The multiple roles of PtdIns(4)*P* – not just the precursor of PtdIns(4,5)P_2

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Summary

The phosphoinositides (PIs) are membrane phospholipids that actively operate at membrane-cytosol interfaces through the recruitment of a number of effector proteins. In this context, each of the seven different PI species represents a topological determinant that can establish the nature and the function of the membrane where it is located. Phosphatidylinositol 4-phosphate (PtdIns(4)*P*) is the most abundant of the monophosphorylated inositol phospholipids in mammalian cells, and it is produced by D-4 phosphorylation of the inositol ring of PtdIns. PtdIns(4)*P* can be further phosphorylated to PtdIns(4,5)P_2 by PtdIns(4)*P* 5-kinases and, indeed, PtdIns(4)*P* has for many years been considered to be just the precursor of PtdIns(4,5)P_2. Over the last decade, however, a large body of evidence has accumulated that shows that PtdIns(4)*P* is, in its own right, a direct regulator of important cell functions. The subcellular localisation of the PtdIns(4)*P* effectors initially led to the assumption that the bulk of this lipid is present in the membranes of the Golgi complex. However, the existence and physiological relevance of 'non-Golgi pools' of PtdIns(4)*P* have now begun to be addressed. The aim of this Commentary is to describe our present knowledge of PtdIns(4)*P* metabolism and the molecular machineries that are directly regulated by PtdIns(4)*P* within and outside of the Golgi complex.

Key words: Phosphoinositides, PtdIns(4)*P*, PtdIns(4)*P*-binding proteins, PI 4-kinase, Golgi complex, Lipid-transfer protein

Introduction

The phosphoinositides (PIs) are derivatives of phosphatidylinositol (PtdIns) that are phosphorylated on up to three of the available hydroxyl groups of the inositol headgroup. PI kinases specifically modify the D-3, D-4 and D-5 positions of the inositol ring in all of the possible combinations. The phosphorylation reactions are reversed by specific PI phosphatases. Seven distinct PI species have been identified and named according to their site(s) of phosphorylation (Fig. 1), and many different isoforms of each of the kinase and phosphatase activities have been identified.

A distinctive feature of PI metabolism is its precise regulation – both in time and space – that is achieved by strict control of the subcellular distribution, membrane association and activity state of each of the different kinases and phosphatases. The balance of the PI kinase versus phosphatase activities can thus be different in different cell compartments, which generates apparently non-homogenous distributions of the distinct phosphorylated PI species among cell organelles (De Matteis and Godi, 2004). This non-homogenous distribution has been deduced from the subcellular distributions of protein domains that recognize the different PIs with different affinities. Although not free from drawbacks (Lemmon, 2007; Lemmon, 2008), this approach has allowed the visualisation of the distinct PI pools in the cell [as for that of PtdIns(4,5)P_2 at the plasma membrane (PM), PtdIns(3)*P* in the endosomal system and PtdIns(4)*P* at the Golgi complex].

PtdIns(4)*P* is the product of PtdIns 4-kinase (PI4K) activity on PtdIns, and it is the most abundant of the monophosphorylated derivatives of PtdIns (Lemmon, 2008). Well before the cloning of the different PI4K activities, it had been realized that, among the different cell-membrane fractions, the Golgi membranes have the highest PI4K-specific activity (Cockcroft et al., 1985). Subsequently, when PI4K activities were categorised into types II and III according to their sensitivities to adenosine and wortmannin, respectively (Balla and Balla, 2006), the Golgi complex was shown to host both activities. Four different PI4K isoforms were then cloned, and two of them were mapped at the Golgi complex: PI4KIIα and type III PI4KIIIβ (Weixel et al., 2005) (Fig. 2).

PtdIns(4)*P* can be further phosphorylated by PtdIns(4)*P* 5-kinases (PIP5Ks) to yield PtdIns(4,5)P_2 and, in fact, for a long time PtdIns(4)*P* was considered to be just an intermediate along the PtdIns(4,5)P_2 synthetic pathway. However, following the pioneering work in yeast that clearly showed that some cell processes that are regulated by PI4Ks can be dissociated from those that are controlled by PIP5K (Hama et al., 1999), it was proposed that PtdIns(4)*P* has its own direct effects in the cell (Hama et al., 1999; Li et al., 2002; Walch-Solimena and Novick, 1999). In support of this concept and of a prominent role for PtdIns(4)*P* at the Golgi complex, many PtdIns(4)*P*-binding proteins have since been identified in yeast and mammals, with the vast majority of these being localised at the Golgi complex (De Matteis and D'Angelo, 2007) (Fig. 2, Table 1). Despite this and other evidence that suggests a Golgi-restricted localisation of PtdIns(4)*P*, a closer inspection of the intracellular distribution of this lipid and of PtdIns(4)*P*-binding proteins supports a wider subcellular distribution (Balla et al., 2008; Balla et al., 2005; Roy and Levine, 2004), which opens questions as to the role of PtdIns(4)*P* outside of the Golgi complex.

In this Commentary we review the main pathways of PtdIns(4)*P* metabolism in yeast and mammals, together with the effectors and roles of PtdIns(4)*P* both within and outside the Golgi complex.

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The cellular role and regulation of yeast and mammalian PI4Ks

Yeast

The use of *Saccharomyces cerevisiae* as a model system has been of outstanding importance in the dissection of the regulation and roles of the PI4Ks. The yeast genome contains three genes that encode PI4Ks (Strahl and Thorner, 2007): *Pik1* (the PI4KIIIβ orthologue that accounts for the production of 45% and 40% of the total cellular PtdIns(4)*P* and PtdIns(4,5)P_2 content, respectively); *Stt4* (the PI4KIIIα orthologue that accounts for the production of 40% and 60% of the total cellular PtdIns(4)*P* and PtdIns(4,5)P_2 content, respectively); and *Lsb6* (the PI4KII orthologue that accounts for the remaining PtdIns(4)*P* content) (Strahl and Thorner, 2007).

Pik1p localises both on cytoplasmic puncta that are positive for the *trans*-Golgi complex marker Sec7p and in the nucleus (Strahl et al., 2005). The localisation of Pik1p in the nucleus is determined by specific karyopherins that regulate both the nuclear import and export of Pik1p (Strahl et al., 2005). The association of Pik1p to the Golgi complex is instead dependent on its binding to Frq1p, an essential 22-kDa N-myristoylated protein that apparently has Pik1p as its only downstream effector (Hendricks et al., 1999). Yeast cells need both nuclear and Golgi-localized Pik1p for viability, because Pik1p mutants that have a restricted nuclear and Golgi localisation cannot reverse the non-viable phenotype in *Pik1Δ*cells (Strahl et al., 2005).

Interestingly, the association of Pik1p with the Golgi complex varies according to nutrient conditions: under starving conditions, together with its non-catalytic subunit Frq1p, Pik1p dissociates from Golgi membranes (Faulhammer et al., 2007) in a manner that depends on its phosphorylation and binding to the 14-3-3 proteins Bmh1p and Bmh2p (Demmel et al., 2008).

The activity of Pik1p, and thus the generation of PtdIns(4)*P* on Golgi membranes, is required for secretion. The first link between PtdIns(4)*P* and secretion was provided by the observation that the levels of PtdIns(4)*P* were markedly reduced in the *Sec14-3*^{ts} mutant that is defective for protein secretion (Hama et al., 1999) and that the growth and secretory defects in *Sec14-3*^{ts} can be rescued by overexpression of Pik1p and by deletion of the PtdIns(4)*P* 4-phosphatase *Sac1* (Cleves et al., 1989; Hama et al., 1999).

Sec14 encodes a PtdIns-transfer protein (PITP), an essential protein that localises to the Golgi and contributes to the transfer of PtdIns (the substrate of Pik1p and precursor of PtdIns(4)*P*) from its production site in the endoplasmic reticulum (ER) to the Golgi complex (Cockcroft, 2007; Schaaf et al., 2008). The loss of function of *Pik1* in *S. cerevisiae* results in strong defects in secretion and in the ultrastructure of the secretory pathway (Audhya et al., 2000; Walch-Solimena and Novick, 1999). The morphological effects of Pik1p loss-of-function closely resemble the effects that are caused by a loss of function of the small GTPase Arf1p (Audhya et al., 2000); in addition, cells that lack *Arf1* produce decreased amounts of PtdIns(4)*P* and PtdIns(4,5)P_2 (Audhya et al., 2000).

Pik1 mutations are synthetically lethal with mutations in *Ypt31*, which encodes an important player in *trans*-Golgi network (TGN)-to-PM transport (Sciorra et al., 2005). However, inactivation of Ypt31p does not result in any reduction of PtdIns(4)*P* or mislocalisation of Pik1p, suggesting that it acts downstream of Pik1p activation.

Stt4p (staurosporine- and temperature-sensitive 4) was originally isolated as a factor responsible for resistance to the protein-kinase inhibitor staurosporine (Yoshida et al., 1994), and is localised to the PM (Audhya and Emr, 2002). This membrane association of Stt4p is mediated by Sfk1p (suppressor of four kinases 1), which is also a multicopy suppressor of *Stt4*^{ts} (Audhya and Emr, 2002). In addition to staurosporine hypersensitivity, loss of function of the *Stt4* gene produces defects in cell-wall stability, an abnormal

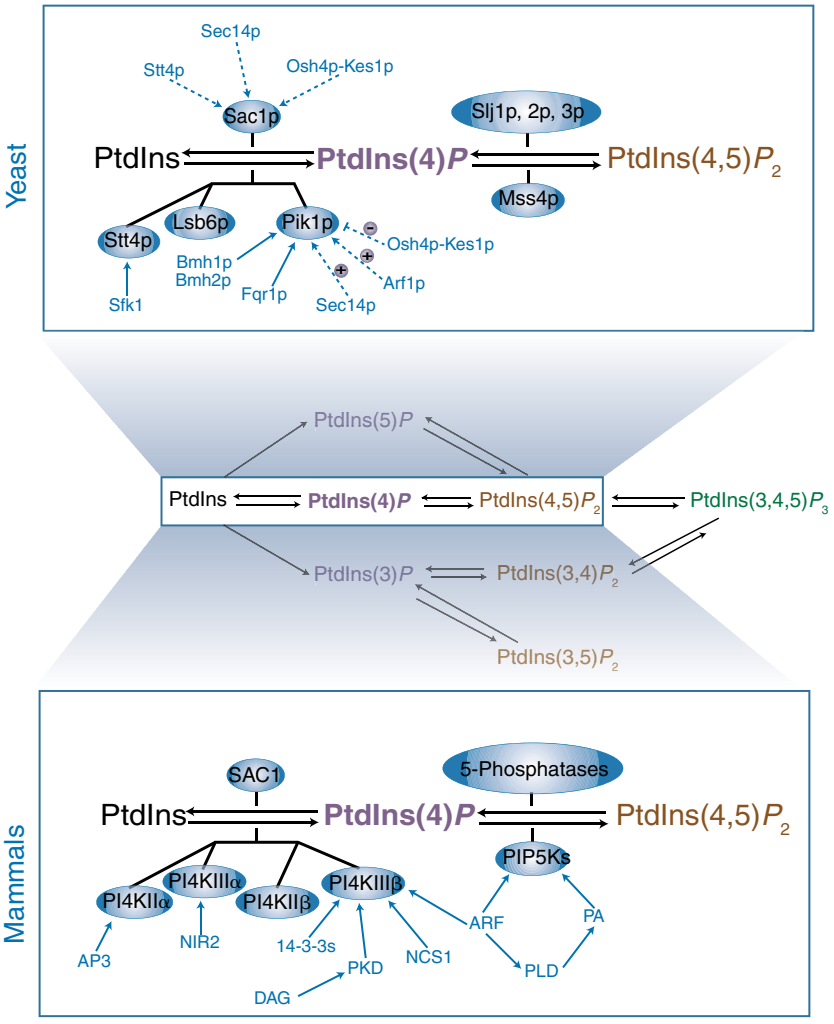

Fig. 1. The metabolic cycle of PtdIns(4)*P* in yeast and mammals. The metabolic cycle of PtdIns(4)*P* in yeast and mammals. PtdIns(4)*P* can be produced through phosphorylation of PtdIns by PI4Ks and through dephosphorylation of PtdIns(4,5)P_2 by PtdIns(4,5)P_2-5-phosphatases (5-phosphatases). These comprise Sjl1p, Sjl2p and Sjl3p in yeast, and several members in mammals, including synaptojanin 1 and synaptojanin 2, OCRL, INPP5B, INPP5E, INPP5F, PAB5PA and SKIP. Enzymes are indicated by ellipses and the regulatory factors are connected to the PI4Ks either by continuous lines (physical interactions) or by dashed lines (functional interactions). See text for details.

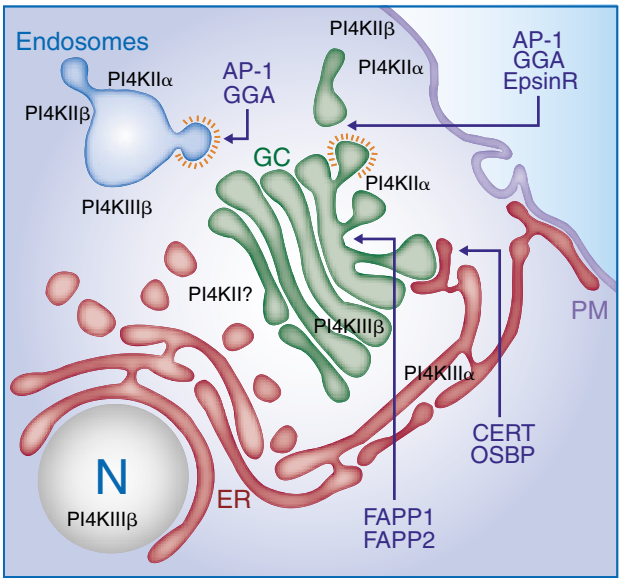

Fig. 2. Intracellular distribution of PI4Ks and PtdIns(4)*P* effectors in mammalian cells. The intracellular localisation of PI4Ks and PtdIns(4)*P* effectors in mammalian cells suggests a central role for the Golgi complex in the synthesis and biological function of PtdIns(4)*P*. N, nucleus; GC, Golgi complex.

actin cytoskeleton, and defective polarised growth and aminophospholipid transport (Strahl and Thorner, 2007).

Both staurosporine hypersensitivity and the cell-wall defects in *Stt4*^{ts} cells can be rescued by expression of the *Mss4* (multicopy suppressor of *Stt4*) gene, which encodes the only yeast PIP5K. *Stt4* and *Mss4* temperature-sensitive mutants fail to correctly organise their actin cytoskeleton, whereas concomitant inactivation of the PtdIns(4)*P* 4-phosphatase gene *Sac1* is sufficient to rescue this phenotype (Foti et al., 2001). These overlapping phenotypes that are seen for *Stt4* and *Mss4* inactivation suggest a role for PtdIns(4)*P* as a precursor of PtdIns(4,5)P_2 in these processes (rather than being a direct effect of PtdIns(4)*P*). Independent studies have indeed shown that the bulk of the PtdIns(4,5)P_2 that is produced at the PM is dependent on *Stt4* (Strahl and Thorner, 2007). However, some of the functions of Stt4p at the PM are not dependent on Mss4p. For instance, the recruitment of the p21-activated kinase Cla4p to the site of polarised growth depends on Stt4p-produced PtdIns(4)*P* and on the GTPase Cdc42, but is independent of Mss4p (and Pik1p) (Wild et al., 2004).

Stt4 has also been shown to be one of the fundamental genes that are involved in phosphatidylserine transport from the ER to the Golgi complex or to the vacuole (Trotter et al., 1998). To accomplish this, Stt4p should be present and active, at least in part, at the ER or Golgi membranes. Indeed, it has been proposed that a pool of Stt4p is localised at the ER via Stt4p interactions with the ER-resident protein Scs2p (suppressor of choline sensitivity 2) (Choi et al., 2006). However, the precise molecular mechanisms that are involved with Stt4p in phosphatidylserine transport remain to be fully elucidated.

Interestingly, the PtdIns(4)*P* pool that is produced by Stt4p appears to be the most sensitive to the activity of the PtdIns(4)*P* 4-phosphatase Sac1p. Sac1p is a type II integral membrane protein, the localisation of which varies according to growth conditions: it is mostly localised at the ER under exponential growth and it shuttles to the Golgi complex during starvation (Faulhammer et al., 2007). Thus, Sac1p (in combination with Pik1p and 14-3-3) is responsible for the adaptation of PI metabolism to different growth conditions (Demmel et al., 2008; Faulhammer et al., 2007). The inactivation of *Sac1* results in an eight- to twelvefold increase in the total cellular PtdIns(4)*P* content, which is accompanied by a shift to the ER of a PtdIns(4)*P* biosensor that usually decorates Golgi membranes (Roy and Levine, 2004); this PtdIns(4)*P* increase in *Sac1Δ* cells can be reversed by inactivation of *Stt4*, but not of *Pik1* or *Lsb6* (Foti et al., 2001). A functional connection also exists in the reverse direction, because the deletion of *Sac1* can suppress the actin phenotype in *Stt4*^{ts} cells (Strahl and Thorner, 2007). Despite their different subcellular locations (the ER and PM, respectively), these intimate functional relationships between *Sac1* and *Stt4* suggest that these compartments must come into close

Table 1. PtdIns(4)*P* metabolic enzymes

Enzyme	Localisation	Molecular mass (kDa)	Gene
PI 4-kinases			
Mammalian			
PI4KIIα	Golgi, TGN, PM, endosomes, synapse	55-56	*PI4K2A, 10q24*
PI4KIIβ	Golgi, TGN, PM, endosomes, ER	55-56	*PI4K2B, 4p15.2*
PI4KIIIα	PM, ER, nucleus	210	*PI4K2A, 22q11.21*
PI4KIIIβ	Golgi, TGN, nucleus, endosomes, exocytic vesicles	110	*PI4KB, 1q21*
Yeast			
Lsb6p	PM, vacuole	70	*LSB6, X*
Stt4p	PM	216	*LSB6, X*
Pik1p	Golgi, nucleus	125	*STT4, XII*
PI 4-phosphatases			
Mammalian			
Sac1	ER, Golgi	64	*SACM1L, 3p21.3*
Synaptojanin 1	Clathrin-coated vesicles (nerve terminals)	145-170	*SYNJ1, 21q22.2*
Synaptojanin 2	Clathrin-coated vesicles (ubiquitous)	140	*SYNJ12, 6q25.3*
Yeast			
Sac1p	ER, Golgi	67	*SAC1, XI*

The main enzymes that are involved in PtdIns(4)*P* synthesis and degradation in mammalian and yeast cells are indicated.

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proximity for Sac1p to act on the PtdIns(4)*P* pool that is generated at the PM by Stt4p.

Finally, Lsb6p (Las17-binding 6) is the most recently identified of the yeast PI4Ks, and is encoded by a non-essential gene. It localises to the PM and the vacuole, behaving similar to an integral membrane protein, possibly due to S-palmitoylation (Han et al., 2002). It has been proposed that, because Lsb6p is the only PI4K that localises to the vacuole (Shelton et al., 2003), it has a role in vacuole fusion by providing the substrate for localised PtdIns(4,5)P_2 production. Lsb6p has also been implicated in endosome motility independent of its kinase activity, with this action proposed to be through its interaction with the actin-filament-polymerisation-promoter protein Las17p, the orthologue of the human Wiskott-Aldrich syndrome protein (WASP) (Chang et al., 2005).

Mammals

Mammalian PI4Ks were originally classified as types II and III (those originally identified as type I PI4Ks were later demonstrated to be PI3Ks), according to their sensitivities to inhibitors. Type II PI4K activity, which is inhibited by adenosine, was seen to be associated with the PM, often in complexes with PM receptors. This activity provides an important contribution to the PM pool of PtdIns(4,5)P_2 (Pike, 1992). Subsequently, when the two type II PI4Ks PI4KIIα and PI4KIIβ were cloned, it was found that PI4KIIα is mainly present in endo-membranes, such as the Golgi complex, endosomal membranes, synaptic vesicles, in compartments that contain the AP-3 adaptor complex and in vesicles that contain Glut4 (Balla et al., 2002; Guo et al., 2003; Salazar et al., 2005; Xu et al., 2006), and PI4KIIβ localises to endosomal and perinuclear membranes under resting conditions (Balla et al., 2002) (Fig. 2). However, a fraction of both type II PI4Ks is found at the PM and, in the case of PI4KIIβ, this fraction increases in a Rac-dependent manner upon stimulation with growth factor (Wei et al., 2002). Consistent with its localisation, PI4KIIα has a role in TGN-to-endosome and TGN-to-PM transport (Wang et al., 2003), in the association of AP-3 with endosomal compartments (Salazar et al., 2005) and in EGF-receptor degradation (Minogue et al., 2006), and it is very likely to have a key role in synaptic-vesicle recycling (Guo et al., 2003). Very little information is available on its regulation, with a hitherto-unspecified role for amyloid-β peptides in PI4KIIα activity reported in the brain (Wu et al., 2004).

Type III PI4Ks, which are sensitive to wortmannin, comprise the 230-kDa PI4KIIIα and the 92-kDa PI4KIIIβ. PI4KIIIα has been reported to be localised on the ER and in a perinuclear compartment (Wong et al., 1997). However, in spite of this reported localisation, PI4KIIIα has been shown to have a role in controlling a PtdIns(4)*P* pool at the PM (Balla et al., 2005). Indeed, a pool of PtdIns(4)*P* that is dependent on PI4KIIIα has been visualised at the PM in response to activation of the angiotensin receptor (Balla et al., 2008), and in the recovery phase following acute depletion of PtdIns(4)*P* and PtdIns(4,5)P_2 (Balla et al., 2005). Interestingly, under these conditions, no change in the intracellular distribution of PI4KIIIα was observed, indicating that the enzyme was filling the PM pool of PtdIns(4)*P* while residing in the ER (Balla et al., 2005). This apparent topological discrepancy can be partially resolved by assuming that the PI4KIIIα-dependent production of PtdIns(4)*P* occurs at the level of the ER-PM contact sites, that is, sites of close apposition between the ER and the PM (Fig. 2). At the PM, PtdIns(4)*P* can be subsequently metabolised to PtdIns(4,5)P_2, which serves as the substrate for agonist-induced Ins(1,4,5)P_3 production, Ca^{2+} mobilisation and signal transduction. Interestingly, and consistent with its role at the PM, PI4KIIIα has been shown to be required to maintain an agonist-sensitive PI pool in this location (Balla et al., 2008).

PI4KIIIβ (the Pik1p orthologue) is mainly associated with the Golgi complex (Godi et al., 1999; Wong et al., 1997), although it is also present on endosomes and in the nucleus (de Graaf et al., 2002) (Fig. 2). PI4KIIIβ is recruited and activated by the GTP-bound form of Arf1 on the Golgi membranes (Godi et al., 1999). It also interacts with neuronal calcium sensor 1 (NCS-1, also known as FREQ), the mammalian orthologue of yeast frq1p, which stimulates PI4KIIIβ activity in vitro (Weisz et al., 2000). Arf1 and NCS-1 are part of a regulatory feedback loop, because their PI4KIIIβ activatory properties are negatively controlled by the direct interactions that Arf1 and NCS-1 establish with each other (Haynes et al., 2005). PI4KIIIβ can be phosphorylated on Ser294 by protein kinases D1 and D2 (PKD1 and PKD2, respectively) (Hausser et al., 2005), both of which are involved in TGN-to-PM trafficking (Yeaman et al., 2004). Phosphorylation of PI4KIIIβ results in its enzymatic activation, and this is stabilised by interactions between PI4KIIIβ and the 14-3-3 proteins (Hausser et al., 2006) – interactions that are evolutionarily conserved from yeast (see above) to humans. Another conserved interaction is that between PI4KIIIβ and the small GTPase Rab11 (the mammalian orthologue of Ypt31p) (de Graaf et al., 2004). Indeed, the GTP-bound form of Rab11 is recruited to Golgi membranes through binding to PI4KIIIβ, where it contributes to the transport of cargo proteins to the PM (de Graaf et al., 2004). Finally, PI4KIIIβ directly interacts with, and is stimulated by, the elongation factor eEF1A2 (Jeganathan and Lee, 2007).

The roles that have so far been ascertained for PI4KIIIβ are exerted mainly – but not exclusively – at the level of the Golgi complex. Indeed PI4KIIIβ is required for regulated exocytosis in mast cells (Kapp-Barnea et al., 2003), pancreatic β-cells (Gromada et al., 2005) and in PC12 cells (de Barry et al., 2006). At the Golgi complex, PI4KIIIβ (alone or in cooperation with the Golgi-localised PI4KIIα) controls important functional and structural processes, such as TGN-to-PM transport of newly synthesised cargoes, the structural architecture of the Golgi complex itself (Godi et al., 1999) and, as shown more recently, the sphingolipid synthetic pathway (D'Angelo et al., 2007; Toth et al., 2006).

Although it is reasonable to assume for some of the above roles of the PI4Ks that the requirement for PtdIns(4)*P* derives from its subsequent conversion to PtdIns(4,5)P_2, for others (such as the control of membrane trafficking and of sphingolipid metabolism at the Golgi complex) it has been specifically shown that this requirement is also due to the direct activity of PtdIns(4)*P* on its effectors.

PtdIns(4)*P* effectors

Two main classes of PtdIns(4)*P* effectors have been characterised to date: adaptor and coat complexes (i.e. AP-1, GGA proteins and epsinR) and lipid-transfer proteins (i.e. OSBP, CERT and the FAPP proteins) (Fig. 3). These apparently unrelated classes of proteins in fact share interesting features. First, they use PtdIns(4)*P* as part of a more complex Golgi-membrane-localisation code that often includes a small GTPase and that can involve separate domains or the sole pleckstrin-homology (PH) domain, as in the case of OSBP and FAPP. Second, by acting as coincidence detectors of specific lipids and proteins, they contribute to the local specialisation of membrane composition and to the definition of distinct membrane domains. Third, they might have important roles in determining the 'geometry' of such membranes, owing to their ability to promote

coat assembly or to directly induce membrane asymmetry by virtue of co-existing curvature-inducing domains [e.g. the epsin N-terminal-homology (ENTH) domain] or, finally, to generate membrane asymmetry by transferring a lipid moiety to the cytosolic leaflet of the membranes.

Adaptors

The first reported example of a coat adaptor that is regulated by PtdIns(4)*P* was that of AP-1 (Wang et al., 2003), which promotes clathrin-dependent TGN-to-endosome trafficking through its ability to interact with clathrin and with specific sorting motifs in the cytosolic tail of endosome-directed transmembrane cargoes (Hirst and Robinson, 1998). AP-1, which binds directly to PtdIns(4)*P*, associates with TGN membranes in a PI4KIIα-dependent manner (Wang et al., 2003). It also binds the small GTPase Arf1, and the coincident binding of PtdIns(4)*P* and Arf1 combined with the direct recognition of cargo molecules represents an effective strategy for the regulation in time and space of the recruitment of AP-1 onto specific domains of the TGN.

A similar strategy has been adopted by another family of clathrin adaptors, the Golgi-localised, γ-ear-containing, Arf-binding proteins (GGAs), which are monomeric proteins that have a C-terminal domain that is related to the appendage or 'ear' domain of the γ-subunit of the AP-1 complex (Nakayama and Wakatsuki, 2003). The GGAs are localised at the TGN – due to cargo recognition and dual binding to Arf1 and PtdIns(4)*P* – and endosomes, and they participate in membrane trafficking between these compartments. The interaction of the GGAs with Arf1 and PtdIns(4)*P* is mediated by their GGA and Tom1 (GAT) domain. Mutant forms of the GAT domain of the GGAs that cannot bind PtdIns(4)*P* show decreased association with the TGN and lose their function in living cells (Wang et al., 2007).

EpsinR is a member of the epsin protein family, a PI-binding protein family in which the members interact with the 'ear' domain of the APs and with clathrin, and can induce membrane curvature (Ford et al., 2002). In contrast to the other family members that interact with AP-2 and PtdIns(4,5)P_2 (Chen et al., 1998; Itoh et al., 2001), EpsinR shows affinity for AP-1 and PtdIns(4)*P* (Mills et al., 2003). Strikingly, the association of EpsinR with membranes is independent of AP-1 but depends on Arf1 (Hirst et al., 2003), adding this protein to the list of the clathrin adaptors that use a dual-key code to localise to the TGN-to-endosome compartment.

Finally, the assembly of COPII at the ER exit sites has been shown to be supported by PtdIns(4)*P*, although the molecular nature of this PtdIns(4)*P* sensitivity remains to be fully elucidated (Blumental-Perry et al., 2006). Indeed, PtdIns(4)*P* has been shown to be required to support maximal binding of COPII components to liposomes upon Sar1 activation (Blumental-Perry et al., 2006). However, no direct binding of any of the COPII components has been reported so far.

The COF family of lipid-transfer proteins

Lipid-transfer proteins of this family contain a distinctive lipid-binding or transfer domain, which is usually located at the C-terminus of the protein, and a conserved N-terminal PH domain that binds PtdIns(4)*P* (Dowler et al., 2000; Levine and Munro, 2002) and, in the case of FAPP and OSBP PH domains, the small GTPase Arf1 (Godi et al., 2004). This protein family includes: CERT (ceramide-transfer protein), which has a steroidogenic acute regulatory (StAR) protein-related lipid-transfer (START) domain; OSBP1 (and the related yeast proteins Osh1p and Osh2p), which has an oxysterol-/cholesterol-binding (OxB) domain; and FAPP2, which has a glycolipid-transfer protein homology (GLTPH) domain (De Matteis et al., 2007).

CERT, OSBP1 and FAPP2 (hereafter, together referred to as the COFs) localise on Golgi membranes through their conserved PH domains (Godi et al., 2004) (Fig. 4). The enzymes that are responsible for the production of the pool of PtdIns(4)*P* that is recognised by the COF PH domains are PI4KIIIβ and PI4KIIα in mammalian cells, and Pik1p in yeast (Balla et al., 2005; Levine and Munro, 2002). In addition, OSBP1 and CERT (but not FAPP2) also bind to integral membrane proteins of the ER (VAP-A and VAP-B) (Kawano et al., 2006; Wyles et al., 2002). The molecular functions of the COFs have been addressed in detail (De Matteis, et al., 2007), which has led to the definition of their central role in the modulation of sphingolipid and sterol metabolism and in membrane trafficking.

CERT

CERT was originally identified as the Goodpasture-antigen-binding protein (GPBP), and was renamed after the demonstration that it is a lipid-transfer protein that is responsible for the non-vesicular delivery of ceramide (ceramide transfer) from its site of synthesis, the ER, to the Golgi complex (Hanada et al., 2003). In the Golgi, the CERT-transported ceramide is used for the production of sphingomyelin through sphingomyelin synthase 1 (SGMS1),

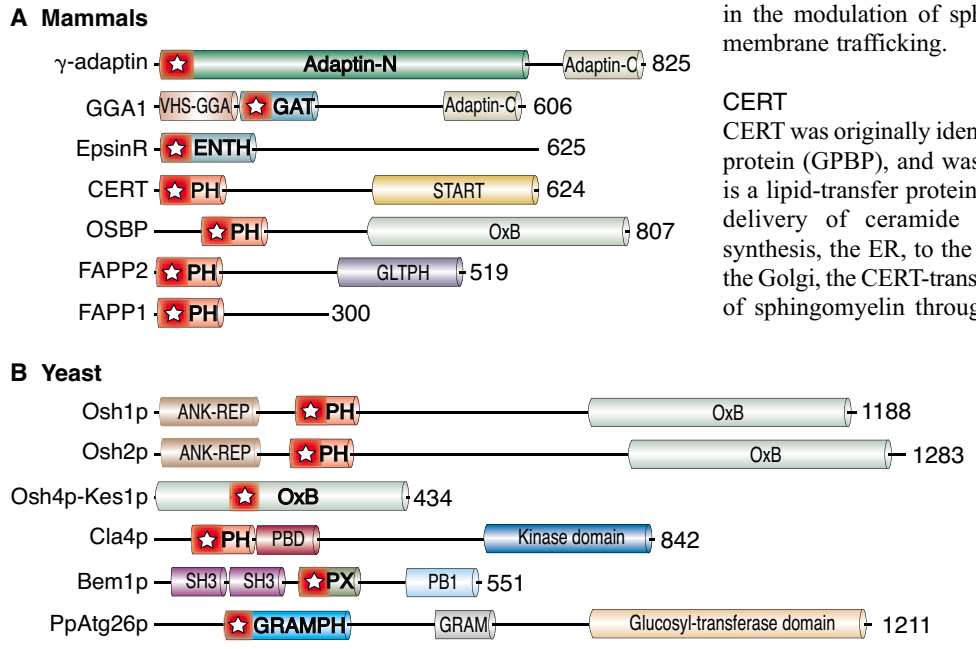

Fig. 3. PtdIns(4)*P* effectors. (A,B) Domain organisation of mammalian and yeast proteins that interact with PtdIns(4)*P*. PtdIns(4)*P* binding sites are indicated by a star. VHS-GGA; (Vps27p/Hrs/STAM-GGA domain); ANK-REP; ankyrin repeats; SH3; Src-homology domain 3; PB1; Phox and Bem1p domain.

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which transfers the choline headgroup from phosphatidylcholine to ceramide (Hanada et al., 2003). The molecular properties that account for the interactions of CERT with both Golgi and ER membranes have been identified: the PH domain targets CERT to the Golgi complex, whereas the FFAT motif mediates its interaction with the ER membrane protein VAP-A (Kawano et al., 2006; Perry and Ridgway, 2006). What remains to be determined is whether CERT shuttles from the ER to the Golgi complex through a cytosolic cycle, or whether it can simultaneously (or in close succession) interact with ER and Golgi membranes, thereby exploiting their close vicinity at the level of contact sites between the ER and the Golgi membrane.

Localisation and activity of CERT is controlled by a phosphorylation-dephosphorylation cycle (Fugmann et al., 2007; Saito et al., 2007; Kumagai et al., 2007). In particular, the phosphorylation of CERT at Ser132 is mediated by PKD and reduces its binding to PtdIns(4)*P*, its membrane association and its ceramide-transfer activity (Fugmann et al., 2007). This regulation contributes a branch to the feedback circuit that connects PKD and CERT at the TGN. PKD is recruited to the TGN through diacylglycerol (DAG) (Baron and Malhotra, 2002), whereas sphingomyelin synthesis is an important pathway that leads to DAG generation at the Golgi complex (Baron and Malhotra, 2002) – a reaction that is operated by SGMS1 and is assisted by CERT. In this way, through DAG generation and PKD recruitment, sphingomyelin synthesis indirectly leads to the inactivation of CERT (Fugmann et al., 2007). Thus, by inactivating CERT [by phosphorylating and activating PI4KIIIβ (Hausser et al., 2005) (see above)] and by interacting with type II PI4Ks (Nishikawa et al., 1998), PKD emerges as a key component of the PtdIns(4)*P*-regulated pathways at the TGN.

The picture that results is one in which two lipid metabolic pathways [the generation of PtdIns(4)*P* and synthesis of sphingomyelin] are regulating each other, as well as being regulated through the activity of the common modulator PKD. Synthesis of sphingomyelin is positively controlled by PtdIns(4)*P* (through CERT), whereas PtdIns(4)*P* synthesis is positively controlled through sphingomyelin synthesis owing to the generation of DAG, and the subsequent phosphorylation and activation of PI4KIIIβ through PKD. At the cellular level, CERT has been reported to have a role in anterograde membrane trafficking along the secretory pathway (Fugmann et al., 2007) and in the ER-stress response (Swanton et al., 2007), whereas at the organism level, CERT has been characterised as a factor that regulates the normal oxidative-stress response and aging (Rao et al., 2007).

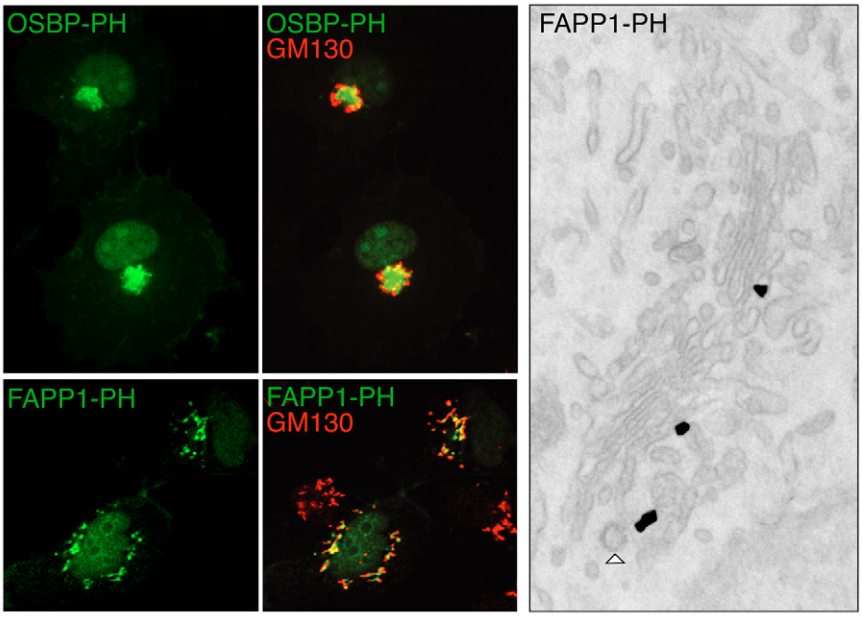

Fig. 4. Golgi localisation of the PH domain of FAPP1 and OSBP1. COS-7 cells expressing the GFP-tagged PH domain of OSBP1 (OSBP-PH) and MDCK cells expressing the GFP-tagged PH domain of FAPP1 (FAPP1-PH) were labelled for the Golgi marker GM130 (red) and processed for immunofluorescence. MDCK cells expressing the GST-tagged FAPP1-PH were processed for immuno-electron microscopy and labelled with anti-GST antibodies (far right panel). Notice the polarised distribution of FAPP1-PH at the *trans*-pole of the Golgi stacks, as indicated by the presence of a clathrin-coated profile (arrowhead). See text for details.

OSBP

OSBP was initially isolated as a cytosolic receptor for oxysterols (Kandutsch and Shown, 1981). To date, 16 OSBP-related proteins (ORPs) have been identified in humans and seven in *S. cerevisiae* (Olkkonen and Levine, 2004), all of which contain an OSBP-related domain that binds oxysterols and other lipids. Although the formal demonstration of sterol-transfer activity has been achieved for Osh4p-Kes1p (Raychaudhuri et al., 2006) (one of the yeast ORPs; see below), there is so far no evidence that any of the mammalian ORPs function as sterol-transfer proteins. OSBP localises to the ER (through its FFAT domain and interaction with VAP-A) and to the Golgi complex (through its PH domain, which binds PtdIns(4)*P* and Arf1) (Godi et al., 2004; Levine and Munro, 2002), with a phosphorylation-dephosphorylation cycle that also regulates its subcellular distribution (Storey et al., 1998).

Several lines of evidence have established bi-directional connections between OSBP and the cell sterols, with reported effects of sterols on OSBP and effects of OSBP on sterol metabolism (Lagace et al., 1997). First, the localisation of OSBP at the Golgi complex is controlled by oxysterols (Ridgway et al., 1992). Second, cellular levels of cholesterol can regulate the association of OSBP to the tyrosine and serine/threonine phosphatases HePTP and PP2A (Wang et al., 2005), forming a complex that keeps extracellular signal-regulated kinases (ERKs) in an inactive state (Wang et al., 2005). Third, OSBP overexpression interferes with cholesterol and sphingolipid metabolism (Lagace et al., 1997; Lagace et al., 1999). Fourth, OSBP sustains the oxysterol-stimulated and CERT-dependent synthesis of sphingomyelin (Kawano et al., 2006; Perry and Ridgway, 2006), with a mechanism that remains to be defined. Thus, OSBP provides a link between sterol metabolism, sphingolipid homeostasis and signal transduction; what remains to be defined is whether this important role is accomplished by the sensing or transferring of sterols.

Seven OSBP-homology (Osh) genes have been identified in yeast, none of which is individually essential, but which together are required for cell viability (Fairn and McMaster, 2008). The different Osh proteins have different sub-cellular distributions and

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distinct partners. Osh4p-Kes1p is the only one of these to have been crystallised (Im et al., 2005), and it has features of a lipid-transfer protein with a hydrophobic cavity that is protected with a flexible lid. Indeed, Osh4p-Kes1p can extract lipids (sterols, and also phosphatidylserine) from liposomes in vitro, and has been shown to be involved in the PM-to-ER transfer of sterols (Raychaudhuri et al., 2006).

Osh4p-Kes1p was originally isolated as a factor that, once suppressed, could rescue some of the defects that were induced by *Sec14* mutations in *S. cerevisiae* (Fang et al., 1996). Sec14p is a yeast PITP homologue that controls the balance of PI and phosphatidylcholine metabolism (Mousley et al., 2007). Mutations in *Sec14* induce a pleiotropic phenotype, which includes severe impairment of cell growth, secretion defects and complex changes in lipid metabolism, including a decrease in the levels of PtdIns(4)*P* (Hama et al., 1999). Interestingly, the inactivation of *Kes1* also partially compensates for defects that are induced by mutations of *Pik1* (Li et al., 2002). Thus, Kes1p appears to be a negative regulator of pathways that involve the generation of PtdIns(4)*P* at the Golgi complex. Other findings that link *Kes1* to PtdIns(4)*P* are: its ability to bind PtdIns(4)*P* in vitro through its non-canonical PH-like domain, the requirement for its PI-binding domain for Golgi localisation, and its de-localisation from Golgi membranes when PtdIns(4)*P* is decreased because of mutations in *Pik1* (Li et al., 2002). A recent analysis of the compensatory mechanisms that are triggered by defects of Kes1p in *Sec14* mutants (Fairn et al., 2007) has led to the conclusion that *Kes1* has a key role in governing the level and availability of PtdIns(4)*P* in the Golgi membranes by inhibiting Pik1p and possibly directly controlling the free pool of PtdIns(4)*P* in these membranes.

Thus PtdIns(4)*P* is central to the activity of Osh4p-Kes1p in the secretory pathway, a role that has also been highlighted by the recent visual screening of genes that are involved in the surface delivery of biosynthetic cargo (Proszynski et al., 2005). However, another interesting feature of Osh4p-Kes1p that is likely to be relevant for its role in membrane trafficking is that it has an ALP motif in its N-terminus, which is a lipid-packing sensor motif that allows proteins to associate with highly curved membranes (Drin et al., 2007).

FAPP2

FAPP2 was originally described as an effector of Arf1 and PtdIns(4)*P* that is involved in TGN-to-PM trafficking (Godi et al., 2004), and has a prominent role in cargo delivery to the apical PM in epithelial polarised cells (Vieira et al., 2005). Recently, FAPP2 has been demonstrated to be a glycolipid-transfer protein that mediates the non-vesicular transport of glucosylceramide from its site of synthesis (the cytosolic leaflet of the Golgi complex) to its site of conversion into more complex glycosphingolipids (GSLs) in the later Golgi compartments (D'Angelo et al., 2007). FAPP2, therefore, is required for synthesis of GSLs, which thus depends on PtdIns(4)*P* production at the Golgi complex and on the small GTPase Arf1. FAPP2 has also been shown to be required for a retrograde pathway of glucosylceramide transport from the Golgi complex to the ER (Halter et al., 2007).

The functionality of the GLTP domain of FAPP2 is required for its role in membrane trafficking from the TGN to the PM, suggesting an involvement of GSL metabolism in TGN function (D'Angelo et al., 2007); however, the ultimate mechanism that connects these two activities of FAPP2 remains to be fully understood. Finally, FAPP2 has been shown to be involved in the formation of primary cilia by promoting the formation of condensed PM domains, which are also enriched in complex GSLs (Vieira et al., 2006).

Other PtdIns(4)*P* effectors

The *Pichia pastoris* PpAtg26 protein has recently been shown to bind PtdIns(4)*P* through its glucosyltransferase Rab-like, GTPase activator and myotubularin (GRAM) domain (Yamashita et al., 2006). PpAtg26 is a UDP-glucose sterol glucosyltransferase that is involved in the autophagic degradation of peroxisomes (a process termed pexophagy). Pexophagy depends on the generation of PtdIns(4)*P* through the PI4Ks PpPik1 and PpLsb6, but not PpStt4. PtdIns(4)*P* is required for the recruitment of PpAtg26 to the micropexophagy-specific membrane apparatus (MIPA) and for membrane elongation of the forming MIPA. As both PpPik1 and PpLsb6 do not localise to the MIPA, their product, PtdIns(4)*P*, is thought to be transferred from its sites of synthesis to the MIPA in a way that is not yet understood (Yamashita et al., 2006).

The p21-activated protein-kinase-related kinase Cla4p can be considered to be a PtdIns(4)*P* effector at the PM in *S. cerevisiae*. Cla4p binds the Rho GTPase Cdc42p through its p21-activated kinase-binding domain (PBD) and the PIs through its PH domain (Wild et al., 2004). Cla4p is recruited to the PM by the Stt4p-dependent pool of PtdIns(4)*P* and – at the PM – is activated by Cdc42p at corresponding sites of polarised growth (Wild et al., 2004). Interestingly, a component of the Cdc42p-centred molecular machinery, the scaffold protein Bem1p, also has a Phox homology (PX) domain that can bind to PtdIns(4)*P* (Stahelin et al., 2007). Bem1p and Cla4p interact with each other and with Cdc42p; Bem1p also interacts with Cdc24 (the guanine nucleotide-exchange factor for Cdc42). Interestingly, Cdc42p and Cla4p are controlled by Sec14p, thus reinforcing their relation with PtdIns(4)*P* and highlighting a role for PtdIns(4)*P* in the assembly of the protein machinery that is involved in polarised growth and yeast budding (Howe et al., 2007).

Concluding remarks and future perspectives

Owing to the work of many laboratories over the past decade or so, PtdIns(4)*P* has emerged as an important and direct regulator of several cellular processes, most of which are conserved from yeast to mammals. The central role of PtdIns(4)*P* in anterograde membrane trafficking at the exit of the Golgi complex, and in sphingomyelin and GSL metabolism makes it a master controller of the protein and lipid fluxes towards the cell surface and, therefore, of the composition of the PM itself.

The list of proteins and protein domains that have affinities for this lipid is steadily expanding, and we have the reasonable expectation of uncovering as-yet-unforeseen general principles of the organisation of living systems. However, our present knowledge of PtdIns(4)*P* regulators and effectors is still fragmentary and future efforts are needed to provide a more comprehensive picture.

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