit of secretory vesicles from the Golgi and where the gene product responsible for these defects was identified as the yeast phosphatidylinositol transfer protein (PITP) [5]. Subsequent studies led to the identification of PITP, the small GTPase ARF, phosphatidylinositol 4-kinases (PI4K), phosphatidylinositol 4-phosphate 5-kinase (PIP5K) and PIP phosphatases as regulators of membrane trafficking along the exocytic and endocytic pathways by virtue of their ability to control PtdIns(4,5)P_2 levels [21, 30, 31, 34, 37, 44, 79].

As for 3-phosphorylated phosphoinositides, their role in membrane trafficking emerged with the identification of the product of the *Vps34* gene (whose mutation was responsible for a defect in vacuolar protein sorting and membrane trafficking) as phosphoinositide 3-kinase (PI3K) which phosphorylates PtdIns to PtdIns(3)P [65, 66]. Since then many studies have provided evidence for a role of PI3Ks and their regulators, such as the small GTPase Rab5, in Golgi to endosomal/vacuole transport and along the endocytic pathway both in yeast and mammals and for the existence of a selective PI(3)P binding domain in some proteins, the FVYE domain [9, 25, 58].

Although the role of inositol lipids in membrane traffic appears to be central in yeast and in mammals, it is also clear that mammalian systems, due to their inherent complexity, have a richer and wider variety of functions where phosphoinositides are important. This is also apparent when one compares the inositol lipid-metabolizing enzymes and the inositol lipids identified in mammalian organisms and in *S. cerevisiae* (*compare* Fig. 1 and Fig. 2 and *see also* Table). PtdIns is the parent lipid from which all the phosphorylated species are derived (Fig. 1). In mammalian cells, PtdIns constitutes between 5–8% of total cellular lipids and the enzymatic

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Key words: Lipid kinases — Phosphoinositides — Phospholipase D — ARF GTPases — Phosphatidylinositol transfer proteins — Membrane traffic

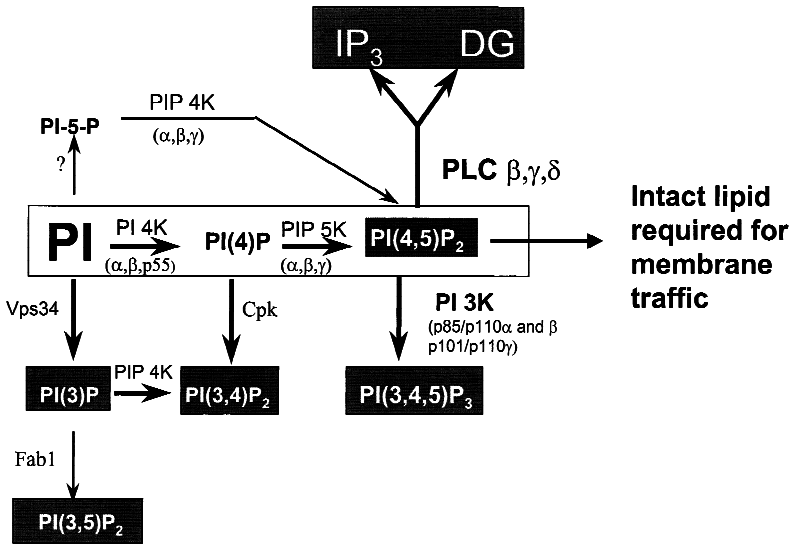

Fig. 1. Phosphoinositides and the enzymes responsible for phosphorylating the inositol ring found in mammalian cells. PI, phosphatidylinositol.

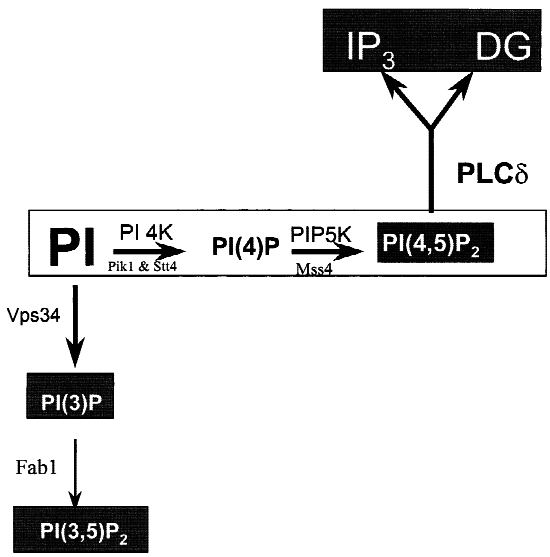

Fig. 2. Phosphoinositides and the enzymes responsible for phosphorylating the inositol ring found in the yeast, *S. cerevisiae.*

machinery for its synthesis is localized at the endoplasmic reticulum (ER) [50, 80]. PtdIns is found on the cytosolic face of most membranes and can be further phosphorylated in the different membrane compartments. The specific distribution of the phosphorylated species of the inositol lipids in the different membrane compartments is governed by the spatial distribution of the specific lipid kinases and phosphatases. Here we review the experimental evidence describing the subcellular distribution and regulation of the lipid kinases and transfer proteins, and the importance of phosphoinositide-dependent mechanisms in several aspects of membrane traffic both in the exocytic and endocytic pathway (Fig. 3).

Phosphatidylinositol Transfer Proteins

In mammalian cells PITPs play complex roles and this includes signal transduction and membrane transport [13]. In signal transduction, PITP is an integral part of the machinery that is required for the receptor-regulated production of the two second messengers, IP_3 and diacylglycerol, by phospholipase Cs (PLCs) [17, 38, 74]. The PLC signalling pathway is a major consumer of PtdIns(4,5)P_2 and the plasma membrane pool of inositol lipids is replenished with PtdIns from intracellular compartments by PITP by virtue of its ability to bind and deliver PtdIns. PITP also participates in the subsequent phosphorylation of PtdIns. Thus, PITP couples PtdIns delivery and PtdIns(4,5)P_2 synthesis in cell signalling.

Two soluble forms of PITP (α and β) and an integral membrane protein (PITPnm) containing the PITP homology domain have been described in mammalian cells [2, 3, 10, 13, 45, 84]. Although both PITPα and PITPβ can support PLC signaling and therefore PtdIns(4,5)P_2 synthesis in studies in permeabilized cells, both proteins are differentially localized, suggesting site-specific functions in vivo. PITPα is cytosolic and is very mobile as judged by its ability to exit out of permeabilized cells very rapidly [17]. GFP-tagged PITPα distributes homogeneously in the cytosol and the nucleus [72]. In contrast, PITPβ, which is 94% homologous to PITPα, localizes to the Golgi, the cellular periphery and the cytosol [19, 72]. On permeabilization, PITPβ also elutes from the cells but incompletely. From its behavior, PITPβ appears to be a functional homologue of the yeast Sec14p. Sec14p (yeast PITP) is localized to the Golgi and is required for the production of secretory vesicles, which carry invertase as cargo. An analogous role for PITP at the Golgi has also been identified in mammalian cells. PITP is

Table. Lipid kinases and PITPs involved in membrane traffic

Mammalian	*S. cerevisiae*	Comments
A. PI 4-kinase		
α form (230 kDa)	STT4	Localized to the Golgi
β-form (92 kDa)	PIK1	Regulated by ARF; Localized to the Golgi
p55 (55 kDa)	??	Not cloned; contributes to the majority of PI4K activity in cells
B. PI(4)P 5-kinases		
α (68 kDa)	Mss4 (90 kDa)	Regulated by PA, Rac and ARF *in vitro*; regulates actin assembly
β (68 kDa)	Mss4	Regulated by PA *in vitro*
γ (87 and 90 kDa)	Mss4	Regulated by PA *in vitro*; participates with PITPα to restore exocytosis in PC12 cells
PITPs		
α (35 kDa)	No homologue present	Localized in the cytosol and nucleus
β (36 kDa)	No homologue present	Localized to Golgi, cell periphery and cytosol
No homologue	Sec14p (35 kDa)	Localized to the Golgi
PITPnm 170 kDa (also known as rdgB or Nir proteins)	No homologue present	Localized to the Golgi; Contains a PITP domain at the N-terminus
PI 3kinases		
HVps34	Vps34	Phosphorylates PtdIns to PtdIns(3)P

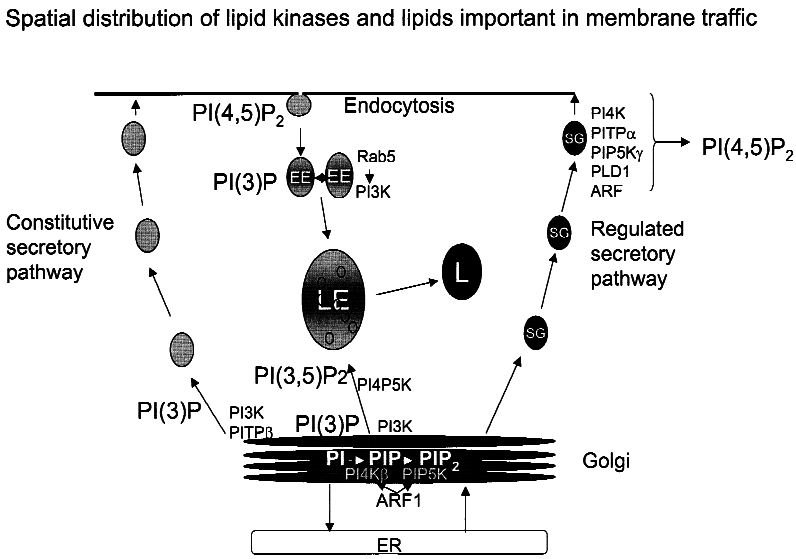

Fig. 3. Membrane trafficking pathways that are dependent on phosphoinositides and discussed in this review. PIP_2, PtdIns(4,5)P_2; PIP, PtdIns(4)P; SG, secretory granule; EE, Early endosome; LE, late endosome; L, lysosome; ER, endoplasmic reticulum.

required for both intra-Golgi traffic and for production of secretory vesicles from the Golgi [56, 59].

Mammalian and yeast PITP share common biochemical properties despite the lack of sequence homology. Interestingly, *Dictyostelium discoideum* contains two homologues of mammalian PITPs (DdPITPI and DdPITPII) and one Sec14p homologue (DdSec14p) [72]. All PITPs bind a single molecule of PtdIns or phosphatidylcholine (PC) and are able to sense the relative concentration of PtdIns and PC and deposit their lipid cargo to the membrane in exchange for the more abundant species. PITPβ can additionally bind and transfer sphingomyelin but the significance of this is not clear [18]. This transfer function is the hallmark of all PITPs. In vitro, PITPs stimulate lipid kinase activity, again a property shared by all PITPs. PtdIns(4)P and PtdIns(3)P production is increased when a PI kinase and PtdIns is used as a substrate in in vitro assays, regardless of the PITP isoform or the lipid kinase used [3, 38, 57, 78]. Thus, these data need to be interpreted with caution due to lack of specificity. An alternative view is that the basic hallmark of lipid binding and lipid transfer are the major determinants that allow lipid kinases to get access to their substrates and that in vivo, specificity is determined by colocalization of the appropriate lipid kinases and PITPs. This interpretation is borne out by studies in

yeast where PI transfer activity is necessary and sufficient for phenotypic rescue of *sec14*^{ts} defects by expression of heterologous PITPα, PITPβ, plant Sec14p homologues or the PITP domain from PITPnm [40, 45, 73]. Likewise, in mammalian cells, Sec14p (*S. cerevisiae* or *D. discoideum*), PITPα or PITPβ are capable of rescuing PLC signaling, exocytosis and vesicle formation [16, 21, 31, 56, 72].

As for the mechanisms by which PITP function is required to guarantee normal Golgi trafficking, analysis performed in yeast had suggested that the major function of Sec14p was to maintain diacylglycerol levels which were essential for the Golgi-to-plasma membrane stage of secretion [39, 60]. In more recent studies the importance of PtdIns(4)P levels are suggested to be an important contributing factor to the secretory defect in *sec14* cells [55]. This conclusion is greatly strengthened by recent studies which couple Sec14p to the action of the PI4K, Pik1 [29, 69, 77].

4 and 5-Phosphorylated Phosphoinositides and the Golgi

Multiple types of PI4Ks exist in mammalian cells [26] and fractionation studies indicate that a substantial fraction of the total PI4K activity is in the Golgi membranes [15, 71, 85]. Two different PI4K isoforms have been localized to the Golgi complex, PI4Kα and PI4Kβ [27, 53, 85] (Table). PI4Kα colocalizes with the membrane form of PITP, PITPnm, at the Golgi, and can be coimmunoprecipitated. Deletion analysis indicates that both an acidic region, which is adjacent to the PITP homology domain, and the PITP homology domain are required for this association. In vitro, PITPnm stimulates the synthesis of PtdIns(4)P [3]. Another pathway for making PtdIns(4)P at the Golgi is via PI4Kβ. PI4Kβ is regulated and localized to the Golgi by ARF1, a small GTPase playing a crucial role in the physiology of the Golgi complex. PI4Kβ is one of the effectors of ARF1 in controlling lipid metabolism, the others being PIP5Kα and phospholipase D1 (PLD1). At the Golgi, ARF1 regulates the synthesis of PtdIns(4,5)P_2 by recruiting from the cytosol, and thus activating, PI4Kβ [27, 35], and does so independently of its effect on PLD1 (*see below*). In vitro, the Golgi PtdIns(4)P is converted to PtdIns(4,5)P_2 by PIP5Kα which is also recruited from the cytosol and is directly regulated by ARF1 [35]. In vivo, however, the subcellular distribution of PIP5Kα (both of the endogenous and transfected protein) appears restricted to the plasma membrane and to a vesicular peripheral compartment, and the Golgi-specific isoform remains to be identified (A. Godi and M.A. De Matteis, *unpublished data*).

Expression of the catalytically inactive form of PI4Kβ disrupts Golgi structure indicating that PtdIns(4)P or PtdIns(4,5)P_2 are required to maintain the Golgi architecture [27] and to mediate at least part of the effects of activated ARF1 on this organelle. On the other hand, phosphoinositides themselves regulate the activation of ARF1 on the Golgi via the ARF-GEFs (guanine nucleotide exchange factors) which are members of a large gene family, which all share a Sec7 domain that is responsible for the exchange of GDP to GTP on ARF proteins [33], and which are able to bind phosphoinositides. Overexpression of members of the ARNO family leads to disassembly of the Golgi complex and inhibition of secretion [23, 51].

As mentioned above, another effector of ARF1 is PLD1 which catalyses the hydrolysis of PC to PA and choline. The activity of all three isoforms of PIP5K (α, β, γ) is increased in vitro, when PA is present at equimolar concentration to their substrate, PtdIns(4)P. Thus PA, derived via the ARF-regulated PLD pathway could be an additional regulator of PtdIns(4,5)P_2 synthesis. While previous studies suggested that PLD1 was localized to Golgi-enriched membranes [43], more recent work clearly illustrates that PLD1 may be primarily localized in intracellular vesicles that may be endosomal/lysosomal [8, 52] and may not be localized to the Golgi [35].

In yeast, two isoforms of PI4K, Pik1 (the homologue of PI4Kβ) and Stt4 have been identified [55]. Genetic elimination of *STT4* leads to reduced PI4K activity and is not lethal, although these strains require osmotic stabilizers for survival. In contrast, genetic elimination of *Pik1* results in a lethal phenotype [24]. *Pik1* mutant strains accumulate Golgi-derived membranes (Berkeley bodies) and show an impaired transport of proteins from the Golgi complex to the plasma membrane (but not the vacuole) [77]. In immunofluorescence studies, Pik1 appears localized both in the nucleus and in the cytoplasm, where it appears enriched at the trans-Golgi, in agreement with its function at the Golgi [77].

Phosphoinositides must play a role at the Golgi as evidenced by acute regulation of PtdIns(4)P and PtdIns(4,5)P_2 levels. How the phosphoinositides exert their function is an active area of research and a precise understanding is dependent on identifying the effectors of the phosphoinositides. PtdIns(4,5)P_2 can specifically interact with a class of proteins containing PH domains, and likely candidates are the oxysterol binding protein and dynamin, which are also Golgi-localized [36, 46], and spectrin, which is also recruited at the Golgi by ARF [28]. Finally, an interesting and hitherto unexplored possibility is that also in mammals, analogous to what is emerging in the yeast secretory pathway, PtdIns(4)P itself (and not PtdIns(4,5)P_2), may exert a direct and major regulatory role on the Golgi structure and function.

A PtdIns(4,5)P_2 Requirement in Regulated Exocytosis in Neuro-endocrine Cells

In mammalian cells, secretory granules and synaptic vesicles are used for storage of chemical mediators, peptide hormones, and neurotransmitters and are discharged upon cell activation. A rise in cytosolic Ca^{2+} is very often the trigger for the release of secretory granules and for fusion of vesicles with the plasma membrane. Evidence that PtdIns(4,5)P_2 plays an important role has emerged from studies in PC12 cells, a neuroendocrine cell-line. Exocytosis of dense core secretory granules from PC12 cells can be subdivided into two stages, an ATP-dependent priming stage followed by a Ca^{2+} trigger stage. PITPα and PIP5Kγ were purified as cytosolic factors that could prime the cells for subsequent stimulation with Ca^{2+} [30, 31]. PITPα synergized with PIP5Kγ for effecting PIP_2 synthesis [30, 31]. The resident PI 4-kinase on the secretory granule was also required for exocytosis and was identified as a p55 PI-4-kinase isoform which has not yet been cloned [82]. Priming thus represents the synthesis of a granule-associated PIP_2 pool that controls the recruitment of a protein effector that regulates the subsequent Ca^{2+}-dependent fusion of the granule with plasma membrane.

An effector protein for PtdIns(4,5)P_2 is a 145 kDa peripheral membrane protein, CAPS (**C**alcium-dependent **A**ctivator **P**rotein for **S**ecretion) which localizes to the granule membrane. CAPS binds PtdIns(4,5)P_2 with high specificity and affinity in vitro, and this binding is mediated by a putative PH domain. CAPS is thought to function as a docking protein that permits CAPS to bring the granule into close association with the plasma membrane for subsequent fusion to take place. CAPS is a Ca^{2+}-binding protein and exhibits a conformational change upon binding Ca^{2+} [4, 7]. The importance of CAPS in neuronal secretion is underscored by genetic mutations in the *Caenorhabditis elegans* CAPS (UNC-31) where neurotransmission defects are observed.

A PtdIns(4,5)P_2 requirement is not restricted to dense-core secretory granule exocytosis but is also required for synaptic vesicle exocytosis [41, 83]. Two proteins that contain two C2 domains and are involved in the exocytic process are synaptotagmin and rabphilin 3A. C2 domains have been identified in many proteins and can bind to Ca^{2+} and phospholipids. One of the C2 domains (C2B) of synaptotagmin interacts with PtdIns(4,5)P_2 in vitro in a Ca^{2+}-dependent manner and it is envisaged that this interaction might influence synaptotagmin's interaction with SNARE molecules involved in the docking/fusion process [64]. Rabphilin 3A is also present on synaptic vesicles and is an effector of the small GTPase, Rab3A. Rabphilin shares regional sequence similarity with synaptotagmin and also contains two C2 domains. In the presence of Ca^{2+} and phosphatidylserine, both these domains bind specifically to liposomes containing PtdIns(4,5)P_2 [11].

A PtdIns(4,5)P_2 Requirement in Regulated Exocytosis in Hematopoeitic Cells

The requirement for PtdIns(4,5)P_2 in secretory function is not restricted to neuronal cells but is also a feature of hematopoetic cells. Cells of the immune system, including mast cells and neutrophils, respond rapidly to extracellular stimulation by secreting proteins, cytokines and histamine that will destroy, or effect the destruction, of infectious agents. Unlike conventional secretory cells, mast cells and neutrophils use a modified lysosome as their storage granule [68]. Early studies have established that the secretory mechanism for degranulation in mast cells and neutrophils is different from neuronal cells: mast cells and neutrophils require not only a rise in cytosol Ca^{2+}, but also the presence of an activating guanine nucleotide [6, 14]. Recent studies on mast cells and neutrophils (and their cultured counterparts, RBL mast cells and HL60 cells) have identified the small GTPase ARF as playing a crucial role in regulating secretion from this organelle [21, 79]. In addition to ARF, Rab3D, present on the secretory granules, is also required for the secretory process [62, 75]. That these cells have an unconventional mechanism of secretion compared to other secretory cells comes from genetic evidence. In Chediak Higashi syndrome, neutrophils have giant azurophilic granules, which are enlarged lysosomes and are resistant to degranulation [42]. Secretion from other hematopoietic cell types is impaired similarly but not from other conventional secretory cells [68].

Another distinguishing feature of stimulated secretory function in mast cells and neutrophils is its inhibition with alcohols [12, 70]. Primary alcohols interfere with the production of PA derived from PC hydrolysis by phospholipase D (PLD), by diverting the PA to the corresponding phosphatidylalcohol, a phenomenon called "transphosphatidylation." Following the identification of ARF as a cytosolic regulator of PLD1, the requirement of ARF and PLD in exocytosis has been analyzed in reconstitution assays using permeabilized cells [8, 21, 34, 79]. Regulated exocytosis is unimpaired in permeabilized cells provided that cytosol, MgATP and μmolar Ca^{2+}, together with an activating guanine nucleotide, are present. Removal of cytosol causes loss of secretory function and activation of PLD, and a cytosolic factor that could restore both secretion and PLD activation was purified and identified as ARF [21]. A second cytosolic factor was also identified as PITP that could only restore secretory function.

ARF and PITP restore secretory function by increas-

ing PtdIns(4,5)P_2 levels [21, 79]. ARF can raise PIP_2 levels either directly by activating PIP5K activity [35] or indirectly via PA produced by PLD. In a recent study, Honda et al. also identified that ARF activates PIP5K in vitro, *provided* PA was present as a costimulus [32]. However, the requirement for PA as a costimulus is dependent on the vesicle composition used in the in vitro assay [35].

Effectors for PtdIns(4,5)P_2 in the hematopoeitic cells have yet to be identified and one possibility is that PtdIns(4,5)P_2 facilitates actin polymerization on the granule membrane. The observation that endogenous membrane vesicles or exogenously added synthetic PtdIns(4,5)P_2 vesicles can be propelled by actin comets in cell extracts [47], raise the possibility of a direct link between PtdIns(4,5)P_2, actin polymerization and transport of vesicles. A recent study with pancreatic acinar cells reports that prior to exocytosis, the secretory granules become coated with actin before fusion and that this actin coating is tightly coupled to the release of Rab3D [76]. A transient coating of secretory granules with actin is thought to facilitate the movement of granules across the subapical actin network and towards their fusion site [76]. PtdIns(4,5)P_2 can induce actin-based movement of vesicles through recruitment of WASP-Arp2/3 followed by *de novo* actin polymerization [63]. Evidence for actin involvement in propelling endocytic vesicles in mast cells has been presented [49].

PtdIns(4,5)P_2 and Endocytosis

Both, the starting step in endocytosis, namely coat assembly, and the subsequent step of endocytic vesicle release, depend on PtdIns(4,5)P_2, as shown by the inhibitory effect of PH domains, which bind with high affinity and specificity to PtdIns(4,5)P_2. The later steps in the endocytic pathway, such as the endosome-endosome fusion step, require instead 3-phosphorylated inositides (*see below*). The above early endocytic events correspond to those requiring adaptor proteins like AP2 (adaptor protein 2) and dynamin, both of which can interact with PtdIns(4,5)P_2 [37]. The importance of the PH-domain of dynamin and of its interaction with PtdIns(4,5)P_2 in clathrin-mediated endocytosis has been reported in many different systems [44] [1]. In addition to directly interacting with the endocytic molecular machinery, PtdIns(4,5)P_2 may affect endocytosis by virtue of its ability to control the local adhesion energy at the plasma membrane, which depends on the state of actin polymerization and the extent of interactions between plasma membrane and cortical cytoskeleton [61]. Indeed, PtdIns(4,5)P_2 regulates both cortical plasma membrane-cytoskeleton interactions by binding cytoskeletal anchoring proteins and actin polymerization by binding proteins, such as profilin and gelsolin, that control actin polymerization.

PtdIns(3)P and Golgi-to-Endosome/ Vacuole Transport

3-phosphorylated inositol lipids took center stage with the identification of two yeast mutants defective in vacuolar protein sorting and membrane trafficking. The vacuolar protein-sorting (*vps*) mutant screen was designed to detect yeast mutants defective in protein-sorting from the Golgi apparatus to the vacuole, the yeast equivalent of the lysosomes. Vps34p and Vps15p turned out to be the catalytic and the regulatory subunits, respectively, of a PI 3-kinase. Vps34p is the only PI 3-kinase in *Saccharomyces cerevisiae* and it only uses PtdIns as a substrate (Fig. 2). Mammalian homologues of Vps34/Vps15 have been identified and function together with PITP (PITPα, PITPβ or Sec14p) to drive the synthesis of PtdIns3P in vitro.

In yeast, PtdIns(3)P may be converted to PtdIns(3,5)P_2 by Fab1p, a FYVE domain-containing protein (*see below*) that appears to control vacuolar homeostasis. In the absence of *Fab*1, the vacuole enlarges and fills up the whole cell. In addition, the cells appear to be defective in the formation of multivesicular bodies (MVBs) [54]. Thus PtdIns(3,5)P_2 somehow facilitates the internalization of the vacuolar membrane for subsequent degradation by acid lipases and proteases. The mammalian homologue of Fab1 is a 235 kDa protein [48] and plays an analogous role in the regulation of multivesicular body formation [22].

3-Phosphorylated Phosphoinositides and Endocytosis

The combined use of PI3K inhibitors, wortmannin and LY2949002, with biochemical approaches led to the demonstration that PI 3-kinase activity is required at the stage of endosome fusion and to the identification of a direct target for PtdIns(3)P in this event, the early endosomal autoantigen 1 (EEA1). EEA1 is specifically found on early endosomes and is released into the cytosol following treatment with wortmannin. EEA1 is a 170 kDa protein which binds to both PtdIns(3)P through a FYVE domain and to the GTP-bound form of the early endosomal small GTPase, Rab5 [67]. By using a dual recognition system, Rab5 and PtdIns(3)P, EEA1 is specifically targeted to endosomal membrane and acts as a tethering device between two Rab5-positive membranes. This is an elegant principle that may apply to membrane recruitment of other proteins by specific lipids [81]. The yeast homologue of EEA1, Vps19p (also known as Vac1p) binds to yeast Rab5 homologue and is also thought to play a role in vesicle docking at the endosome.

Future Directions

The case for phosphoinositides in allowing recruitment of specific proteins is now well established. In addition

to recruitment, proteins may undergo conformational changes and thus modify their activity state. The challenge lies in the development of new methodology that can allow for monitoring changes of specific phosphoinositides in living cells both spatially and temporally. Our knowledge of phosphoinositide-based signalling will expand as our knowledge of the lipid kinases and their regulators will grow. The lipid phosphatases are beyond the scope of this review but will be equally important in dictating the flux of individual phosphoinositides through the different organelles. Future work will no doubt include gene knockouts of specific isoforms of the lipid kinases and their regulators. It is clear that phosphoinositides will impinge on many aspects of biology and will keep the labs busy for many years as we move rapidly into the postgenomic era where new and novel targets for PtdIns(4,5)P_2 will be increasingly identified based on sequence analysis.

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